Outcome of Pregnancy in Epileptic Women

A Prospective Evaluation of Genetic and Environmental Risk Factors

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ABSTRACT

A prospective study of 116 pregnancies in epileptic women was carried out in order to assess the distribution of newborn growth parameters, and the risk of abnormal outcomes (spontaneous abortions, perinatal deaths, developmental anomalies, deformations or hernias). The putative risk factors associated with these outcomes were assessed by multivariable analyses. Major congenital anomalies were increased and head circumference was decreased relative to the general population. Maternal plasma phenytoin or phenobarbital levels were positively associated with developmental anomalies and total abnormal outcomes. An increase in maternal seizures and/or anticonvulsant drugs, as well as detoxification defects of phenytoin arene oxide metabolites were correlated with decreased head circumference, while family history of seizures was associated with reduced birth weight and/or length. Decreased blood folate levels were correlated with spontaneous abortion and developmental anomalies. The present study suggests that adverse pregnancy outcomes in epileptic women may have heterogeneous multifactorial etiologies and mechanisms.

RÉSUMÉ

Une étude prospective de 116 femmes épileptiques enceintes est réalisée afin d'évaluer la distribution des paramètres de croissance des nouveau-nés et les risques d'issues anormales (avortements spontanés, décès périnatals, anomalies du développement, déformations ou hernies). Les facteurs de risque hypothétiques associés à ces issues sont évalués à l'aide d'analyses multivariables. L'auteur note une hausse des anomalies congénitales graves et une diminution du périmètre crânien par rapport à l'ensemble de la population. Les taux plasmatiques maternels de phénytoïne ou de phénobarbital sont associés à des anomalies du développement et à l'ensemble des issues anormales. Une augmentation des convulsions chez la mère et(ou) une hausse de la posologie d'anticonvulsivants ainsi qu'une biotransformation insuffisante des métabolites de l'oxyde arene de la phénytoïne sont associées à une réduction du périmètre crânien, tandis que des antécédents familiaux de convulsion sont liés à un moindre poids et(ou) à une taille plus petite à la naissance. On constate également une corrélation entre la réduction des taux sanguins de folates et les avortements spontanés et les anomalies du développement. A la lumière de cette étude, il semble que les issues indésirables des grossesses observées chez les femmes épileptiques pourraient s'expliquer par des étiologies et des mécanismes plurifactoriels hétérogènes.

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CONTRIBUTION TO ORIGINAL KNOWLEDGE

Many of the previous studies of the outcome of pregnancy in epileptic women were carried out retrospectively and assessed outcome only at birth. The present study carried out prospectively, carefully collected a large body of data concerning putative risk factors in epileptic women prior to knowledge of the outcome of pregnancy. This method limited information bias. Follow-up of the outcome of pregnancy extended beyond the neonatal period, thereby increasing the likelihood of detecting major congenital anomalies which may have been asymptomatic or missed at birth.

Several types of pregnancy outcome were evaluated including newborn growth parameters, spontaneous abortions, and major congenital anomalies. Unlike previous studies, congenital anomalies were divided into developmental anomalies, arising from errors during morphogenesis, as well as isolated congenital postural deformities or hernias. In this way, attempts were made to assess which type(s) of risk factors might be related to purely developmental anomalies and/or to anomalies in which mechanical factors may play a role. Specific and total abnormal pregnancy outcomes were each evaluated in relation to the same potential risk factors. Thus the possibility of heterogeneous, multifactorial etiologies for different pregnancy outcomes could be explored.

Univariate analysis showed a highly significant association between plasma anticonvulsant levels (phenytoin and /or phenobarbital) and

abnormal pregnancy outcome, including spontaneous abortion, developmental anomalies and decreased head circumference. Frequency of maternal seizures was also significantly negatively associated with decreased head circumference. This is the first human study to provide evidence for these relationships.

Most previous studies have evaluated only one or a few potential risk factors for abnormal pregnancy outcome in epileptic women In the present study, a large number of putative risk factors were evaluated by multivariable analyses. In particular dose and plasma levels of specific anticonvulsant drugs and the frequency of maternal seizures in each trimester of pregnancy and for the entire pregnancy were assessed in relation to pregnancy outcome, after having controlled for the possible confounding effects of family history of seizures and major congenital malformations, and other factors. This is the first study to show that developmental anomalies are positively correlated with plasma phenytoin levels in the first trimester following multivariable analyses. Furthermore, this is the first study to show that head circumference at birth is negatively correlated with maternal plasma phenytoin levels and/or the frequency of maternal seizures following multivariable analyses. These findings provide a strong argument for the teratogenic role of these factors

In the search for possible mechanisms for the teratogenic effects associated with anticonvulsant use, blood folate levels were evaluated in relation to pregnancy, number and dose of anticonvulsants, and

pregnancy outcome in epileptic women. The results suggested that a decrease in blood folate levels periconceptionally or early in pregnancy may be related to the occurrence of spontaneous abortion and developmental anomalies Detoxification defects for arene oxide metabolites of phenytoin were also correlated with abnormal pregnancy outcome This provides a means for predicting which families are genetically predisposed to having abnormal fetal outcomes. This is the first study to have provided evidence for one or both of these mechanisms (folate and detoxification defects) in epileptic women.

In summary, the major contributions of the present study are: developmental anomalies and decreased head circumference at birth are correlated with maternal plasma phenytoin levels and/or the frequency of maternal seizures following multivariable analyses, and abnormal pregnancy outcomes are significantly associated with decreased maternal blood folate levels and an inherited defect in the detoxification of phenytoin arene oxide metabolites.

Risk factors found to be important in the occurrence of abnormal pregnancy outcomes in the present study might in future be used to develop models of prediction, providing probabilities of the occurrence of abnormal pregnancy outcome in the individual epileptic woman.

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I. INTRODUCTION

Prior to the development of modern epileptology, epilepsy was regarded as a disabling, progressive, and intractable disease, usually associated with mental and genetic deficiency. At various times, superstitious ideas concerning the origin of epilepsy also prevailed, and an epileptic seizure was thought to be a manifestation of supernatural forces. These beliefs stigmatized the epileptic and led to the enactment of eugenic laws limiting his or her right to marry and reproduce. Apart from legal and social sanctions, the likelihood that the epileptic would marry and have children has also depended on the nature, severity and duration of the disease.

In the last century, scientific and medical developments have contributed much to the understanding and treatment of epilepsy. In the late 1800's, a neurophysiological basis for the disorder was established with John Hughlings Jackson's description of a convulsion as an excessive neuronal discharge. The discovery of the electroencephalogram by Hans Berger in 1929 led to the correlation of specific EEG patterns with particular types of seizure disorders, an important diagnostic prerequisite for present-day selective drug therapy.

The first breakthrough in the treatment of epilepsy occurred when the bromides were introduced in 1857. The less toxic and more effective phenobarbital was introduced for clinical use in 1912. A major landmark in the history of epilepsy was the discovery of phenytoin in 1936, which proved to be effective in many cases of generalized and partial epilepsies. In the past three decades, many other anticonvulsant drugs have been developed, most importantly

carbamazepine and valproic acid. Monitoring of serum concentrations of anticonvulsant drugs has become an important adjunct in the pharmacological control of seizures. These developments, together with advances in the neurosurgical treatment of intractable seizures, have markedly improved the prognosis of this disease.

Through the studies of William Lennox and others, the concept that there is a high risk of epilepsy in the offspring of all epileptics has been discounted in recent decades. Rather, the epilepsies are seen as a heterogeneous group of disorders, only a small proportion of which are caused by single mutant genes, such as tuberous sclerosis, and by chromosomal aberrations, such as trisomy 13-15. However, the vast majority are due to the interaction of genetic predisposition and various environmental factors, resulting in relatively low recurrence risks in the offspring.

It is estimated that about 3.3 per 1000 births are to women with epilepsy (Kelly, 1984a). This number is probably increasing, because in recent times, progress in medical treatment and in the public understanding of the disease resulting in greater social acceptance of the epileptic, have afforded epileptics wider opportunities to marry and have children (Dansky et al., 1980). In approximately 75% of epileptic patients, symptoms of the disorder occur before the age of 20 (Lennox, 1960), and treatment is usually longterm, continuing well into the reproductive years. These developments, along with a greater awareness, in general, of the potential dangers of drugs and chemicals on the unborn child, heralded concerns about the potential teratogenic effects of anticonvulsant medication.

In the past two decades there have been an increasing number of

studies showing an association between in utero exposure to anticonvulsant medication and an increased risk of both major and minor congenital malformations, as well as abnormalities of growth and development. Sabin and Oxorn (1956) were the first to report the occurrence of major congenital malformations in the offspring of epileptic women. In Germany, Müller-Kuppers (1963) decribed a boy with microcephaly, cleft lip and palate and mental retardation who had been exposed to mephenytoin in utero. The possible effects of maternal anticonvulsant therapy on the unborn child were then formally investigated in Germany, after the teratogenic effects of thalidomide became evident (Janz and Fuchs, 1964). Meadow (1968, 1970) and Speidel and Meadow (1972) in England subsequently described a pattern of major and minor anomalies involving the orofacial, cardiovascular and skeletal systems in children exposed in utero to substantial doses of anticonvulsant drugs. Following these reports, most epidemiological studies showed that offspring of epileptic women taking anticonvulsant medication during pregnancy have a 2-3 fold increased risk of congenital malformations as compared with controls or the general population. However, some studies have also found an increased prevalence of congenital malformations in offspring of untreated epileptic women and of epileptic men.

Since many of these findings are multifactorially determined, as is epilepsy itself, the interaction of genetic and environmental factors must be considered in the etiology of aberrant growth and development There has been much discussion as to whether constitutional factors associated with epilepsy itself, maternal seizures or other complications of pregnancy, rather than anticonvulsant medication,

may be responsible. Some authors believe that many of the aberrant features in offspring of epileptic women are related to the genetic background of the epileptic, and that anticonvulsant drugs increase the occurrence of abnormality in those individuals who are genetically predisposed (Janz, 1982a,c) Still others believe that it is the severity of the disease rather than its treatment which may be the important factor (Majewski et al., 1981) It has been difficult to attribute abnormalities of growth and development to any one factor, particularly anticonvulsant drugs, due to the complex interrelationship among possible antenatal risk factors, both genetic and environmental.

If anticonvulsant drugs are teratogenic, one would suspect this effect to be determined in part by the amount of drug reaching the embryo. Although animal studies have demonstrated a dose-response relationship, human studies have so far been controversial. In many of these studies, plasma anticonvulsant levels were not monitored. Moreover, it is well known that patients with epilepsy do not always comply with anticonvulsant treatment, and that they may exhibit large interindividual variation in plasma levels at any given dosage.

Further studies are needed to investigate what genetic and environmental factors determine adverse pregnancy outcomes in some epileptic women, using methods which will take into account the contribution of possible confounding variables The role of type(s) and dose of anticonvulsant drugs needs to be separated from the part played by epilepsy itself Putative mechanisms for the potential teratogenic effects of anticonvulsant medication should be considered, such as the possible effects of drug-induced deficiency states and the contribution of genetic factors which determine susceptibility to congenital

malformations, and pharmacological differences in the metabolism of anticonvulsant drugs.

The present study utilizes univariate and multivariable analyses in order to more fuliy assess the multifactorial etiology of adverse pregnancy outcome in epileptic women including spontaneous abortion, major congenital anomalies and altered growth parameters at birth Major factors assessed are dose and plasma levels of anticonvulsant medication, and putative mechanisms for the teratogenic effects of anticonvulsant medication, including blood folate levels and detoxification defects of phenytoin metabolites. Other possible risk factors such as family history of congenital malformations, type of maternal epilepsy, frequency of seizures and other complications during pregnancy are also analyzed.

II. REVIEW OF THE LITERATURE

A. Abnormal Pregnancy Outcomes in Epileptic Women

Table II.1 shows a number of abnormal pregnancy outcomes of women with epilepsy, many of which have been shown to be increased as compared with nonepileptic women. The present thesis will mainly review the literature concerning prenatal and postnatal mortality, congenital malformations and somatic parameters at birth The literature concerning the condition of the newborn, and postnatal growth and development will also be discussed

A general review of normal and abnormal growth and development of the embryo and fetus is presented in sections II B to F General reviews of the etiology of congenital malformations and of abnormal growth in the general population are presented in sections G and H,

Table II.1

Abnormal Pregnancy Outcomes in Women with Epilepsy

Spontaneous abortion

Perinatal death

Neonatal and infant death

Prematurity

Hypoxia

Coagulation disorder

Drug withdrawal

Feeding difficulties

Major congenital anomalies (e.g. cleft lip ± cleft palate)

Minor congenital anomalies (e.g. digital hypoplasia)

Craniofacial dysmorphic features

Prenatal growth deficiency

Postnatal growth deficiency

Developmental delay

Carcinogenesis (e.g. neuroblastoma)

Seizures

respectively. Methodological strengths and weaknesses of various epidemiological approaches to evaluating pregnancy outcomes in epileptic women, and of experimental studies are discussed in section I. This is followed by the results of studies in epileptic women concerning the occurrence of congenital anomalies and patterns of anomalies in the offspring and possible etiological factors in sections J and K, respectively. Experimental animal studies of congenital anomalies induced by anticonvulsant drugs are discussed in section L. The results of studies of somatic parameters at birth in children of epileptic patients are reviewed in section M and postnatal growth is discussed in Section N. The effects of anticonvulsant medication on growth in experimental animal studies is presented in section O. This is followed by the findings of studies in epileptic women and experimental studies concerning mental development in section P. Other outcomes of pregnancy: spontaneous abortion, perinatal death, infant death, prematurity, and the condition of the newborn are presented in section Q. Finally putative mechanisms for the teratogenic effects of anticonvulsants are reviewed in section R.

B. Manifestations of Abnormal Growth and Development

From gametogenesis to birth, certain stages of development are more vulnerable to specific teratogenic effects than others. The type and severity of effects that a potential teratogen will have on reproduction will depend in part on the teratogen and on the stage of development. Abnormal reproductive responses to a teratogen can range from infertility, spontaneous abortion, growth retardation, congenital defect (malformation, deformation) and stillbirth to functional abnormalities.

The following presentation briefly reviews the normal growth and development of the embryo and fetus, and the types of abnormalities that may be produced by teratogenic agents at different stages of development.

C. Development of the Embryo and Fetus

Four sensitive periods of prenatal development may be defined, involving gametogenesis, blastogenesis, embryogenesis, and the fetal period of growth and maturation.

1. Gametogenesis

Spermatagonia develop in the fetus and remain dormant until puberty when they begin to divide to form mature sperm. Transformation from immature to mature sperm then proceeds continually and the entire maturation process takes about 64 days (Goldman, 1979).

The length of time involved in the development of the egg is much longer than that for the sperm. In the ovary of the fetus, eggs enter the first division of meiosis and remain in late prophase of the first meiotic division until just before ovulation. Thus, maturation of the

ovum occurs at the earliest at puberty, and may be delayed until the very late reproductive years.

The prolonged prophase of the ova is one of the explanations postulated for the increased occurrence of chromosomal abnormalities in offspring of older mothers, such as in Down's syndrome. The ova that are released in later reproductive years are thought to be subjected for many years to potential adverse environmental factors, such as chemicals, radiation, and drugs which may increase the risk of chromosomal non-disjunction when the ovum resumes meiosis (Goldman, 1979).

It has also been suggested that drugs or chemicals excreted into the semen may induce adverse pregnancy outcome. For instance, thalidomide was observed to have penetrated into the semen of male rabbits pretreated with the drug, and a high incidence of fetal loss was subsequently noted (Lutwak-Mann et al., 1967). Other agents such as methadone, phenytoin, and valproic acid have also been detected in semen (Goldman,1979; Swanson et al., 1978a,b).

Toxic insults during the period of gametogenesis may result in abnormal morphology and motility of the sperm or egg, death of the sperm or egg, and chromosomal defects in the sperm or egg leading to spontaneous abortion, growth retardation or congenital defects of the embryo (Goldman, 1979).

2. Blastogenesis

In humans, the fertilized ovum is transported down the fallopian tube to the uterine cavity where it implants into the endometrium about day 7 after fertilization. During this time, the fertilized egg remains in a

relatively undifferentiated state as it develops from a unicellular zygote to a multicellular blastocyst, by a series of mitotic divisions .

The preimplantation blastocyst lies free within the fallopian tube and uterus and receives its nutrition from their secretions. Although congenital defects have been induced in the preimplantation stage in animals, it is not known whether this can also occur in humans. For example, in animals, drugs that interfere with nucleic acid synthesis, such as actinomycin D, have been shown to produce congenital defects when administered very early in gestation. A number of drugs such as nicotine and caffeine have been shown to penetrate into the tubular or uterine fluid and into the pre-or post-implantation blastocyst, causing growth retardation or degeneration . Furthermore, implantation of the embryo can be prevented by the administration of estrogens (Persaud, 1985a; Goldman, 1979).

Despite these examples, it is thought that the early embryo is generally resistant to the induction of congenital malformations. Typically, because of the relative omnipotency of the cells of the early embryo, the effect of an embryotoxic agent during the preimplantation stage is an all-or-none phenomenon: either the damage affects so many cells that the embryo dies and spontaneous abortion follows, or so few cells are affected that the embryo is capable of repairing the damage (Persaud, 1985a)

Although drugs administered either prior to conception or early post-conception may not have a direct action on the early embryo, it seems possible that some drugs may later exert teratogenic effects indirectly by altering the maternal environment, or because of long half-lives, by later affecting the critical period of embryonic

development (Hutchings,1983).

3. ***Embryogenesis***

In the second week following fertilization, there is rapid proliferation and differentiation of the trophoblast with the beginning of a uteroplacental circulation, and formation of a bilaminar embryo. Several chemicals and drugs in animals (e.g. retinoic acid) have been shown to be teratogenic during this stage, but it is not known whether this is also the case in humans (Persaud,1985*a*).

The period extending from the formation of the three primary germ cell layers, through cell and tissue differentiation to the completion of organogenesis (3-8 weeks of gestation) is considered the most susceptible period for the development of congenital malformations. Embryonic death and growth retardation may also occur at this time.

During this period, the biochemical and morphological differentiation of specialized cells into tissues and organs of the body involves the processes of cell replication, cell migration, the interaction of cells and tissues, cell degeneration and cell death. These processes result from the selective activation and inactivation of genetically preprogrammed instructions. Different organs and systems develop at varying times and rates, so that critical periods of differentiation are recognized for different organs. These critical periods vary among different species (Tuchmann-Duplessis, 1983, Persaud, 1985*a*)

A wide variety of genetic and environmental factors can interfere with this developmental program and induce congenital defects. Wilson (1977a) has formulated a model whereby teratogenic agents initiate a sequence of pathogenic events, beginning at the cellular level:

Interference with fundamental cell processes such as nucleic acid synthesis, microtubule formation, or enzyme activity, leads for example to disruption of cell migration or cell death. The different types of pathogenesis converge "into a relatively narrow channel of maldevelopment, one that would lead ultimately to insufficient cells or cell products to carry out morphogenesis or to carry on function at the site of the final defect".

The type of defect will depend on the effect of the agent on embryonic cells and the stage of organogenesis at the time of exposure to the harmful influence (Wilson, 1977a). Thus, a specific teratogen may induce more than one type of defect, if given at a time when there is an overlap in the developmental timing of different organs, and different agents given at the same time of gestation may induce the same defect, if they have similar effects on morphogenesis.

4. Fetal Period

By the 9th week, the major period of organogenesis is completed, and the likelihood of a major structural malformation has diminished. Then follows the fetal period characterized by the processes of growth, histogenesis, functional maturation closely paralleling the process of histogenesis, and the storage of energy substrates (Tuchmann-Duplessis, 1983).

Environmental agents at this stage of development may result in spontaneous abortion, prematurity, or malformations of late-developing structures (for example, hypospadias). There may be injury to a previously normally developed tissue or organ (for example, necrosis of fetal brain due to maternal infection with toxoplasmosis). As the fetus

tends to outgrow the uterus late in gestation, uterine constraint may result in deformations of a normally formed fetus (for example, clubfoot). As well, failure to achieve functional competence might result from fine structural defects due to toxic insults during the process of histogenesis (Hutchings, 1983; Perrin, 1982).

The processes of growth, histogenesis, and functional maturation are by no means completed with birth. The nervous system as well as some other systems continue to mature in infancy and childhood. The extent to which these relatively late-maturing processes are vulnerable to harmful influences is largely unexplored (Perrin, 1982).

D. Growth of the Embryo and Fetus

During the course of some 280 days the human zygote increases in weight by a factor of several billion and from a single cell about one tenth of a millimeter in diameter to a complex organism (Widdowson, 1968)

1. Definition and Components of Growth

Growth may be defined as an increase in size or body mass and as such is reflected by an increase in weight and body dimensions (MacMillan, 1970).

At the cellular level, growth occurs in three sequential stages: a hyperplastic stage involving a rapid increase in deoxyribonucleic acid synthesis and cell replication, a combined or intermediate phase of increase in cell number (now slower) and cell size, and a final stage of hypertrophic growth involving only an increase in cell size.

Growth occurs continually throughout gestation , but the rate of

growth is not constant. In the first two or three weeks of in utero development, growth takes place entirely by cell division. As the embryo develops and its tissues differentiate, there is a slowing down of cell division and as this occurs the cells begin to increase in size, as a manifestation of the accretion of protein and in some cases lipids. Differentiation of tissues also contributes to the process of growth by the cellular production of substances forming intercellular matrices, such as cartilage and connective tissue (Widdowson, 1981; Winick, 1971).

The end of the hyperplastic phase and the relative contribution of cell number and cell size to growth varies with the organ and tissue. For instance, the potential for the addition of glial cells in the brain extends up to the second postnatal year, while that for muscle cells continues through adolescence (Brasel and Gruen,1986; Enesco and Leblond, 1962).

2. *Susceptible Period to Growth-Retarding Influences*

It has been suggested that during the time of rapid growth, the embryo and fetus would be most susceptible to growth-retarding influences (Dobbing 1970; Winick, 1971). The following sections review growth patterns of the human embryo and fetus with respect to weight, length and head circumference. As well, some aspects of brain growth are also described.

3. *Increase of Weight, Length, and Head Dimensions during Gestation*

In the first week after conception during which the fertilized ovum

undergoes cleavage forming a blastocyst of about 100 cells there is relatively little increase in size (Southgate and Hey, 1976). From the second week to the end of embryogenesis, the blastula increases in size from around 0.1 mm to an embryo 27 to 31mm long weighing about 2.7 grams (O'Rahilly and Müller, 1986). From the early fetal period to about mid-gestation, weight increases to about 300 grams and crown-rump length to 160 mm (Birkbeck, 1976), while in the latter half of gestation length increases to 51 cm. and weight to 3500 grams (Usher and McLean, 1969).

From animal data, growth or increase in size of the embryo and fetus during gestation is shown to follow a sigmoid or S-shaped curve and it appears that the human embyo and fetus follows a similar pattern (Campbell, 1976).

Some measurements of the early human embryo are available from preserved specimens (O'Rahilly and Müller, 1986) Measurements have also been made ex utero on fresh fetal material without obvious abnormalities obtained through spontaneous abortion or terminated pregnancies, and on term and preterm liveborn and stillborn neonates (Birkbeck, 1976; Brenner et al., 1976; Davies, 1981; Jones et al., 1986; Usher and McLean, 1969). Curves showing embryonic and fetal size and fetal growth velocity (increment in size per unit time) relative to gestational age have been calculated. In addition, ultrasound has proved a valuable means of obtaining measurements of length and head dimensions of the embryo and fetus and of evaluating the growth velocity of these different dimensions in utero (Campbell, 1976; Meire, 1986).

In the embryonic period, there is a curvilinear relationship between

embryonic length and gestational age (O'Rahilly and Müller, 1986). The charts of Usher and McLean (1969) showing fetal length in relation to gestational age indicate that the increase in fetal length is almost linear in the latter part of pregnancy with flattening beginning at about 38 weeks of pregnancy. Crown-rump length has been shown to increase rapidly from the earliest time it can be measured by ultrasound (6 weeks) to 14 weeks postmenstrual age, with a doubling of size every 10 days and a linear increase in growth velocity or constant acceleration of 1.4 mm/week during this period, reaching a peak of 14mm/week at 14 weeks postmenstrual age. This indicates that the embryo is growing at its maximum rate. From late fetal material ex utero, it has been found that growth velocity also increases steadily, although not as steeply, from 10 mm/ week to 13 mm/ week between 25 and 31 to 32 weeks, remaining constant until 33 to 34 weeks. A gradual slowing down of growth velocity then occurs until term (Davies 1981).

Growth of the head shows different patterns according to the index of head growth. Growth of the biparietal diameter and head circumference is almost linear from the earliest time these dimensions can be measured in utero (11 to 12 weeks) until 30 to 32 weeks of pregnancy. Then growth slows down, more so after 36 weeks to term (Campbell, 1976; Jones et al, 1986; Meire, 1986; Pearce and Campbell, 1983).

Growth of fetal head volume computed from multiple head measurements or head circumference in relation to gestational age gives a clearer picture of the actual pattern of head growth. The curve of head volume in relation to gestational age is approximately sigmoid

in shape. From 13 to 28 weeks of gestation, there is a linear increase in growth velocity of head volume. The velocity then reduces abruptly at 30 weeks of gestation and progressively decreases to term (Meire, 1986).

The intrauterine deceleration of growth of head dimensions towards term , does not appear to reflect an underlying slowing down of brain growth, as the curve for growth of brain weight increases steadily over the same period (Dobbing and Sands, 1978) Thus measurement of external dimensions of the head may not entirely reflect brain growth (Davies, 1981).

Fetal weight cannot be recorded directly in utero. Charts of weight in relation to gestational age derived from measurements on the fetus outside the uterus (age 10 weeks to term) show a sigmoid curve In the third trimester, fetal weight increases from about 1000 gm to 3500 gm (Southgate and Hey, 1976; Usher and McLean, 1969). Between 34 and 38 weeks postmenstrual age, the rate of fetal weight gain reaches its peak at 200-225 gm/week (Brenner et al. 1976). After reaching the maximum weight gain, the growth rate declines to term.

There is some evidence to indicate from ultrasonic measurements that the growth of the fetus may not be uniform during gestation and that periods of acceleration and deceleration occur at intervals during gestation as they have been noted to occur during postnatal somatic growth (Meire, 1986).

Why the rate of increase of different fetal growth indices decelerates towards term has remained a matter of speculation. As the placenta grows more slowly than the fetus late in gestation resulting in a decreased placental: fetal weight ratio towards term it has been

postulated that the reduction in growth rate of the fetus towards term is due to declining nutritional support from the placenta (Gruenwald, 1963). However, it is unlikely that placental weight alone accurately reflects placental function (Gluckman and Liggins, 1984; Meschia, 1978).

In summary, growth of various dimensions of the fetus encompasses all of gestation The rate of growth varies with different dimensions and with the period of gestation, but essentially is most rapid in the first two trimesters and early third trimester of pregnancy for head dimensions and body length, whereas weight gain is most rapid in the latter part of the third trimester due to the accumulation of important components of fetal mass such as fat at this time. It is often assumed that since most of fetal weight gain is in the third trimester, that the origin of low weight gain must be in the third trimester. However, an unusually low weight gain may be due to the fact that fetal growth was compromised earlier in pregnancy, and the fetus entered the third trimester with a below normal complement of cells (Wynn and Wynn, 1981).

4. Brain Growth

As head size gives some indication of brain growth it would be important to review some aspects of fetal and postnatal brain development. The total mass of the brain increases rapidly from 4 to 5 months gestation until the end of the first postnatal year, moderately fast during the second year and at a slower rate until at least the age of 8 years (Dobbing and Sands, 1973).

DNA estimations of whole brain have revealed two rapid periods of cell division. The first occurs from 10 to 18 weeks of gestation and is

thought to represent neuronal multiplication. The greatest number of brain cells are glial and these divide after the neuronal population, from 30 weeks of gestation into the first postnatal year, with a peak at 3 months of postnatal age Increases in DNA in whole brain plateau at approximately 18 months of age, prior to the cessation of total brain growth (Dobbing 1970; Dobbing and Sands, 1973).

Concurrently with glial proliferation there is also growth of the number and length of cell processes and the consequent establishment of synaptic connections which continue until several years postnatally Myelination occurs from 9 months of gestation until several years postnatally (Dobbing , 1970; Dobbing and Sands, 1973)

Not all these changes occur at the same time or rate in different regions of the brain In particular, growth of the cerebellum compared with that of the forebrain begins later at 30 weeks postmenstrual age, and finishes earlier by the end of the first postnatal year (Dobbing and Sands,1973).

In view of the pattern of human brain development, prenatal adverse influences may have different effects depending on the timing of the insult. For instance, adverse factors operating early in pregnancy would be expected to interfere with neuronal multiplication, while adverse factors in the mid to late fetal period and postnatally could interfere with glial multiplication, the development of neuronal processes and myelination, particularly during their periods of fast development Thus retardation of human brain growth might involve either effects on cell multiplication, or non-mitotic processes or both (Dobbing 1970; Dobbing and Sands, 1973).

E. Abnormal Intrauterine Growth

Intrauterine growth retardation has been most commonly considered in terms of a reduction in birth weight and this has been defined in two general ways. The first definition refers to all infants with birth weight less than or equal to 2500 grams irrespective of gestational age: both preterm infants whose weight is appropriate for their gestational age and preterm and term infants with birth weight reduced for gestational age are therefore included. In developed countries, 5.9% of children are born weighing 2500 grams or less and about one third of these are born at term (at or after 37 weeks of pregnancy) (Gould, 1986; Gruenwald, 1963) and are therefore small for their gestational age.

The second definition refers to infants whose birth weights are at or below the 10th or 3rd percentile for their gestational age, as determined with reference to weight for gestation charts of a standard population. This definition encompasses both preterm and term infants who appear to be underweight for their gestational age. These infants are presumed to have grown at an abnormally slow rate. The frequency of infants small for gestational age has ranged from 2 to 8% depending on the definition used (Abdul-Karim, 1975; Gruenwald, 1963; Low and Galbraith, 1974).

There are several limitations inherent in the use of either definition. For example, in the first definition, the infants are a heterogeneous group including those that are small at birth solely because they are preterm, those that are genetically small, and/ or those who are small for pathological reasons. In addition, the use of cut-off points excludes infants who may not have reached their growth potential but whose birth weights happen to fall above the cut-off points. On the other hand,

an assumption in weight for gestation charts calculated from birth weights of infants born at each gestational age is that infants born preterm are normal which may not be correct.

Despite these limitations, definitions such as these have been of value in delineating deviations in fetal growth and the sequelae of low birth weight. For example, infants with low birth weight for gestational age have been found to have higher perinatal and infant mortality than infants with appropriate weight for their age. They have also been found to be at risk for intrapartum asphyxia, neonatal complications such as hypoglycemia and hypothermia, congenital malformations, and longterm problems such as reduced postnatal growth, neurological abnormalities, and poor intellectual outcome. (Cook, 1977; Low and Galbraith, 1974 ; Pearce and Campbell, 1983).

Depending on the time of pregnancy when growth is disturbed, growth retardation will involve a decrease in the number of cells in the body, a decrease in cell size, and/or failure to deposit energy stores. As such, two main clinical types of intrauterine growth retardation have been described in which the infant is symmetric or asymmetric in its body dimensions. In early onset of growth retardation in the first trimester, there is a proportionate retardation of weight, length and head circumference, while in late onset growth retardation there is a reduction in weight, there may be some reduction in length, and head circumference may be spared or mildly reduced (Warshaw, 1979; Gould, 1986). The extent to which length will be affected in addition to weight in the latter type of growth retardation appears to be related to how late in gestation the insult occurs. When the insult occurs late in pregnancy velocity of length increase will have reached a minimum but

velocity of weight increase will have reached a maximum, and there will be a limitation in the amount of muscle, fat, and hepatic glycogen laid down (Gould, 1986; Miller and Merritt, 1979).

The extent to which the head may be affected in late gestation may depend on the velocity of head growth at the time of insult and/or on whether there is any "sparing effect" on brain growth. For example, experimental induction of placental insufficiency in sheep results in disproportionate growth retardation, with effects on the brain mitigated by a redistribution of blood flow to the fetal brain (Campbell, 1976).

In underdeveloped countries where there is a high prevalence of chronic malnutrition most small-for-gestational-age babies are proportionately growth retarded, while in developed countries only 40-50% of such babies are proportionately growth-retarded (Gould, 1986).

The two main patterns of human growth retardation have been confirmed on ultrasound (Campbell, 1976). In the first growth retardation pattern, there is a persistently low growth rate of biparietal diameter from early in the second trimester when this parameter can first be measured, with usually no cessation of growth. In this type, the brain to liver ratio is usually normal. Infants with this type of growth pattern are usually those with a decreased genetic growth potential including those with chromosomal abnormalities, those exposed to severe chronic malnutrition, or other extrinsic adverse factors such as viral infection, maternal smoking, heroin abuse or alcoholism (Gould, 1986; Pearce and Campbell, 1983). In the second type, there is an abrupt slowing and eventual cessation of growth rate

in late pregnancy after a period of normal growth. This type is associated with substrate deprivation which is usually secondary to placental dysfunction (e.g. toxemia); increased brain to liver ratio, and a wasted appearance (Campbell, 1976; Gould, 1986; Pearce and Campbell, 1983).

The infants with the two types of growth patterns in utero have been shown to differ with respect to longterm physical and mental development. The longer the decrease of head growth in utero, the greater were the deficits in postnatal somatic growth and intellectual development (Campbell, 1976).

There are other types of abnormal intrauterine growth: these include fetal overgrowth and disproportionate growth between head size and body length (Miller and Merritt, 1979). An example of fetal overgrowth is the heavy birth weight associated with maternal diabetes. More commonly, large babies result from non-pathological pregnancies in which the mothers are older, of higher parity, taller, heavier, and sometimes show excessive weight gain during pregnancy as compared with mothers of normal weight babies (Davies, 1981).

Normally at birth, head size is proportionate to length, but in 5-10% of babies in the general population, head size is small or large relative to length. The clinical significance of this disproportion has not been investigated (Miller and Merritt, 1979). However, in some forms of familial microcephaly, there may be no neurological impairment, dysmorphic features or mental retardation (Ramirez et al., 1983).

F. Growth Retardation and Congenital Malformations

There is a close association between fetal growth retardation and congenital malformations. In animals, a teratogen will often produce both growth retardation and congenital malformations, either in different fetuses of the same litter, or in the same fetuses. In humans, as well, known teratogens produce both prenatal onset growth retardation as well as a spectrum of congenital malformations (Hanson, 1983).

In man, the strength of the associations between low birth weight and malformations in children differs with the type of malformation, being strongest for those of the central nervous system, somewhat less so for malformations of the digestive system , skeletal system and heart, and least for cleft lip or palate. (Wynn and Wynn, 1981).

It has been suggested that interference with cell replication leading to depletion of cells at a critical time in development is one mechanism for the production of congenital malformations (Wilson, 1977a). Wynn and Wynn (1981) pointed out that fetal growth retardation and malformations have a reduced number of cells as a common factor in most cases (a reduction in the number of cells in the congenitally malformed heart, for instance). They postulated that factors which depress cell replication contribute to the multifactorial causation of both growth retardation and congenital malformations.

G. Etiology of Congenital Anomalies in the General Population

Figure II.1 outlines factors known to be responsible for congenital anomalies in man. Etiologically, congenital anomalies can be subdivided into five categories: those which are due primarily to simple Mendelian

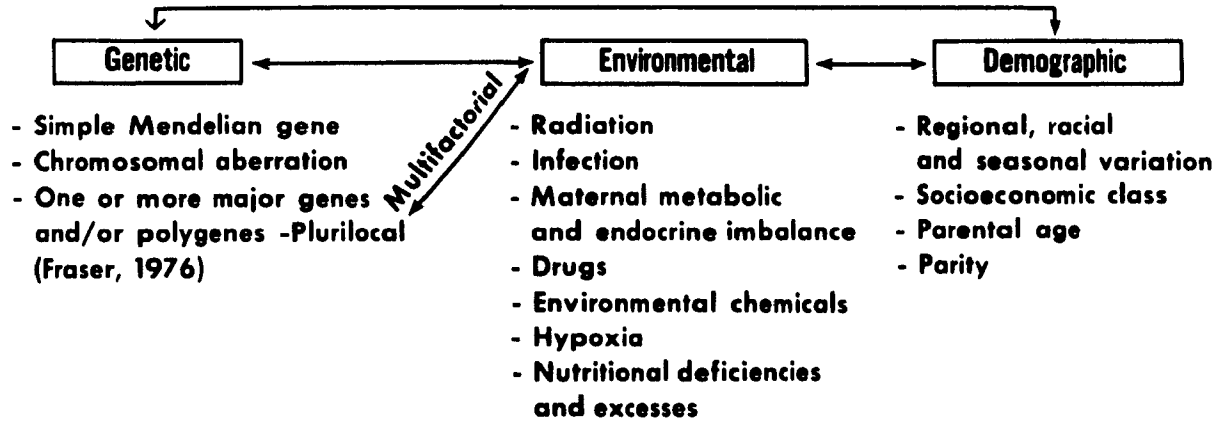

Figure II.1

Etiology of congenital malformations in man. Various etiological factors known to contribute to the development of congenital malformations are listed. Only a small proportion of major congenital malformations in man (about 20%) may be attributed to a single etiology, e.g. a simple Mendelian gene, a chromosomal abnormality, or a single environmental factor. The vast majority are due to a complex interaction of various factors-genetic, environmental, and demographic-to produce the malformed phenotype. A combination of one or more genes and/or polygenes with one or more environmental factors is termed multifactorial inheritance. Frequently, the specific genetic and/or environmental factors implicated in the etiology of congenital malformations are not readily identifiable (Dansky, 1978; Andermann et al., 1981)

variation (5%), those associated with chromosomal abnormalities (10%), those largely determined by a known environmental agent (5%), those for which there is evidence of multifactorial etiology (20%), and finally the vast majority of anomalies for which no cause has been identified (60%) (Fraser, 1977a). The proportions of congenital disorders in each category are crude estimates, and represent what is presently known about the etiology of human congenital anomalies.

1. ***Simple Mendelian Gene***

Some congenital malformations can be attributed to a simple Mendelian gene from one or both parents. On rarer occasions, a mutant gene arising sporadically through new mutation in the maternal or paternal germ cell may result in a congenital anomaly. Most congenital disorders caused by single deleterious mutant genes are rare (fewer than 1 in 100,000), because there has been strong selection against them. Congenital malformations transmitted as simple Mendelian traits show either dominant or recessive inheritance, and may be autosomal or sex-linked.

2. ***Chromosomal Aberrations***

Morphologically detectable abnormalities in the chromosomes consisting of numerical changes (e.g. aneuploidy) or structural anomalies (e.g deletion, translocation) are often associated with multiple congenital malformations or malformation syndromes, but are a relatively rare cause of isolated congenital malformations. The majority of chromosomal aberrations arise sporadically, although on rare occasions, a chromosomal anomaly is inherited. Because most

aberrations lead to intrauterine loss, only a few chromosome disorders have a prevalence of 1 in 1000 or more at birth (e.g. Down's syndrome).

3. ***Environmental Factors***

Environmental interference with normal development is usually considered to occur between conception and birth, in contrast to genetic interference, which is determined prior to fertilization. However, mutant genes and chromosomal aberrations are also caused by environmental agents such as radiation, viruses, drugs and chemicals (mutagenesis). Sensitivity to the dysmorphogenic effects of environmental agents (teratogenesis) is most evident during the organogenetic period in the first trimester of gestation.

Many epidemiological studies have been carried out in various countries to elucidate the environmental factors involved in the development of common congenital malformations. Demographic factors such as race, social class, parity, maternal age, calender year, season of birth have all been associated with the incidence of these disorders in various studies. However, these factors have often been found to be interrelated , and it is very difficult to draw any clearcut conclusions regarding these findings. Furthermore, in most cases, the specific environmental agents which are operating to produce these differences have not been identified

Four main classes of environmental agents have been identified as responsible for developmental abnormality in man. These are physical agents (e.g., radiation , nuclear and therapeutic); mechanical factors (eg., intrauterine molding); infection (e.g., rubella virus); maternal metabolic and endocrine imbalance (e.g., maternal phenylketonuria,

endemic cretinism); environmental chemicals (e.g., organic mercury) and drugs (Wilson, 1977b).

Reviews of one or more of these factors are given by Wilson, 1977b; Shepard, 1986 and Hanson, 1983. A brief summary of one exogenous agent, drugs, will be given below.

The prenatal use of drugs is surprisingly common. In several surveys done in different geographical areas and spanning the last two decades, 40 to 90% of women took at least one drug during pregnancy and each woman took an average of 3 to 10.3 drugs. Analgesics, iron, vitamins, antacids, sedatives, antiemetics, antibiotics, antihistamines, and diuretics were the most frequently used drugs (Persaud, 1985b)

Based on criteria for proof of human teratogenicity , Shepard (1986) classified drugs and chemicals according to the likelihood of their producing teratogenic effects in humans. Among 18 known teratogens were thalidomide, which causes the well-known syndrome of limb defects (amelia, phocomelia) associated to varying extents with facial, heart and intestinal defects; androgenic hormones and synthetic progestins causing virilization of female offspring; folic acid antagonists (aminopterin, methotrexate) producing mainly embryolethal effects, as well as various structural anomalies including craniofacial and skeletal anomalies, and functional defects. Other drugs and chemicals included diethylstilbestrol; organic methylmercury; warfarin; alcohol; lithium; retinoic acid (accutane); and several anticonvulsants (trimethadione, phenytoin, and valproic acid).

At present, only 4 to 6% of human congenital anomalies have been etiologically related to single drugs or chemical entities (Wilson, 1977 b,c). However, experimental evidence in animals suggests that many

human malformations may be multifactorially determined, involving combinations and synergistic interactions of genetic and/or environmental factors, particularly drugs and chemical agents (Wilson, 1973,1977b).

The effects of a teratogen may range from mild effects such as single major abnormalities of morphogenesis which may not be specific for a particular agent to more severe and specific effects, such as a characteristic pattern of altered growth and dysmorphogenesis. When all exposed and affected individuals are considered, single major abnormalities may be more common than a clear pattern or syndrome of abnormalities (Hanson,1983). Some authors believe that the malformation syndromes described for specific agents (eg., fetal hydantoin syndrome, fetal alcohol syndrome) may represent a clinical continuum, rather than being agent specific (Hill, 1976).

Not all individuals exposed to a teratogen will exhibit abnormality or all the specific features of a particular drug-induced syndrome. There are several factors which may account for variability in the effects of a specific teratogen on morphogenesis (Hanson,1983). These are the dose of an agent, timing of exposure, genetic sensitivity (e.g. maternal and/or fetal genetic metabolic differences, liability to specific congenital malformations in one or more organ systems, in the embryo and fetus) and interactions with other environmental agents.

4. *Multifactorial / Threshold Etiology*

The small minority of congenital malformations which are caused by simple Mendelian, chromosomal or major environmental factors may be part of recognizable syndromes. For example, encephalocoele is one

feature of the autosomal recessive Meckel's syndrome; cleft lip and palate is a feature of trisomy 13, and congenital heart disease, such as patent ductus arteriosus, is a common manifestation of the rubella syndrome. However, not all cases of these syndromes show these specific defects. Such clinical variability indicates that the effects of a particular genetic defect or environmental teratogen may be modified by other subtle genetic and/or environmental factors as depicted in Figure II.1.

Apart from usually rare malformation syndromes due to major etiological factors, there are an increasing number of common congenital anomalies, usually involving only a single anatomical system, which show evidence of complex determination. These anomalies, including those of the heart, the neural tube, the face, the gastrointestinal tract and the skeleton, frequently show a familial pattern but pedigree and sib analyses do not fit classical Mendelian ratios. Chromosomal abnormalities are also not involved. Both preconceptional (genetic) and intrauterine environmental factors may be important in determining whether or not an individual will have the disorder.

Many of these malformations have been shown to have similar patterns of expression in relatives (Carter, 1965, 1969, 1976, 1977; Fraser, 1970; Nora et al., 1970). The main features are :

(1) The prevalence of malformation in first degree relatives is much lower than that for Mendelian inheritance (i.e., 25-50%), but higher than that in the general population.

(2) The prevalence of malformation in first-degree relatives approximates the square root of the prevalence in the general

population.

(3) The proportion of affected children is as high as the proportion of affected siblings.

(4) The proportion of affected relatives decreases sharply between first- and second-degree relatives, and less sharply between second- and third-degree relatves.

(5) If one sex is affected more frequently than the other, the proportion of affected relatives is higher when the index patient is of the less frequently affected sex.

(6) The recurrence risk in first-degree relatives increases with the number of near relatives affected as well as with the severity of the condition in the index patient.

(7) The risk of malformation in relatives increases with the presence of parental consanguinity.

These features are not observed in conditions with known simple Mendelian inheritance or major environmental etiology. However, they can be explained in terms of a multifactorial / threshold model in the form proposed by Falconer (1965). According to this model, the likelihood of developing a disorder (liability) is a continuously distributed variable with a threshold value above which individuals manifest the trait. Both genetic and environmental factors contribute to the liability, thus rendering the system multifactorial, as discussed by Fraser (1976). The nature of the genetic element is uncertain for the majority of human congenital malformations. Various models have been proposed ranging from a major gene with variable penetrance to polygenes (many genes, each with a small effect, acting additively). Environmental factors contributing to liability may also be multiple.

For example, in experimental studies of cleft palate in the mouse, (Fraser, 1970, 1977b), an important developmental variable appears to be the stage at which the palate shelves overcome the resistance of the intervening tongue and achieve a horizontal position above the tongue. The threshold for the development of cleft palate is the latest stage at which the shelves can meet each other and fuse, when they do move to this position. The probability of an embryo falling beyond the threshold may be influenced by several factors such as head width, tongue size, tongue mobility, mandible growth, shelf width, shelf force, etc. Both genetic and environmental factors may act on these variables to determine the position of an embryo relative to the threshold. Thus mouse strains in which the palate normally closes early in development are more resistant to cortisone-induced cleft palate. For cleft lip, the nature of the predisposing factors is less clear; however, experimental observations suggest that this malformation may be related to embryonic face shape (Trasler, 1968).

There is indirect evidence that similar biological variables may be involved in the development of human cleft malformations. In Finland, both the width of the head and the frequency of cleft palate increase from East to West (Saxén, 1975). The palate closes later in development in female than in male embryos (Burdi and Silvey, 1969), and this is compatible with the higher frequency of cleft palate in females As well, certain differences in facial topography observed in relatives of probands with cleft lip are consistent with the face-shape hypothesis (Fraser and Pashayan, 1970).

Certain postural deformities such as clubfoot and congenital dislocation of the hip also appear to be due to a combination of genetic

and environmental factors (Dunn, 1976). For example, there is evidence that congenital dislocation of the hip is determined in part by genetic predisposition to joint laxity, and acetabular dysplasia (Wynn-Davies, 1970). Similarly, there is some evidence for an association between clubfoot and a generalized laxity of connective tissue (Wynn-Davies, 1964). These conditions have also been associated with first pregnancies in which there is a higher incidence of uteroplacental insufficiency and hypertension. The uteroplacental insufficiency may be responsible for oligohydramnios and breech presentation leading to deformation of the fetus (Dunn, 1976)

Similar developmental variables are probably also operative in the etiology of congenital malformations affecting other systems, but these have not been clearly worked out.

H. Etiology of Abnormal Intrauterine Growth in the General Population

Intrauterine growth appears to be regulated by a multifactorial system of constraining and stimulating factors which act on the conceptus' genetically programmed drive to growth (Gluckman and Liggins, 1984). Some of these factors are common to all pregnancies (for example: fetal sex, endocrine factors), while others occur as pathological features in a minority of pregnancies (for example: chromosomal abnormalities, toxemia). This section reviews some of the important factors associated with growth retardation in the general population. In order to classify diverse causes of intrauterine growth retardation, factors influencing embryonic and fetal growth can be divided into those originating in the fetus, placenta or mother (Table II.2). This division is arbitrary as certain factors may induce growth retardation by affecting, for example, both the fetus and placenta (as in the case of rubella, see below).

I. Fetal Factors

a. *Genetic Factors*

The term fetus is the result of 42 successive divisions of the fertilized ovum. It is not entirely clear how the timing and total number of cell divisions is controlled. A genetic clock or counting mechanism inherent in the base sequences of DNA has been postulated to explain how cell differentiation can be programmed to occur following a specified number of cell divisions (Gluckman and Liggins, 1984; Holliday and Pugh, 1975)

Experiments in the mouse (Snow, 1986) in which observations have

Table II.2

Etiology of Intrauterine Growth Retardation in the General Population

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1. ***Fetal Factors***
   a. Genetic factors
   b. Antigenic differences
   c. Fetal anomalies
   d. Endocrine factors
   e. Radiation
   f. Infection

2. ***Placental Factors***
   a. Endocrine factors
   b. Placental size
   c. Placental anomalies
   d. Placental pathology (infarction, separation)

3. ***Maternal Factors***
   a. Maternal constraint
   b. Nutrition
   c. Maternal Illness
   d. Temperature
   e. Altitude
   f. Drugs
   g. Socioeconomic status

been made on the intrauterine development of strains differing in their adult size, and on the growth of embryos whose size has been deliberately altered, indicate that the growth rate of the early embryo is under genetic control and that differences in growth rate first appear at early postimplantation. Differences in size are due primarily to variation in cell number.

Birth weight has been shown to differ markedly among different population groups of the same socioeconomic status, suggesting that genetic factors are important in the regulation of birth size (Eveleth, 1986).

Inbreeding has been shown to be associated with some reduction in birth weight. This is presumably due to increased homozygosity in the fetus (Morton, 1955).

Prenatally, males grow larger than females (Jones et al., 1986). Sex differences in biparietal diameter and crown-rump length have been noted as early as the first trimester (Pedersen,1980). This may be related to higher levels of testosterone or other hormonal differences between the sexes which are genetically determined (Naeye and Tafari,1983). Ounsted (1971) has suggested that Y chromosome antigenicity interacting with the maternal XX system leads to greater invasiveness of the trophoblast, and incorporation of more decidua in the placenta, resulting in a larger placenta and conceptus.

Syndromes involving abnormalities of chromosome number with the exception of the poly-X (Reisman, 1970; Roberts, 1986), Klinefelter's syndrome (XXY) (Reisman, 1970), and XYY (Roberts, 1986) have consistently been noted to be associated with a decreased potential for growth. Growth arrest is most obvious for Trisomy 18 and D1

(Reisman,1970). A slowing down of cell division has been noted in cultures of fibroblasts or fetal cells in the case of Down's syndrome and other chromosomal abnormalities (Gropp, 1978). In Trisomy D, E and G, the deficit in growth has been shown to be a subnormal number of cells in different organs, and the reduction in cell number in different organs varies according to the chromosomal anomaly (Naeye and Tafari, 1983). Defects of chromosome structure (e.g. cri du chat syndrome involving a deletion of chromosome 5) and single gene abnormalities (e.g. galactosemia) have also been associated with growth retardation (Reisman, 1970). Thus this type of crude analysis indicates that the genetic loci influencing fetal growth are scattered widely on different chromosomes (Roberts, 1986; Wright,1976), supporting polygenic inheritance.

An analysis of the genetic contribution to the variation in fetal size has been made only in terms of birth weight. The normal continuous distribution of birth weight can best be understood in terms of multiple genetic factors (polygenes each with small effect, acting at independent loci and additive in effect) and /or environmental factors determining birth weight. Studies of the resemblance of birth weight in different classes of relatives which share different degrees of genetic and environmental relationship have enabled a partitioning of the total variation in birth weight into genetic and environmental sources of variation (Morton, 1955; Nance et al.,1983; Penrose,1954). The classic study of Penrose (1954) found that 38% of the variation in birth weight could be attributed to heredity, and this was made up of 16% from fetal hereditary factors other than sex, 2% from fetal sex, and 20% from the maternal genotype. The remaining 62% of the variance was attributable

to environmental sources, of which 18% were derived from the mother's general health and nutrition, 6% from her health during each particular pregnancy, 7% from her parity and 1% from her age, and the remaining 30% from unknown intrauterine factors. Thus, the contribution of the fetal genotype to the variation in birth weight is small relative to maternal genetic and environmental influences (Roberts, 1986).

The contribution of the paternal genotype to birth weight is smaller than that of the maternal genotype. The paternal genes contribute only to the fetus' genetic make-up including sex, while the maternal genotype influences the fetal environment (Gluckman and Liggins, 1984). However, Ounsted (1971) has suggested that when the maternal restraining influence is relaxed in the case of large birth weights (see maternal constraint below), factors which increase birth size such as increased paternal or maternal size and parity explain more of the variance in birth weight.

b. Antigenic Differences

It has been suggested that antigenic differences between the mother and fetus might influence the depth of trophoblastic invasion, and placental and fetal size Some evidence for this has come from mouse experiments, showing that maternal tolerance of fetal antigens reduces fetal weight while immune reactions enhance the weight (Benirschke, 1978) In humans , this type of mechanism might be one explanation for the lower birth weight observed with inbreeding (Morton,1955; Ounsted, 1978), as well as the positive correlation noted between parity and increasing birth weight (birth weight increases with increasing sens'tization to paternal antigens) (Ounsted ,1978). On the

other hand, there are data suggesting that ABO incompatibility is associated with a relatively smaller placenta and HLA compatibility with higher birth weight (Ounsted, 1978).

c. *Fetal Anomalies*

The association between growth retardation and congenital anomalies in some infants has been discussed previously (section II. F). In some instances, the congenital abnormality may contribute to or cause a failure in growth. For example, absence of the pituitary results in underdevelopment of the end organs, such as thyroid hypoplasia, while transposition of the great vessels leads to increased cell size of the abdominal organs (Naeye and Tafari, 1983). It is not known what induces the marked intrauterine growth retardation associated with renal agenesis. One hypothesis is that the resulting oligohydramnios may impair fetal growth (Dunn, 1976).

d. Endocrine Factors

The genetic potential for fetal growth is influenced by a number of hormones originating and/or operating in the mother, placenta and fetus. The production of these hormones can be quite complex, involving communication among the maternal, placental and fetal units to provide the necessary precursors for hormonal synthesis.

These hormones are thought to either stimulate growth directly or support growth indirectly by maintaining nutrient substrates Endocrine regulation of fetal growth may involve effects on the rate of cell division, the transport of glucose and amino acids into the cell, and placental transfer of nutrients. Hormones may also trigger the

differentiation of tissues. There are several recent reviews of the current knowledge of endocrine influences on fetal growth (D'Ercole and Underwood, 1986; Gluckman and Liggins, 1984; Sizonenko and Aubert, 1986), and certain aspects will be summarized briefly in this section, and in the section concerning placental factors.

The peptide hormones insulin and somatomedins (insulin-like growth factors) have important influences on fetal growth. They promote cell replication and the transport of amino acids and glucose into cells, and these biological actions are thought to be initiated by binding to insulin and somatomedin receptors. These hormones are not transmitted across the placenta. Insulin is produced by the fetus by 10 weeks of gestation. It has also been shown that fetal tissues synthesize somatomedins. Somatomedin and insulin receptors are present in many tissues of the fetus from early in gestation. Insulin and somatomedin receptors are also present in the placenta, and it is hypothesized that they may promote placental growth or transfer of nutrients across the placenta (D'Ercole and Underwood, 1986; Gluckman and Liggins, 1984; Sizonenko and Aubert, 1986). Cord somatomedin levels have been positively correlated with birth length, weight and head circumference corrected for gestational age (Gluckman and Brinsmead, 1976), and cord insulin levels have been positively correlated with birth weight (Sizonenko and Aubert, 1986). Decreased binding of somatomedins to fetal hepatic tissue has been reported in growth retardation resulting from maternal alcoholism (Guyda et al., 1980).

The influence of thyroid hormone on fetal growth varies with different species. Fetal hypothyroidism is associated with marked growth retardation in sheep and monkeys in contrast to man.

Hypothyroid infants are generally of normal birth weight and length, although skeletal maturation is delayed. It has been suggested that the differences among species may be due to differences in the placental transfer of thyroid hormone; the placenta in sheep, for example, is impermeable to thyroid hormone. In man, it is not clear how much of the hormone might be required for normal fetal growth and whether infants with congenital hypothyroidism may have sufficient thyroxine levels to sustain fetal growth. It is also not clear to what extent thyroid hormone crosses the placenta in man, and whether small quantities passing through the placenta may mask the true effects of fetal hypothyroidism (D'Ercole and Underwood, 1986; Gluckman and Liggins, 1984).

In the human fetus, concentration of iodide and synthesis of hormone by the thyroid gland occurs at about 10 weeks of gestation. In contrast to its apparent limited role in somatic growth, thyroxine plays a key role in fetal brain development. It stimulates the synthesis of protein, cerebral gangliosides, and neuronal RNA and DNA. The effects of thyroxine on brain differentiation may be mediated through nerve growth factor which has been shown to promote the growth and maturation of adrenergic neurons and the sympathetic nervous system (D'Ercole and Underwood, 1986; Gluckman and Liggins, 1984).

Glucocorticoids promote differentiation of cell function, and depending on the cell type either stimulate or inhibit replication of fetal cells in culture. In rodents, synthetic glucocorticoids induce fetal growth retardation (D Ercole and Underwood, 1986). Cortisol has been shown to cause a decrease in cell number in the lung and decreased cell replication and dendritic growth in rat brain (Bohn, 1984).

The role of estrogen and progesterone in regulating fetal size is not known. In rabbits, estrogen inhibits growth in a dose-related manner (Abdul-Karim et al., 1971). Progesterone and estrogen stimulate the secretion of chorionic somatomammotropin and may induce maternal physiological changes during pregnancy (Longo, 1985) (see section II.H.2., Placental Factors).

e. Radiation

In experimental studies, radiation causes embryonic death, structural malformations and intrauterine growth retardation; the frequency of these abnormalities depends on the dosage and timing of radiation. These effects have been shown to be due to the direct effects of radiation on the embryo consisting of mitotic delay, cell death, and inhibition of cell migration. In humans, in utero exposure to radiation therapy results in growth retardation and central nervous system effects such as microcephaly, eye malformations, and mental retardation. Medical diagnostic radiation (less than 5 rads) has been observed to have no measurable increased teratogenic risk (Beckman and Brent, 1986; Brent, 1986).

f. Infection

Growth failure is one of the recognized effects of intrauterine infection with a number of viral infections including rubella, cytomegalovirus, herpes simplex, and varicella-zoster, and certain protozoan (toxoplasmosis) and bacterial infections (syphilis) (Beckman and Brent, 1986; Hanson, 1983; Hughes, 1970; Naeye and Tafari, 1983). In all of these conditions except syphilis, infection of the nervous

system may result in microcephaly. Growth arrest following congenital infections has been attributed to direct invasion of fetal tissues, causing inflammation of fetal tissues and cell death, inhibition of normal mitotic rates, and vascular damage that reduces blood flow to fetal tissues. Chronic ischemia due to vascular lesions in the infected placenta may further compromise the fetus (Hanson, 1983; Hughes, 1970; Naeye and Tafari, 1983).

2. Placental Factors

a. *Endocrine Factors*

The placenta is important in supporting growth of the fetus by carrying nutrients and oxygen to the fetus and removing waste products from the fetus. In addition, the placenta has complex endocrine functions, synthesizing a number of protein and steroid hormones, which either directly or indirectly stimulate fetal growth and differentiation (Longo, 1985). For example, estrogen and progesterone produced by the placenta may be responsible for maternal adaptations to pregnancy such as the increase in blood volume, cardiac output and uteroplacental blood flow which influence fetal growth. Chorionic somatomammotropin synthesized by the syncytiotrophoblast is secreted primarily into the maternal circulation and it is hypothesized that this hormone influences maternal metabolism ensuring a supply of nutrients to the fetus (Gluckman and Liggins, 1984; Longo, 1985).

b. *Placental Size*

Placental size may be a limiting factor in fetal growth. Normally, the placenta in early gestation grows faster than the fetus but towards

term it grows more slowly than the fetus. In general, there is a positive correlation between placental and fetal weight toward term. The fall-off in fetal growth observed in the latter part of the third trimester has been attributed to a diminution in the growth of the placenta and transport of nutrients (Gruenwald, 1963). There is experimental evidence to indicate that placental mass has a critical relationship to fetal growth. Experimental reduction of placental mass in animals has been shown to lead to fetal growth retardation. The retardation may be due to restriction of oxygen and nutrients to the fetus and altered placental hormone production (Gluckman and Liggins, 1984; Owens et al., 1985).

c. *Placental anomalies*

In cases of intrauterine growth retardation, the placenta may exhibit changes that are intrinsic to it or more commonly are indications of fetal abnormality or maternal disease (Shanklin, 1970).

A few structural anomalies of the placenta can result in low birth weight. These include anomalous cord insertion such as battledore and velamentous insertion, circumvallate placenta, chorangioma, single umbilical artery and parabiotic transfusion in a monochorial twin placenta (Benirschke, 1978; Gluckman and Liggins, 1984; Shanklin, 1970).

d. *Placental Pathology*

The placenta is composed of fetal tissue and as such growth disorders which affect the fetus may also affect the placenta (Naeye and Tafari, 1983). For example, placental pathology may be a reflection

of some teratogenic effect (e.g. rubella) acting on the whole conceptus. In the latter case, the placenta is not the primary cause of the growth disorder, although placental pathology may contribute to a limitation in fetal growth.

Any external factor which alters placental function may have an adverse effect on fetal growth. The placenta may undergo changes in response to diseases or malnutrition in the mother or exposure to teratogenic agents, and in turn this may affect growth of the fetus. For instance, underperfusion of the placenta due to lesions of the uterine spiral arteries in preeclampsia can lead to placental infarctions, which could limit the placental area available for respiratory exchange and nutrient transfer (Novy, 1978). Women who smoke are found to be at increased risk of placenta praevia, abruptio placentae and placental infarcts (Wynn and Wynn, 1981), all which have been associated with fetal growth retardation (Hibbard and Jeffcoate, 1966; Naeye and Tafari, 1983; Shanklin, 1970).

3. ***Maternal Factors***

a. *Maternal Constraint*

A classic experiment by Walton and Hammond in 1938 illustrated the importance of maternal influence on fetal growth Birth weights of hybrid embryos in reciprocal crosses between Shire horses and Shetland ponies were related to the size of the mother breed. This was particularly evident for the hybrid fetus in the small dam. Further experiments in mammals using embryo-transfer to place "small" genotype embryos into "large" genotype mothers and vice versa indicated the importance of the maternal uterine environment in

influencing growth, regardless of fetal genotype. In mice, the differences between the descendants of transplants and the parent strains persisted for a number of generations, even though no further transplants were done. This phenomenon, termed maternal constraint, is transmitted from the mother to her daughters and influences the birth weights of all her offspring of both sexes (Hull et al., 1978; Ounsted, 1971; Snow, 1986).

There is indirect evidence of maternal constraint in humans. The correlation of birth weight is higher between half-sibs with a common mother than between half-sibs with a common father (Morton, 1955), and higher between maternal first degree cousins than between paternal first degree cousins (Robson, 1978).

The mechanism underlying maternal constraint is not known. Some possible factors are maternal nutrition and its reflection in maternal weight and stature, maternal placental perfusion and growth of the placenta (Gluckman and Liggins,1984; Metcoff, 1986), all of which have been correlated with birth size (Naeye and Tafari, 1983).

b. Nutrition

Experimental studies in animals have clearly shown that a reduced diet from early in pregnancy results in proportionate growth retardation with reduced cell numbers in placenta and fetal organs, including brain In humans, it is generally thought that, on a world scale, malnutrition is the most common cause of intrauterine growth retardation, and that the effects of malnutrition are most pronounced in populations with chronic malnutrition prior to pregnancy (Campbell, 1976; Gould,1986). It is difficult, however, to show the effects of malnutrition on fetal growth

in developed countries where overt malnutrition is uncommon and the diet variable. As well, the effects of other factors that are related to birth size such as socioeconomic class, infection, cigarette smoking , drugs, and so on must also be taken into account before conclusions can be made about the influence of nutrition (Metcoff, 1986)

Evidence for the effects of malnutrition on fetal abnormality has come in part from studies of the effects of acute famine on previously well-nourished populations in times of war, and nutrition intervention studies in chronically undernourished populations in underdeveloped countries Wynn and Wynn (1981) reviewed the findings on malformations and low birth weight in children born to previously well-nourished women in times of food shortage in Europe They emphasized the occurrence of both congenital malformations and low birth weight in children of women who conceived at the time of the food shortage. Infants of mothers who experienced food shortages only late in gestation also had reduced birth weights, but no increase in the prevalence of congenital malformations. Studies in Guatemala of villagers of low socioeconomic status found that food supplements during pregnancy resulted in increased birth weights and decreased perinatal mortality (Lechtig et al., 1975).

Other studies have looked at certain parameters of nutrition in developed countries in relation to fetal growth and have found that maternal height, ponderal index, prepregnant weight, and/or weight gain during pregnancy are correlated with one or more fetal growth parameters (Miller and Merritt, 1979, Naeye and Tafari, 1983) For example, in the U.S Collaborative Perinatal Project, birth weights were positively correlated with maternal pregravid weights. The latter has

been shown to have a positive correlation with maternal blood volume expansion during pregnancy , and a large blood volume expansion correlates with a large uteroplacental blood flow, which in turn has a positive influence on fetal growth (Naeye and Tafari, 1983).

Specific nutrient deficits have been shown to cause growth retardation in animals, and such deficits may also have an influence on human fetal growth. Cell division has been shown to depend on the concentration of specific nutrients, such as folate, with the rate of division increasing with increasing concentration until it reaches a maximum rate, and a threshold below which the cell no longer divides and dies (Wynn and Wynn, 1981). Negative associations between maternal and/ or neonatal red cell folate levels and birth weight and length have been found (Baker et al., 1977; Ek, 1982).

Maternal zinc levels in blood and tissue have been shown to be negatively correlated with birth weight (Metcoff, 1978).

The well-developed placenta is capable of concentrating essential nutrients, including folate, for the benefit of the fetus. It has been argued that the embryo is more dependent on the availability of nutrients in the maternal blood than the fetus, because the embryo does not have the ability of the developed placenta to extract nutrients from the maternal blood, and to synthesize other compounds needed by the fetus. Thus it has been suggested that the most sensitive period for the effects of nutrient deficiencies is in early pregnancy or around the time of conception (Wynn and Wynn, 1981).

c. Maternal Illness

Maternal illness during pregnancy may influence fetal growth. Low

and Galbraith (1974) found an increased risk of low-birth-weight babies in pregnancies complicated by severe preeclampsia, chronic hypertensive vascular disease, or chronic renal disease. About 30-40% of mothers with severe preeclampsia or toxemia have babies with intrauterine growth retardation (Metcoff, 1978). Uteroplacental vascular insufficiency resulting in a decrease in placental transport of oxygen or nutritional substrates, or interference with placental endocrine function, is thought to be the cause underlying these disorders (Novy, 1978). This type of insult results in a disproportionate type of growth retardation in humans and animals (see section II.E).

Women with severe congenital or acquired heart disease, or sickle cell trait, may give birth to babies who are small-for-gestational age. The supply of oxygen is probably the limiting factor in fetal growth. Moderate to severe anemia is also associated with a reduction in fetal weight. Whether this is due to hypoxia and/or poor nutrition in the mother is not clear (Benirschke, 1977; Novy,1978).

Certain maternal genetic disorders are associated with fetal abnormality. For example, in maternal phenylketonuria, intrauterine growth retardation, microcephaly and mental retardation are the most common features (Hanson, 1983).

Women with insulin-dependent diabetes give birth to babies with increased length and weight due to the growth-promoting effects of insulin (maternal hyperglycemia produces fetal hyperglycemia leading to hypersecretion of insulin by the fetal pancreas), although in a minority of cases the newborn will be growth retarded due to the vascular lesions of long-standing diabetes (Beckman and Brent, 1986; Naeye and Tafari,1983). Glucose intolerance during pregnancy has been

associated with increased placental weight, birth weight, and length (Metcoff, 1978).

d. Temperature

Heat, either environmental or in the form of fever associated with maternal illness, may be a potential risk factor for abnormalities of growth and morphogenesis. In experimental studies in sheep, increased environmental temperature results in decreased fetal weight and in malformations in the white matter of the cerebral hemispheres (Challis, 1978; Gluckman and Liggins, 1984). In other experimental models, heat has been found to interfere with neural tube development, and there have been reports of defects of neural tube closure and neuromigrational errors in the offspring of women exposed to hyperthermia during pregnancy (Hanson, 1983).

e. Altitude

It has been observed that babies born at high altitude have lower weights than those born at sea level (Lichty et al., 1957). Experimental studies suggest that in such circumstances the supply of oxygen may limit fetal growth (Novy, 1978).

f. *Drugs*

For most drugs commonly taken during pregnancy, little is known about their effects on human fetal growth, and only a few frequently taken drugs have been associated with intrauterine growth retardation. Nevertheless, if tobacco smoke and alcohol are included, drugs are among the most common causes of intrauterine growth retardation.

In general, the evaluation of drug-related intrauterine growth retardation is complicated by the presence of potential confounding factors which might influence growth such as other medications taken concurrently, nutrition, and maternal diseases for which the drug under study may have been prescribed.

The retardation associated with cigarette smoking affects all body measurements (D'Souza et al., 1981; Naeye and Tafiri, 1983), and therefore has its origins early in pregnancy. The degree of retardation increases with the number of cigarettes smoked (Persson et al., 1978). Decreased nutritional intake in smokers has not been shown to explain the association between smoking and growth retardation. (Gluckman and Liggins, 1984; Naeye and Tafari, 1983; Persson et al., 1978). Several mechanisms have been proposed to explain the growth-retarding effects of maternal smoking, including fetal hypoxia due to uterine vasoconstriction and the production of carboxyhemoglobin. There are over 4000 chemicals present in cigarette smoke, few of which have been investigated for their effects on fetal development (Gluckman and Liggins, 1984; Naeye and Tafari, 1983).

Alcohol consumption during pregnancy has been associated with a constellation of abnormal features in exposed infants involving growth deficiencies (prenatal and postnatal), central nervous sytem dysfunction including microcephaly and mental retardation, characteristic facial abnormalities, and variable major and minor anomalies (Redmond, 1979). Growth inhibition appears to be of the proportionate form, and there may also be a paucity of subcutaneous tissue. The number and severity of these anomalies appear to be dose-related with the most severely affected children often being born to chronic alcoholic women. As few

as two drinks/per day have been associated with recognizable although milder features of this syndrome (Hanson, 1983). Twenty-seven percent of heavy drinkers have been found to give birth to small-for-gestational age babies, as compared to 7 to 8% of light or moderate drinkers. The mechanism by which alcohol induces abnormalities has not been determined. Both ethanol and its metabolite acetaldehyde are embryotoxic and teratogenic in animal studies. Although the growth retardation associated with maternal alcohol consumption has been shown to be independent of such factors as socioeconomic class, smoking or maternal food intake, more subtle nutritional deficits might be important (Beckman and Brent, 1986; Redmond, 1979).

Other drugs which have been associated with intrauterine growth retardation include narcotics (e.g. heroin, pentazocine); the cardiovascular drug propanolol; chronic high doses of aspirin; warfarin ; glucocorticoids (e.g. prednisone); anticancer agents (e.g. aminopterin, methotrexate); and antithyroid drugs.

In addition, industrial chemicals such as polychlorinated biphenyls and heavy metals such as lead and mercury have also been associated with intrauterine growth retardation (Gluckman and Liggins, 1984; Hanson, 1983; Naeye and Tafari, 1983; Redmond, 1979).

g. Socioeconomic Status

Low socioeconomic class has been associated with a higher frequency of low-birth-weight babies. When other factors related to low birth weight and low socioeconomic class are taken into account, such as cigarette smoking, the association is no longer observed (Ounsted, 1971).

I. Methodological Considerations

Studies of the outcome of pregnancy in relation to maternal epilepsy and anticonvulsant drugs have been conducted using different methods such as case reports and case series, case-control, and cohort studies The following sections 1 to 5 review the strengths and weaknesses of these approaches (Fletcher et al., 1988, MacMahon and Pugh, 1970) Then, in the following sections, the results of studies concerning pregnancy outcome in epileptic women will be discussed in light of these methods.

1. *Case Reports and Case Series*

Case reports and case series describe a case or series of malformed children who have been exposed to a putative teratogen These studies are a means to delineate clinical phenotype and to generate hypotheses about etiology and pathogenesis They are particularly susceptible to bias in that they have been ascertained on the basis of outcome, and may emphasize unusual presentations of the case(s) Such studies do not permit analysis of the frequency of the findings nor of the relationship between outcome and possible risk factors or mechanisms In contrast, case-control and cohort studies allow computation of the risk of abnormality associated with exposure to the teratogen

2. *Case-Control Studies*

In case-control studies, cases with a specific abnormal outcome and controls are selected, and the frequencies of exposure to a putative teratogen in utero are compared between the two groups The data is used to estimate the relative risk of abnormality related to exposure The advantages of case-control studies are that they are a fast and

efficient method to study the risk of both common and rare abnormalities associated with exposure.

The limitations of this type of study are that investigation of a group of cases with a specific abnormality does not provide any information on whether or not exposure is associated with other abnormalities. Furthermore, data on exposure and possible confounding variables can be incomplete because it is collected in a retrospective manner. The potential for bias is a very important consideration in these studies, because data on exposure is collected after the outcome is known. For example, if exposure is collected by interview, women with abnormal children may recall their pregnancy history differently than women with normal children. One way to circumvent this bias has been to select as controls mothers of children with some other type of abnormality.

Ensuring the comparability of the control group to cases is a difficult problem in these studies. In general, controls are chosen from the same setting as cases (e.g. hospital patients). The controls and cases should be similar on criteria related to exposure and outcome (confounding variables, eg., socioeconomic group) as differences in such criteria would bias the results concerning relative exposure of cases and controls.

3. *Cohort Studies*

In cohort studies, the study groups are mothers (or fathers) exposed or unexposed to a putative teratogen and they are followed in time either prospectively or historically to observe pregnancy outcome. In a prospective cohort study, the investigation begins before the outcome

is known, and exposure data is recorded concurrently with the study. In an historical cohort study, the investigation often begins after the outcome is known, but the subject(s) ascertained are the parent(s), and their pregnancy histories are recreated from interviews or medical records.

The advantages of cohort studies are that they provide a means to evaluate both the absolute and relative risks of total and specific abnormalities associated with a particular exposure, as compared with those in unexposed controls. In the prospective cohort study, exposure data is recorded before the outcome is known and specifically for the purposes of the study , thus limiting bias. This, however, may not be the case in historical cohort studies, where exposure data is collected from the past, and may be poorly documented.

The major disadvantage of both retrospective and prospective cohort studies is the number of pregnancies required to detect a significant difference in infrequent pregnancy outcomes between exposed and unexposed groups. For example, given that a specific congenital malformation occurs with a frequency at birth of 3 per 100 in the unexposed group, about 2000 pregnancies would have to be studied in order to detect a 2-fold increase of congenital malformations in the exposed group as compared to the unexposed group (Karon, 1980). However, fewer pregnancies would be required , if length of follow-up of the offspring was increased to detect abnormalities postnatally.

Cohort studies may also be susceptible to bias if pregnancy outcomes are not assessed equally for exposed and unexposed groups.

4. *Controlling for Confounding Variables*

In case-control and cohort studies, confounding variables can be controlled for by matching controls on these factors. However, matching can only be done for a few variables at one time, and once one matches on a factor, the contribution of this factor to the outcome cannot be assessed. There is also the danger of overmatching, as for example, in case-control studies where matching on factors that are correlates of exposure may reduce the differences in exposure between cases and controls.

Confounding factors can also be controlled for in the analysis of results, such as by stratification or standardization. However, these methods can only deal with a few characteristics at one time. Multivariable analysis techniques such as multiple regression analysis or multiple logistic regression analysis are the most efficient means of adjusting for differences in a large number of factors related to outcome (Breslow and Day, 1980; Fletcher, 1988).

5. *Experimental Animal Studies*

Experimental studies in vivo or in vitro have the advantage that exposure to a teratogen is under the control of the investigator, and the experimental unit can be randomly assigned to exposed and unexposed groups. Thus single risk factors or combinations of risk factors can be studied under randomized controlled conditions, minimizing the problem of confounding variables as found in human studies of exposure to potential teratogens.

The primary problem with animal studies is that, although every demonstrated human teratogen has been shown to be teratogenic in

laboratory animals, the findings in animals are not necessarily reproducible in man. Variability among species in the response to a teratogen may be due to differences in genetic susceptibility to maldevelopment and to pharmacokinetic differences resulting in different exposures of the embryo to the teratogen (Nau, 1987).

Experiments in animals may not be adequately carried out to assess potential human toxicity, due to such factors as mode of administration and lack of drug concentrations. For example, the intraperitoneal mode of administration can result in higher drug concentrations in the embryo and more extensive teratogenic effects than other modes (Nau, 1986a; Stevens and Harbison, 1974) due to the fact that there is extraplacental transfer of the drug. Furthermore, drug testing once or twice a day in experimental animals results in drug pharmacokinetics different from that in humans, because of the faster metabolic rate of experimental animals. Large fluctuations involving a series of narrow and high peak drug levels are observed in experimental animals as compared to lower steady-state therapeutic concentrations in man (Nau, 1986a). Thus, the experimental situation may not accurately reflect the clinical situation

Dose regimens in a number of experimental studies have resulted in adverse effects on the mother (eg. poor weight gain), and without proper controls, it is not clear whether the adverse effects in the offspring are drug-induced *in vitro* studies may not accurately reflect conditions *in vivo* Thus the teratogenic risk in humans can only be accurately predicted from human exposures

J. Results of Epidemiological Studies of Congenital Anomalies in Offspring of Epileptic Patients

1. Case-Control Studies

There have been several case-control studies in which the prevalence of epilepsy and anticonvulsant drug (AED) use among parents of infants with specific major congenital malformations is compared to that among parents of a comparison or control group of infants. Some of these studies have addressed maternal epilepsy only, whereas others have also examined the role of paternal epilepsy.

The series of infants with congenital defects have either been all infants seen at a particular hospital over a specified period of time, or all infants with the defect found in a geographic area over a period of time. Thus the former method may be subject to the bias that may arise when affected individuals are seen at a particular specialized medical facility. In these studies, either a history of AED use in general or of specific AED during pregnancy have been investigated. Often, however, the precise timing of in utero exposure to AED in these studies was unclear. Information about a history of maternal epilepsy or exposure to AED in cases and controls have been obtained either from medical records, registries, personal interviews, or from questionnaires There may be potential sources of bias in the selection of cases and controls and in the collection of information on exposure For example, if reporting to a birth defects registry is voluntary, malformed infants may be reported because of exposure to valproic acid (VPA) during pregnancy. The fact that a mother has a malformed child may influence the recollection of events obtained in questionnaires or interviews. Some studies have attempted to control for such bias by using other

birth defects as the control group.

a. *Studies of Congenital Anomalies in General*

Greenberg and co-workers (1977) in England analyzed data from interviews with doctors about drugs used in the first trimester for 836 mothers of malformed infants and their matched controls. They found that 28 mothers had consumed barbiturates in the malformation group as compared with 13 in the control group. This was mainly accounted for by women with epilepsy: of 15 epileptic case mothers , 11 were taking phenobarbital (PB) in the first trimester, as compared with one of 3 epileptic control mothers.

In the Finnish birth register of congenital malformations from 1963 to 1972 (Shapiro et al., 1976), 2784 children with selected congenital malformations (central nervous system, skeletal and/or craniofacial defects) were reported, of whom 8 were associated with a history of maternal epilepsy and AED use during pregnancy (0.39%). This contrasts with 2 epileptic mothers identified among normal controls (birth immediately before case child in same district) (0 08%) With respect to specific AED, a 2-3 fold increased frequency of phenytoin (PHT) and/or PB exposure was found among malformed children as compared to normal children, with little difference among the drugs used alone or in combination. In contrast to these findings, there was no difference in exposure to PB for indications other than epilepsy This suggested that the association between this drug and malformations in maternal epilepsy was not causal.

In Italy, the Multicentric Registry on Birth Defects (Bertollini et al.,

1985; Mastroiacovo et al., 1983) was utilized to evaluate the association between birth defects and maternal epilepsy Fourty-one cases of maternal epilepsy (AED used in 37) were identified among 7607 malformed babies registered in the period 1980-1983. Controls were babies with malformations not in the case group. The overall relative risk of having a malformed baby among pregnant epileptic women was 1.8. Significant associations were found for Trisomy 18 (odds ratio, 24.6); diaphragmatic hernia (7.7); congenital heart disease (7.6); spina bifida (6.2); and orofacial clefts (5.0). There were no significant differences between cases and controls with respect to maternal age, socioeconomic status, and consumption of other drugs.

b. *Studies of Orofacial Clefts and/or Congenital Heart Disease*

(1) Investigation of exposure to anticonvulsants in general

Erickson and Oakley (1974) identified nearly all infants with cleft lip and/or palate born in a U.S county over 9 years and matched these to controls on several variables, such as maternal age. Three mothers with a history of AED use during pregnancy were identified among 203 infants with cleft lip and/or palate, whereas none were noted among 103 cases with isolated cleft palate. One control mother with a history of seizure disorder was also identified. All epileptic mothers had taken PHT with or without other AED during pregnancy. Based on these data, the authors calculated that the risk for a woman with a seizure disorder of having a child with cleft lip with or without cleft palate is 6 times greater than the risk for women without seizure disorders, or about 1%, given the general population prevalence of 0.2% for this congenital defect.

Pashayan and colleagues (1971), found 4 mothers with a history of AED use during pregnancy in a survey of 100 randomly selected infants with orofacial clefts. No comparison group was mentioned In 3 of the 4 cases the mother was taking PHT alone, and in 2 cases a first- or second-degree relative also had orofacial clefts

Carter and colleagues (1980) investigated the association between maternal consumption of AED in the first trimester and orofacial clefts in the offspring, by utilizing hospital medical records and questionnaires. Controls were newborns born on the same dates as cleft patients. Of 10 mothers who had children with isolated cleft palate, 2 (20.0%) had taken AED during pregnancy as compared to none of 37 mothers of children without this defect The mothers did not have a family history of cleft defects

In Denmark, Friis (1979), ascertained all children with facial clefts reported to the national registry in 1973 to 1976 , and then by questionnaires identified 18 of 391 probands (2.3%) with a history of maternal or paternal epilepsy. This represented a 3-fold increased prevalence of parental epilepsy as compared with the expected prevalence for fertile males and females. The increase was similar for epileptic mothers and fathers, thus suggesting that epilepsy per se was a factor in the production of orofacial clefts However, a teratogenic role for AED could also not be ruled out because three times as many mothers as fathers were taking AED at conception or during pregnancy

Anderson (1976) identified 18 cases with cardiac malformations with a history of maternal epilepsy and AED use during pregnancy among 3000 pediatric patients (0 60%) attending a large university-based hospital over a period of 12 years. Phenytoin with or without other AED

had been taken during 13 of the pregnancies. The number of women ascertained who took AED represented a 2-3 fold increased frequency over the general population prevalence of maternal AED therapy (0.33%) in pregnancy The author noted among the 18 cases, a preponderance of ventricular septal defects with or without pulmonary stenosis, as well as noncardiac defects such as cleft defects, and / or growth retardation. It should be pointed out that 4 of the 18 cases including the ones with cleft defects were exposed to oxazolidinediones (trimethadione, paramethadione), anticonvulsants which have been shown to produce a high frequency of cleft and heart defects. If these cases are omitted, the frequency of exposure to AED among 14 cardiac cases is reduced to 0.47%.

(2) Investigation of exposure to specific anticonvulsants

In a study searching for drugs which were related to the birth of children with specific defects (ascertained from a regional birth defects program), Safra and Oakley (1975) found from interviews of 278 women of malformed children that a history of diazepam exposure was 4 times more frequent among 49 infants with cleft lip ± cleft palate (14.3%), than among 229 infants with other defects (3.9%).

This was not confirmed by studies in Hungary (Czeizel, 1976; Lakos and Czeizel 1977), utilizing questionnaires sent to parents of children with cleft lip and/or palate ascertained from the Hungarian Congenital Malformation Register. However, in these studies, 11 of 413 cases (2.7%) with cleft lip and/or cleft palate were associated with maternal use of AED, including PHT and its derivatives, as compared to 0.2% for mothers of infants with neural tube defects. No association was found

for PB.

In England (Hill et al., 1988), prescription data were examined for the three months before the last menstrual period (LMP) and for the first trimester of pregnancy in 676 mothers of children with cleft lip and/or palate born during a 12 month period (1983-1984) and who were notified to the congenital malformation surveillance system of the Office of Population Censuses and Surveys. Controls consisted of the next normal infant born in the same general practice as the abnormal child within 3 months of the latter's birth. Mothers of children with oral clefts were prescribed AED (any type) significantly more often than control mothers, throughout the whole study period [Odds Ratio (OR) = 5.3 for the 3 months prior to the LMP; OR = 8.5 for the first trimester]. Significant differences were noted for PHT (OR = 4.6 for the three months prior to the LMP) and PB (OR = 10.1) (but these were the only drugs for which there were sufficient numbers of mothers for statistical analysis). Although a family history of malformations was 3 times more frequent in mothers of children with oral clefts than in controls, and more case than control mothers were from the manual class, no adjustment was made for these factors.

c. *Other Anomalies*

Hoyt and Billson (1978) reported 7 cases of maternal epilepsy and AED therapy during pregnancy among 67 cases (10.7%) of optic nerve hypoplasia, identified at a pediatric hospital or in private practice. Four of the cases also had other anomalies including microcephaly, cardiac and urogenital anomalies. Four of the mothers had taken PHT alone and the other three had taken PHT with PB or diazepam.

d. *Valproic Acid and Specific Major Congenital Malformations*

(1). Spina bifida

The case-control study has been invaluable in assessing the association between VPA exposure in utero (a relatively rare exposure in view of its recent introduction) and specific major malformations, both common (e.g. congenital heart disease) and rare, such as spina bifida.

Robert and Gibaud's report (1982) from the birth defects monitoring system for the Rhone-Alpes region of France was the first epidemiological case-control study to implicate VPA as a human teratogen Among 140 cases of spina bifida reported to the birth defects registry in the period 1976-1982, 10 epileptic mothers were identified, 9 of whom had received VPA (6.2%) in the first trimester of pregnancy (alone or combined with other AED). Dosages of VPA were relatively high, one gram or more in 7 cases. Twenty-one (0.32%) of the mothers of 6616 infants with other malformations registered had taken VPA in the first trimester. These data yielded a highly significant odds ratio of 20 6 (an estimate of relative risk in case-control studies). This finding could not be explained by the seizure disorder itself or other drug therapy, as the results were similar when VPA exposure was compared between epileptic mothers of infants with spina bifida, and epileptic mothers of infants with other defects. Based on these data and the prevalence of spina bifida in the general population, it was estimated that the epileptic mother taking VPA has a 1.2% risk of having a child with spina bifida, which is similar to the recurrence risk for a woman with a previous affected child (Centers for Disease

Control,1982)

Further follow-up in France involving nearly a total of 10,000 birth defect cases, including 190 spina bifida cases, and 38 VPA exposures (Centers for Disease Control,1983; Robert and Rosa, 1983) confirmed these initial findings Confirmatory evidence also came from the Netherlands where Lindhout and Meinardi (1984) ascertained, from various medical and registry facilities, 10 spina bifida cases with a history of maternal use of VPA during pregnancy, which exceeded the expected number of cases for the entire Dutch population

When the results of other birth registries of the International Clearinghouse for Birth Defects Monitoring, excluding the one from Rhone-Alpes, are pooled (Bjerkedal et al, 1982; Castilla,1983; Rosa, 1984), 14 cases of VPA exposure were identified among 1963 cases of spina bifida The exposure rate (0.7%) lower than than that seen in France, may be due to a lower usage rate in other countries (Bjerkedal et al., 1982). For example, Robert and associates (1984) calculated an exposure rate to VPA of 28% in pregnancies of epileptic women in France, whereas the exposure rate was 1 in 42 or 2 4% in a report from Latin America (Castilla, 1983).

(2). Congenital heart disease and orofacial clefts

In the French study from Rhone-Alpes (Robert and Rosa, 1983), VPA exposure was also significantly associated with orofacial clefts and congenital heart disease. The VPA exposure rate was 0 71% among 1541 heart defects and 0.88% among 566 cleft cases as compared with 0.16% for control defects (odds ratio 4.3 and 5.4 for heart and cleft defects, respectively). The data suggested that VPA alone or with PB

was associated with these defects.

Studies in Italy (Bertollini et al., 1985; Mastroiacovo et al.,1983) analyzed the relationship between different drug regimens and specific congenital malformations within maternal epilepsy, using the data on 40 epileptic mothers and 96 epileptic mothers of malformed infants ascertained in the Italian Multicentric (Bertollini et al., 1985; Mastroiacovo et al ,1983) and Rhone-Alpes birth registries (Robert and Rosa, 1983), respectively For each type of malformation, the frequency of exposure to specific AEDs was compared with the frequency of exposure to all other AED therapies. The only significant association found was VPA exposure and spina bifida. The authors concluded that the association between maternal epilepsy and malformations found in the Italian or French studies (Trisomy 18; diaphragmatic hernia, orofacial clefts; congenital heart disease) might be attributed to the confounding effect of maternal epilepsy per se. However, their findings might also be explained by non-specific teratogenic effects of AED, in which different drugs induce more than one type of defect (Bertollini et al., 1985).

e. *Summary*

In summary, case-control studies have shown a 2-3 fold increased prevalence of maternal or paternal epilepsy among offspring with major congenital malformations as compared with the prevalence of epilepsy in the general population. Calculation of odds ratios for the risk of specific malformations in different studies suggests that, overall, women with seizure disorders have a 4 to 12-fold increased risk of having a child with a cleft defect, as compared to women without

seizure disorders, whereas congenital heart disease is increased 4 to 5 fold. The role of epilepsy per se, other potential risk factors, and of specific AED in the formation of these defects is unclear. Valproic acid appears to be specifically associated with the occurrence of spina bifida: the risk for women taking this drug in the first trimester is in the order of 1%

2. *Historical and Prospective Cohort Studies*

Over the past twenty-five years, an increasing number of historical and prospective cohort studies from various parts of the world have reported the prevalence of congenital malformations in the offspring of epileptic women. Tables II.3 and II.4 summarize the location, methods of ascertainment of epileptic women and their offspring, methods of data collection, and the results of these studies.

More historical cohort[a] (n=42) than prospective cohort studies (n=24) have been carried out. The strenghs and weaknesses of these types of studies have been outlined in section II. I.

Subjects were ascertained either from large pregnancy or birth populations, or they were identified through special centres for the treatment of epilepsy or certain hospitals. Thus, the latter studies may be biased towards the inclusion of women with more severe epilepsy. In the ascertainment of large birth populations, greater use has been made in recent years of birth registries (Bertollini et al., 1987; Bjerkedal and Bahna, 1973; Bjerkedal, 1982b; Kallén, 1986; Stanley et al., 1985) in which epileptic women have been identified from data in the registry.

The majority of studies gave information on AED usage during pregnancy. Some studies did not use control groups; others calculated malformation prevalences for total birth populations or small nonepileptic control groups (matched or unmatched).

The studies also differed with respect to the definition of congenital malformations. Some studies only included major congenital

[a] Historical cohort studies of epileptic women have usually been referred to as retrospective studies in the literature

Table II.3: Occurrence of Congenital Anomalies in Offspring of Epileptic Women in 42 Historical Cohort Studies

Author(s), year	Ascertainment, Location	Data Collection[a]	AED Taken[b]	MA/MI[c]	Total with Epilepsy		AED		NO AED		CONTROLS	
					No. of Births[d]	% Malf	No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf
Baptisti, 1938	Records 3 obstet. clin., Baltimore, U.S.A	br,cr	?	?	37	0.0	-	-	-	-	-	-
Burnett, 1946	Hosp. births 3 obstet. clin. 1939-45, Middlesex, Eng.	br,cr	?	?	19	0.0	-	-	-	-	-	-
Sabin and Oxorn, 1956	Hosp. births, 1945-54, Montreal, Can.	br,cr	1,2,7&?	MA	56	5.4	-	-	-	-	-	-
Janz and Fuchs, 1964	Epilepsy clinic, Heidelberg, W. Ger.	h	1,2,3, 6,7,8	MA	358	1.4	225	2.2	120	0.0	-	-
Maroni and Markoff, 1969	Hosp. births 1959-68, Zurich, Switz.	br, cr	?	MA	35	2.9	21	4.8	14	0.0	-	-
German et al., 1970b	Hosp. live births 1946-68, New York, U.S.A.	h, br	1,2,3,7, 8	MA,MI	237	5.9	-	-	-	-	-	-
Mountain et al., 1970	Hosp. births 1968-69, Treated epilepsy, Melbourne, Austral.	br	1,2,3,4 6,7,8	MA	16	6.3	16	6.3	-	-	-	-

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy No. of Births	Total with Epilepsy % Malf	AED No. of Births	AED % Malf	NO AED No. of Births	NO AED % Malf	CONTROLS No. of Births	CONTROLS % Malf
Elshove and Van Eck, 1971	All hosp. live births 1959-68, Groningen, Neth.	h, br	1,2,3,7 & ?	MA,MI	65	15.4	65	15.4	-	-	12,051	1.9
Pritchard et al., 1971	Inpatient hosp. records 1958-70 Dallas, Texas	br	1,& ?	MA	69	2.9	69	2.9	-	-	-	-
Watson and Spellacy, 1971	Hosp. births 1960-70, Treated epilepsy, Miami, U.S.A.	br	1,2,3	MA,MI	52	5.8	52	5.8	-	-	51	0.0
Speidel and Meadow, 1972	Epileptic women, Neurol. and obstet. depts., Births 1948-72, Leeds, Eng.	h, br, cr aut	1,2,3 6,7,8	MA	388	4.4	329	5.2	59	0.0	448 94,474	1.6 2.7
Fedrick, 1973	Hosp. and home births, Oxford Record Linkage Files, 1966-70, Eng.	br, cr	1,2,3,4 6,7,8	MA,MI	217	13.8	162	14.8	19	10.5	649	5.6
Koppe et al., 1973	All hosp. births 1963-68, Amsterdam, Neth.	br	1,2,3,7	MA	197	6.6	125	8.8	67	3.0	12,455	2.9

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy No. of Births	Total with Epilepsy % Malf	AED No. of Births	AED % Malf	NO AED No. of Births	NO AED % Malf	CONTROLS No. of Births	CONTROLS % Malf
Lowe, 1973	All births 1965-71, Cardiff, Wales	br, cr	1,2,3,6	MA,MI	245	4.9	134	6.7	111	2.7	31,877	2.8
Meyer, 1973	Neurol. Dept., Univ. of Heidelberg, W. Ger.	h	1,2,3,4 6,8	MA,MI	323	17.3	199	18.6	124	15.3	-	-
Niswander and Wertelecki, 1973	All live births 1965-71, U.S. A. Air Force Hosps.	br	?	MA,?	413	4.1	-	-	-	-	347,097	2.7
Barry and Danks, 1974	Antenatal clinic, live births 1967-72, Melbourne, Austral.	h, ce	1,2,3,4 8	MA MI MA,MI	93 93 93	5.4 8.6 14.0	73 73 73	6.8 11.0 17.8	20 20 20	0.0 0.0 0.0	-	-
Goujard et al., 1974	Antenatal clinic, 13 Matern. hosps., 1963-69, France	h	?	MA	49	6.1	-	-	-	-	?	2.4
Higgins and Comerford, 1974	All hosp. births 1959-73, Treated epilepsy, Dublin, Ire.	br	1,2,3,4 8	MA	115	3.5	115	3.5	-	-	-	-

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy No. of Births	Total with Epilepsy % Malf	AED No. of Births	AED % Malf	NO AED No. of Births	NO AED % Malf	CONTROLS No. of Births	CONTROLS % Malf
Loiseau et al., 1974	Neurol. Clin., Univ. Bordeaux, France	h	1,2,3,4 6,7,8	MA	60	6.7	60	6.7	–	–	–	–
Starreveld-Zimmerman et al., 1974	Outpatient epilepsy clinics, Neth.	h, cr	1,2,3,4 6,7,8	MA,MI	297	7.4	279	7.2	18	11.1	–	–
Visser et al. 1976	Obstet. dept., Groningen, Neth.	br	?	?	65	15.4	–	–	–	–	12,051	1.9
Tsuboi and Endo, 1977	? Japan	?	?	MA	273	1.1	–	–	–	–	–	1-2%
Weber et al., 1977	? Nancy, France	h	1-8	MA,MI	655	3.8	569	4.0	86	2.3	5,011 11,220	2.2 1.8
Annegers et al., 1974,1978	Medical records linkage, Mayo Clinic, Births 1939-76, Rochester, U.S.A.	h,br,cr aut	1,2,3,6 7,8, and ?	MA	259	8.1	177	10.7	82	2.4	748	3.5
Martin, 1978	? Switzerland	?	1-8	MA	109	6.4	78	7.7	31	3.2	–	–
Nakane et al., 1980	11 Neuropsych. centres, Collaborative study, Births prior to 1974, Japan	h, br, cr	1-8	MA,MI	638[a]	9.9	478	11.5	129	2.3	–	–

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy		AED		NO AED		CONTROLS	
					No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf
Majewski et al., 1981	Outpatient records, Neurol. Univ. Hosps., Tübingen & Düsseldorf, W. Ger.	h, br, cr, ce	1-6,8	MA[f]	184	17.9	138	18.8	46	15.2	-	-
				MA,MI[g]	192	5.2	146	6.8	46	0.0		
Beck-Mannagetta et al., 1982	Dept. of Neurol., Free Univ. of Berlin, 1969-72	?	?	MA,MI	397	15.4	268	19.8	129	6.2	-	-
Bjerkedal, 1982b	Medical Birth Registry 1967-1978, Norway	br	?	MA, MI	3879	4.4	-	-	-	-	3879	3.5
Densky et al., 1982a	Outpatient records, Seizure Clinic Montreal Neurol. Hosp., Montreal, Can.	h, br, cr, ce	1,2,3 6,7,8	MA	134	12.7	88	15.9	46	6.5	94,474	2.7
Deblay et al., 1982 Vert et al., 1979	Consecutive admissions neonatal unit, Regional maternity unit, Univ. of Nancy, 1973-1980, France	br, ce	1,2,4,5 6,8	MA	115	6.9	115	6.9	-	-	-	-
Giraud et al., 1982	Obstet. service, Poitiers, France	br	1,2, and?	MA,MI	83	14.5	-	-	-	-	3050	2.1

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy		AED		NO AED		CONTROLS	
					No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf
Biale et al., 1975,1984	Obstet. clin., Treated epilepsy, Beersheva, Israel	br	1,2,3,7	MA	66	15.2	66	15.2	-	-	-	-
Satish and Kalyanaraman, 1984	Neurol. Clin., Instit. of Neurol., Madras Med. College, India	h,br,cr	1,2,3	MA	94	1.1	48	2.1	46	0.0	98	1.0
Sviggs, 1984	Treated epilepsy, Hosp. deliveries 1968-79, Adelaide, Australia	br	1-5,8	MA	76	6.6	76	6.6	-	-	75	1.3
				MI	76	3.9	76	3.9	-	-	75	2.7
				MA,MI	76	10.5	76	10.5	-	-	75	4.0
Stanley et al., 1985	Public Health Dept., All births 1980 -82, West. Austral.	h,br,cr	1,4,8	MA	244	2.9	-	-	-	-	-	-
		malf. register	and ?	MA,MI[h]	244	3.7	-	-	-	-	62,265	3.4
Kallen, 1986	Swedish Medical Birth Register, 1973-81	br, cr	1-8	MA	644	3.7	551	3.6	93	4.3	-	1.8[i]
				MI	644	3.1	551	3.3	93	2.2	-	-
				MA,MI	644	6.8	551	6.9	93	6.5	-	-

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy No. of Births	Total with Epilepsy % Malf	AED No. of Births	AED % Malf	NO AED No. of Births	NO AED % Malf	CONTROLS No. of Births	CONTROLS % Malf
Robert et al., 1986	EEG records, hosp. neurol. and neurosurg., 3 matern. wards, 1976-1983, Lyon	h,br	1-6,8	MA MI MA,MI	147 147 147	5.4 12.2 17.7	112 112 112	7.1 12.5 19.6	35 35 35	0.0 11.4 11.4	- - -	1.4 - -
Akhtar and Millac, 1987	Matern. hosp. records, 1977-1981, Leicester, Eng.	br	1,2,4,5	MA,MI	188	2.6	152	2.6	36	2.8	-	-
Bertollini et al., 1987	Records of French Chapter of Int. League Against Epil.; EEG service and 3 matern.units, Lyon; Ital. Malf. Register; Swedish Birth Register, 1973-1985	birth, malf, registries, cr	1-6,8	MA	557	3.5	557	3.5	-	-	-	1.3-1.8
Holmes et al., 1987	Newborns exposed to PHT in utero, identified in cohort study of newborns, Boston, U.S.A	h,cr,ca	1 and ?	MA	38	5.3	38	5.3	-	-	6977	3.6

LEGEND TABLE II.3

[a] Data Collection: h-history; br-birth records; cr-clinical records; ce-clinical examinations; aut-autopsy reports.

[b] Type(s) of anticonvulsant drugs taken during pregnancy in each study irrespective of pregnancy outcome. The actual single and multiple drug therapies are not indicated. 1=phenytoin; 2=phenobarbital; 3=primidone; 4=carbamazepine; 5=valproic acid; 6=succinimides; 7=trimethadione; 8=other.

[c] MA-major congenital anomalies; MI-minor congenital anomalies.

[d] Viable births or live births

[e] Not known whether the epileptic women took anticonvulsants during 31 pregnancies.

[f] Children who had major congenital malformations and no dysmorphic features.

[g] Children with hydantoin-barbiturate embryopathy

[h] Includes three children with features of the fetal hydantoin syndrome, of whom two did not have major congenital malformations.

[i] From Bertollini et al., 1987

Table II.4: Occurrence of Congenital Anomalies in Offspring of Epileptic Women in 24 Prospective Cohort Studies

Author(s), year	Ascertainment, Location	Data Collection[a]	AED Taken[b]	MA/MI[c]	Total with Epilepsy		AED		NO AED		CONTROLS	
					No. of Births[d]	% Malf	No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf
Melchior et al., 1967	Obstet dept., Admissions for delivery 1962-65, Copenhagen, Denmark	int,br	1,2,3 6,7	MA	32	6.3	32	6.3	-	-	-	-
Cvetko, 1969	Epilepsy clinic, Yugoslavia	int,br	1,2,3 7,8	MA	35	8.6	35	8.6	-	-	-	-
South, 1972	3 Maternity units, 1969-70, London, Eng.	int,br	?	MA	31	6.5	22	9.1	9	0.0	7896	2.4
Kuenssberg and Knox, 1973	All pregnancies 1965-67, Scotland	int,br	1,2,3,6	MA,MI	?	?	48	10.4	?	0.0	14,668	3.1
Miller and Nevin, 1973	Neurology dept., 1969-73 Belfast, Ire.	int,br	1,2,3 4,6	MA,MI	110	6.4	110	6.4	-	-	32,227	3.8
Monson et al., 1973	Collaborative Perinatal Study, U.S.A.	int,br,cr, ce,aut	1 and?	MA,MI	306	4.6	98	6.1	101[e]	3.0	50,591	2.5

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy No. of Births	Total with Epilepsy % Malf	AED No. of Births	AED % Malf	NO AED No. of Births	NO AED % Malf	CONTROLS No. of Births	CONTROLS % Malf
Goujard et al., 1974	Antenatal clin., 13 Matern. hosps., France	int, ce	1,2,3,4	MA	42	2.4	39	2.6	3	0.0	12,764	1.6
Knight and Rhind, 1975	Neurol. and obstet. clinics, 1953-73, Sheffield, Eng.	int,br	?	MA	140	3.6	97	4.1	43	2.2	69,000	3.7
Hanson et al., 1976	Children exposed to PHT in utero, identified from Collaborative Perinatal Project, U.S.A.	int, br, cr ce, aut	1,2,3 and?	MA,MI	104	11.0[f]	104	11.0[f]	-	-	100	0.0
Shapiro et al., 1976	Collaborative Study, U.S.A.	int,br,cr ce,aut	1,2,3 and?	MI	305	3.9	102	6.9	97[e]	3.1	49,997	3.7
				MA,MI	305	10.5	102	11.8	97[e]	11.3	49,997	6.4
Dansky et al., 1981,1982b	Seizure Clin., Montreal Neurol.Hosp., Private neurol. and obstet practices, Montreal, Can.	int,br,cr ce,aut,lab	1,2,3, 4,6	MA	52	19.2	52	19.2	-	-	-	-

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy		AED		NO AED		CONTROLS	
					No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf
Granström and Hiilesmaa, 1982a	Regional matern. units, Outpat. clin., Helsinki Univ. Central Hosp., 1976-79, Finland	int,br,ce, aut, lab	1,2,3, 4,5	MA,MI	150	5.3	134	6.0	16	0.0	-	-
Hill et al., 1974, 1982	Admissions for delivery 1968-80, Baylor Med. College, Houston, Texas	int,br,cr, ce	1,2,3 and?	MA	59	18.6	59	18.6	-	-	252	2.0
Nakane, 1982	11 Neuropsych. centres, Collaborative study,1974-77, Japan	int,br, cr,ce	1-8	MA,MI	151	13.9	151	13.9	-	-	-	-
Bossi et al., 1982a,1983	Milan Collaborative Study,1977-80 Italy	int,br,ce lab	1-6,8	MA,MI	49	8.2	46	8.7	3	0.0	-	-
Biale and Lewenthal, 1984	Obstet clinic, Beersheva, Israel	int,br	1,2,4 5,8	MA	33	0.0	33	0.0	-	-	-	-
Kaneko et al., 1984a	Hirosaki Univ. Hosp.,1975-83 Japan	int,ce	4,5 and?	MA	112	4.5	97	5.2	15	0.0	-	-
				MI	112	12.5	97	12.4	15	13.3	-	-
				MA,MI	112	15.2	97	15.5	15	13.3	-	-

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy		AED		NO AED		CONTROLS	
					No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf
Kelly, 1984b	Epilepsy and private clinics, Dept. of Neurol., Univ. of Virginia, 1977-82, U.S.A.	int,br,cr ce	1-6,8	MA	171	3.5	150	3.3	21	4.8	-	-
Miyakoshi and Seino,1984	National Epilepsy Centre, 1975-84 Shizouka,Japan	int,br,cr ce	1-5, 7,8	MA,MI	132	15.2	123	16.3	9	0.0	-	-
Lindhout et al., 1982, 1985b	Epilepsy clinic outpatients, Netherlands	int, ce, lab	1-6,8	MA,MI								
	Cohort A: 1972-1979				165	9.7	151	9.9	14	7.1	-	-
	Cohort B: 1980-1985				186	7.5	172	7.6	14	7.1	-	-
Koch et al. 1982b,1983 Rating et al., 1987a,b	Antenatal visit, 5 Obstet clinics, 1976-84, West Berlin, W. Ger.	int,br,cr ce,lab	1-6,8	MA	150	8.7	116	8.6	34	8.8	116	6.0
Buchanan, 1988	Obstet. and epilepsy clinics, Sydney, Australia	int,br,ce	1,2,4 end?	MA	26	0.0	26	0.0	-	-	-	-
				MI	26	7.7	26	7.7	-	-	-	-

Author(s), year	Ascertainment, Location	Data Collection	AED Taken	MA/MI	Total with Epilepsy		AED		NO AED		CONTROL	
					No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf	No. of Births	% Malf
Kaneko et al., 1988	3 Univ hosps. 1978-84, Japan	int,ce	1-5,8	MA,MI	172	14.0	172	14.0	-	-	-	-
Jones et al., 1989	Women who voluntarily contacted California Teratogen Registry, U.S.A	int, cr, ce	2,3,4, 5,6	MA	54	13.0	54	13.0	-	-	70	7.1

LEGEND TABLE II.4

[a] Data Collection : int-personal interview; br-birth records, cr-clinical records; ce-clinical examinations; aut- autopsy reports; lab-monitoring of plasma anticonvulsant and or blood folate levels

[b] Type(s) of anticonvulsant drugs taken during pregnancy in each study irrespective of pregnancy outcome The actual single and multiple drug therapies are not indicated 1=phenytoin; 2=phenobarbital; 3=primidone, 4=carbamazepine; 5=valproic acid; 6=succinimides; 7=trimethadione; 8=other

[c] MA-major congenital anomalies, MI-minor congenital anomalies

[d] Viable births or live births

[e] Births to women not taking phenytoin. The women may have been taking other anticonvulsants

[f] Children exhibiting a pattern of malformation consistent with the fetal hydantoin syndrome

malformations, while other studies also included minor anomalies such as digital hypoplasia or facial dysmorphism. In Tables II.3 and II.4 malformation prevalences for major and minor anomalies are given separately, when possible.

Information on exposures during pregnancy, for example, type of AED, and on the occurrence of malformations in the offspring were collected from one or more of the following sources: maternal history recorded in questionnaires or personal interviews after completion of pregnancy in historical cohort studies (h); personal interviews carried out concurrently with prospective cohort studies (int); birth records (br); clinical records (cr); autopsy reports (aut) and clinical examinations which may have included specialized investigations (ce). Use has also been made of linkage to malformation registries, in order to obtain information on pregnancy outcome (Robert et al., 1986). Only a few prospective cohort studies may also have included monitoring of plasma AED and/or folate levels during pregnancy (lab) (Bossi et al., 1982a; Dansky et al., 1981, 1982b; Granström and Hiilesma, 1982a; Hiilesmaa et al., 1983; Koch et al., 1982b, 1983; Lindhout et al.,1985b; Rating et al., 1987a,b).

The types of AED taken by epileptic women in these studies generally follow the prescribing practises of different countries during different periods of time. In earlier studies, the types of AED taken during pregnancy were mainly PHT, PB and primidone (PRM), and in some cases trimethadione. In later studies, the latter drug was no longer used due to its indictment as a potent human teratogen, while carbamazepine (CBZ) and VPA were used more frequently. The different prevalences of total and specific malformations in the various studies may therefore in

part reflect differences in AED use.

In general, data collection in prospective studies (Table II.4) has involved more sources than in historical studies (Table II.3). In many historical cohort studies, assessment of the occurrence of malformations has been confined to the neonatal period. However, two studies (Annegers et al., 1974,1978; Fedrick, 1973) utilized record linkage systems for extensive follow-up of the children. In most of the prospective studies, follow-up of pregnancy outcome, involving clinical examination of the offspring to detect malformations, extended to early childhood or beyond. Studies not conducting follow-up after birth may have underreported the occurrence of specific malformations such as congenital heart disease which may be asymptomatic at birth.

Even though the various studies differed with respect to methodology and geographic location, their findings show a common trend: epileptic women have a higher prevalence of congenital anomalies in their children than controls. Twenty historical cohort studies and 11 prospective studies have compared the prevalence of malformations in the offspring who were and were not taking AED during pregnancy. As most of the epileptic women were treated during pregnancy, numbers of untreated epileptic women in the individual studies have generally been small. The majority of the studies, however, show that the prevalence of congenital anomalies is higher in the treated group.

Table II.5 gives a summary of the prevalence of children with congenital anomalies born to epileptic women who were taking AED during pregnancy and those not taking AED, summed over the studies in Tables II.3 and II.4. This summary is only a rough measure of the overall occurrence of anomalies in the offspring of epileptic women, as it

Table II.5

Occurrence of Congenital Malformations in Offspring of Epileptic Women (28 Historical Cohort and 15 Prospective Cohort Studies)

	Cohort Studies			
	Historical		Prospective	
Population	No. of Births[a]	% Malf	No. of Births[a]	% Malf
Total epileptic	7,104	5.31	891	8.75
vs. Nonepileptic	698,950	2.74	172,402	4.28
Treated epileptic	1863	7.57	647	9.12
vs. Nonepileptic	176,134	2.65	186,970	4.19
Treated epileptic vs.	4,206	9.38	1101	8.44
untreated epileptic	1,311	4.96	178	5.06
Untreated epileptic	516	2.71	89	4.49
vs. Nonepileptic	156,980	2.67	89,776	3.29

[a] Viable births or live births

includes studies of differing methodologies and locations. The overall results reflect what most authors have found: the prevalence of congenital malformations among offspring of epileptic women taking AED is 2- to 3-fold that in the general population, whereas the corresponding prevalence among the offspring of untreated epileptic women is generally not increased.

These results suggest that AED have an etiologic role in dysmorphogenesis; however, in many cases the comparability of the treated and untreated epileptic groups, particularly with respect to seizure type and frequency, is not known.

In general, the overall prevalences of congenital malformations in the epileptic and nonepileptic groups are somewhat higher in the prospective as compared with historical cohort studies, but the relative increase in the prevalence of anomalies in the epileptic as compared to nonepileptic groups are similar in the historical and prospective cohort studies. The higher prevalence of congenital anomalies in the prospective studies may reflect inclusion of anomalies detected later than the neonatal period during clinical examination of the offspring, which may have been missed at birth (eg. facial dysmorphism, digital hypoplasia, inguinal hernia).

Although the relative risk of all congenital malformations in the offspring of epileptic women as compared to the general population is in the order of 2 to 3, the relative risk of specific anomalies may be much higher (see the following section concerning type of congenital anomalies).

a. *Type of Major Congenital Anomaly*

Several authors at various times have summarized the prevalence of specific major malformations over a large number of prospective and historical cohort studies, totalling 2000 or more births to epileptic women (Bossi, 1983; Dansky, 1978; Hill et al., 1974; Janz, 1982a; Schardein, 1985). These summaries show that cardiovascular malformations and orofacial clefts have been the most common malformations observed (each in about 2% of births to epileptic women). Anomalies of the skeletal, nervous, urogenital and gastrointestinal systems have also been reported in the offspring of epileptic women but usually less frequently (each in about 1% or fewer of births) and not as consistently as defects of the heart or orofacial region.

In a Japanese collaborative study, Nakane (1982) showed that cardiovascular anomalies, cleft lip and/or palate, and skeletal anomalies each occurred in 2 to 3% of 478 liveborn offspring exposed to AED in utero, while defects of other systems were found in 1% or fewer of live births.

Table II.6 gives the relative frequencies of cardiovascular anomalies and orofacial clefts for all historical and prospective studies combined (the prevalence of these malformations summed separately for historical cohort and prospective studies were similar). The specific relative frequencies are compared for epileptic women according to AED use and to the corresponding values in two large population studies. It can be seen that cardiovascular anomalies and orofacial clefts occur more frequently in offspring exposed to AED in utero, whereas they are not increased in the untreated epileptic group. Although cardiovascular abnormalities are more common than cleft lip with or without cleft

Table II.6

Occurrence of Cardiovascular Malformations and Orofacial Clefts in Offspring of Women with Epilepsy as Compared to the General Population (29 Historical and 21 Prospective Cohort Studies)

	Cardiovascular Malformations		Orofacial Clefts		
Population	No. of Births	% Malf	No. of Births	%CL±CP	%CP
Treated Epilepsy	6,582	1.90	5,854	1.23 (88%)[a]	0.16 (12%)[a]
Untreated Epilepsy	1,360	0.66	1,360	0.15	0.00
General Population:					
Leck et al., 1968	94,474	0.42	94,474	0.14	0.06
Heinonen et al., 1977	50,282	0.80	50,282	0.10 (63-70%)[b]	0.06 (30-38%)[b]

[a] Proportion of all facial clefts in offspring of treated epileptic women

[b] Proportion of all facial clefts in general population

palate in offspring of epileptic women taking AED, the relative risk of cardiovascular defects in epileptic women as compared to the general population (2 to 4) is less than that for cleft lip with or without cleft palate (9 to 12).

Table II.6 also shows the relative frequencies of specific facial clefts, cleft lip with or without cleft palate and isolated cleft palate, as these are embryologically and genetically distinct malformations in the general population (Nora and Fraser, 1989). As can be seen, the relative risk (RR) of isolated cleft palate (2.7) is much lower than that for cleft lip with or without cleft palate. The data also show that infants exposed to AED in utero differ from the general population in the distribution of specific facial clefts, there being a preponderance of cleft lip with or without cleft palate in the epileptic group.

In contrast, there does not appear to be any major deviation for cardiac lesions, the most common defect being isolated ventricular septal defect as in the general population (Janz, 1982a). An exception appears to be a preponderance of defects related to truncoconal development (e.g. Tetralogy of Fallot) in association with in utero exposure to trimethadione (Feldman et al., 1977; German et al., 1970a,b).

In an historical cohort study in Norway utilizing a large birth registry (Bjerkedal, 1982b), the overall risk of congenital malformations among newborns of epileptic women (n=3879) was only 1.25-fold higher than that for a matched control group . However, no differentiation was made according to AED exposure in utero. The relative risks for specific malformations were greater, 8.0 for atresia/stenosis and other malformations of the digestive canal, 2.6

for cleft lip with or without cleft palate, and 1.8 for hypospadias. However, only the difference for digestive canal malformations was statistically significant ($p < 0.001$). Although in this study, controls were matched on a number of variables, they were not matched for socioeconomic status, and therefore the relative risk of specific and total malformations may be smaller than observed in this study.

Kida (1984) compared the prevalence of malformations in offspring of mothers treated with AED in the first trimester of pregnancy who were ascertained in a collaborative study at eleven neuropsychiatric facilities in Japan (Nakane et al., 1980; Okuma et al., 1980), with control data derived from a monitoring system of congenital anomalies in 16 Tokyo Hospitals. There was an increase in malformations of the nervous system (anencephaly, RR=7, spina bifida, RR=6); the cardiovascular system (all malformations, RR=8; ventricular septal defect, RR=6); orofacial clefts (cleft palate, RR=3; cleft lip with or without cleft palate, RR=17); torticollis (RR=9); and hypospadias (RR=4).

Speidel and Meadow (1972), in their historical cohort study, reported that the most common malformations among 329 offspring exposed to AED in utero were congenital heart disease (1.8%); cleft lip with or without cleft palate (0.9%) and microcephaly (0.9%). The risk of congenital heart disease was 9 times the control figure, whereas each of the other specific anomalies occurred 4 times more often than expected. Diaphragmatic hernia and hypospadias were also frequent (0.6%, RR=6).

In an uncontrolled study, Barry and Danks (1974) reported a high prevalence of diaphragmatic hernia (3.2%) among 93 offspring exposed to AED in utero.

Giraud et al. (1982), in an historical cohort study in France, observed an increase in cardiac defects (RR=12), genitourinary anomalies (RR =6), and dysmorphic craniofacial features (RR=150) among 83 offspring of epileptic women as compared to the entire birth population. Orofacial clefts (RR=12) and defects of the central nervous system (RR=4) were also increased. It is not known to what extent knowledge of a diagnosis of epilepsy in the mother may have inflated the prevalence of certain anomalies such as facial dysmorphism in the offspring, as compared to the general population (such defects may not have been sought for in a child of a nonepileptic mother).

In an historical cohort study of a total birth population in the U.S., Niswander and Wertelecki (1973) noted a significant increase of cleft lip and/or palate (RR=4.8) and congenital heart disease (RR=3.0), and a trend towards an increase of clubfoot (RR=2.7), among 413 newborns of epileptic women, compared to the entire birth population. An uncontrolled historical cohort study in France (Deblay et al., 1982; Vert et al., 1979) also reported a high prevalence of clubfoot (2.6%). In contrast, Lowe (1973), who conducted a study of 245 births to epileptic women in Cardiff, Wales, derived from a total birth population, did not find a significant association between AED and any one malformation, although there was some suggestion that the specific rates for orofacial clefts and neural tube defects might be raised.

In some studies, congenital dislocation of the hip was noted to occur in about 0.9-3% of the offspring born to mothers who took AED during pregnancy (Deblay et al.1982; Granström and Hiilesmaa, 1982a; Hiilesmaa et al., 1983; Nakane et al., 1980). The corresponding values in the general population are in the order of 0.03 to 0.18% (Heinonen et al.,

1977; Leck et al., 1968; Nakane et al., 1980).

In an historical cohort study of 88 offspring exposed to AED in utero (Dansky et al., 1982a), the relative frequencies of congenital heart disease (6.8%), cleft lip with or without cleft palate (3.4%) and ventral hernia (2.3%) were significantly elevated relative to the general population frequencies.

Several prospective or historical cohort studies noted frequent occurrence of inguinal hernia, ranging from 4.3% to 10.7% of offspring exposed to AED in utero (Deblay et al., 1982; Hill et al., 1974; Miyakoshi and Seino, 1984). In one study, the relative frequency among control offspring of nonepileptic parents was 4.0% (Hill et al., 1974), which is higher that found in the U.S Collaborative Perinatal Study (1.4%) (Heinonen et al., 1977).

In recent years, as discussed under case-control studies, spina bifida has been specifically associated with in utero exposure to VPA during the first trimester.

Despite the consistency with which most studies have shown an increase in cardiovascular defects in epileptic women, two large studies have cast some doubt about the relationship between maternal epilepsy and congenital heart disease in the offspring. In the large birth registry study in Norway (Bjerkedal, 1982b), anomalies of the heart and great vessels were not increased among offspring of epileptic women as compared with matched controls. However, this study was limited to cardiac defects noted at birth, and did not differentiate according to the maternal use of AED during pregnancy.

Friis and Hauge (1985) found a similar prevalence of congenital heart disease among 1062 liveborn offspring of epileptic males (0.75%) and

1399 females (0.71%), which was similar to that for the general population. The occurrence of congenital heart disease was also not related to AED exposure in utero. The authors attributed their discrepant findings to possible selection bias in previous studies, which may have included a limited number of patients with more severe epilepsy, or to bias in the reporting of studies which only showed positive results.

b. *Anticonvulsant Syndromes and Minor Anomalies*

In 1963, Müller-Küppers reported a single case of a boy with submucous cleft palate, microcephaly and mental deficiency, whose mother had epilepsy treated with mephenytoin throughout pregnancy.

In a letter to the Lancet in 1968, Meadow described 6 children with cleft lip and palate, and other abnormalities including congenital heart disease, minor skeletal defects and unusual facies and skulls, born to epileptic women who took AED during pregnancy. He asked for details of other babies with cleft lip and palate associated with treated maternal epilepsy. From the replies, he compiled a selected series of 32 children with similar multiple major and minor malformations, born to epileptic women who received high doses of PHT and barbiturates in combination therapy (Meadow 1970). In 1972 in Leeds England, Speidel and Meadow published the results of an historical cohort study of the outcome of pregnancy in epileptic women. They noted, among 324 offspring exposed to AED in utero, 4 children wih craniofacial and multiple minor anomalies, who were described as "odd" in appearance; two of the children were mentally subnormal. A total of 6 children or 1.5% of the offspring born to epileptic women were noted to be mentally

subnormal (5 of these were on medication), compared with the findings of one similarly affected child in the control group (0.2%). These findings prompted the authors to postulate that the major and minor abnormalities observed in the offspring of epileptic women constitute a syndrome with variable expressivity. When fully expressed, the syndrome consists of congenital heart disease, cleft lip and palate, trigonocephaly or microcephaly, and various minor anomalies which include hypertelorism, low-set abnormal ears, short neck with low posterior hairline, bilateral single transverse palmar creases, and minor skeletal abnormalities. Mental subnormality may also be a feature.

In 1973, Loughnan and colleagues described seven selected infants with similar skeletal anomalies (hypoplasia and irregular ossification of the postaxial phalanges) who were born to mothers taking PHT and barbiturates. In view of the association of major malformations, particularly diaphragmatic hernia, in 5 of the 7 infants, the authors suggested that the skeletal changes may serve as "markers" indicating more serious teratogenic effects.

Since these reports, a number of investigators have reported craniofacial abnormalities and hypoplasia of the distal phalanges and nails of the hands and feet. Individual features may occur alone or in combination, and in association with prenatal and/or postnatal growth deficiency. Similar patterns of anomalies have been seen in offspring exposed to hydantoins, PB, PRM, trimethadione, and recently with CBZ and VPA, thus showing that there is great overlap in the syndromes associated with these anticonvulsants. Many of the features also occur in relation to other teratogenic agents such as alcohol (Hill, 1976). More detail concerning the individual features associated with specific

AED are given in Section II.K2b(3)(a), Type of Anticonvulsant).

Most controlled cohort studies of the frequency of minor anomalies in the offspring have not been carried out blind to drug exposure , and thus may be subject to observer bias. Minor anomalies may also be difficult to assess, involving more subjectivity on the part of the observer than with major congenital anomalies. For example, not all investigators did measurements to determine true hypertelorism, judging this anomaly by inspection alone. A flat nasal bridge may give the optical illusion of hypertelorism. The criteria for certain minor anomalies, for example epicanthus, was not described in most of the studies, and therefore it is difficult to compare the results of different studies. Most studies did not evaluate similar anomalies in the parents, and therefore the extent of the genetic basis for these anomalies is not known. The importance of genetic factors in the determination of these anomalies was suggested by reports of minor anomalies such as epicanthus, low-set ears, and dermal arches in epileptic patients themselves, before the advent of modern day AED therapy (Janz,1982a).

In 1974, Hill and colleagues published the results of a carefully carried out prospective study, in which 28 newborns exposed in utero to PHT and/or barbiturates and 165 control infants born to nonepileptic mothers were followed from birth to 36 months of age. The majority of the epileptic mothers were from a middle socioeconomic group. Examination of the infants was performed without prior knowledge of drug exposure. Children of epileptic mothers had a higher prevalence of disfiguring or life-threatening malformations (25.0% vs 4.2%, respectively), as well as a higher prevalence of a variety of minor anomalies, including large fontanelle, low hairline; broad alveolar ridge,

broad nasal bridge, Sydney line, hypoplastic distal phalanges and nails, digital thumb, and pilonidal sinus. In addition, failure to thrive and developmental delay were found more frequently in the treated group. Hill and colleagues concluded that " the clustering of physical findings observed in the infants exposed to anticonvulsant agents makes them an identifiable population among neonates." In this investigation, however, the method of analysis did not distinguish between two possible etiological factors, epilepsy and anticonvulsants, because they occurred in the same individuals.

In an update of this study, Hill and colleagues (Hill and Tennyson, 1982b; Hill et al., 1982), reported that only 13% of 48 live-born infants of epileptic mothers had no minor anomalies. The number of minor anomalies was greater in infants with major malformations (range 8 to 23) than in those without major malformations (range 2-8). Infants of epileptic mothers had a two-fold increased prevalence of minor anomalies as compared to infants of nonepileptic mothers, and minor anomalies were particularly frequent in infants exposed to a combination of PHT and PB in utero.

The prevalence of digital and/or nail hypoplasia in offspring exposed to AED has ranged from 11.0% to 51.4% in various studies (Andermann et al., 1981b, 1982a; Barry and Danks, 1974; Dieterich et al., 1980; Gaily et al., 1988a; Hill et al., 1974; Kelly, 1984b; Rating et al., 1987a,b). The prevalence of this anomaly has ranged from 0 to 16% in offspring of nonepileptic controls, 0 to 20% in offspring of untreated epileptic women, and 0 to 5.3% in offspring of epileptic fathers (Barry and Danks, 1974; Hill et al., 1974; Dieterich et al., 1980; Gaily et al., 1988a; Koch et al., 1983 ; Kelly, 1984b; Rating et al., 1987a,b). Although the digital and

nail hypoplasia were initially attributed to the specific effects of PHT, these have also been observed following exposure to other AED including PRM, VPA, CBZ, and PB (Barry and Danks, 1974; Dieterich et al.,1980; Jones et al., 1989; Kelly, 1984b; Rating et al., 1987a,b). Kelly (1984b,c) observed that digital hypoplasia occurred in 43.9% of children exposed to PHT alone in utero as compared to 23.1% of children exposed to PB alone. The frequency and severity of distal digital hypoplasia did not differ between children exposed to PHT alone or combined with other AED.

Andermann, Dansky and colleagues (1981b,1982a) found a high prevalence of digital hypoplasia with or without nail hypoplasia (22%) in children exposed to AED in utero, whereas this anomaly was not observed in unexposed children of epileptic women. Dermal arch patterns on the fingers occurred more frequently in the treated (18.7%) as compared to the untreated group (2.1%), and were particularly evident on hypoplastic phalanges (34.5%). These patterns were not significantly elevated in the untreated group as compared with controls. The authors suggested that dermal arches may serve as a sensitive indicator of the teratogenic effects of AED on the fetus.

Lindhout and co-workers (1985a) confirmed the findings of Andermann et al. (1981b,1982a) concerning the occurrence of dermal arches in the offspring of treated epileptic women, and also investigated the contribution of genetic factors to the occurrence of dermal arch patterns. The authors found that the teratogenic effects of AED may not be the only explanation, and that constitutional factors, some of which may be related to epilepsy itself, also appear important in the determination of dermal arch patterns.

In a prospective West German study (Koch et al., 1983; Rating et al.,1982a, 1985,1987a,b), minor anomalies occurred significantly more often in children exposed to AED in utero as compared to nonepileptic controls, while anomalies in offspring of untreated epileptic women and in offspring of epileptic fathers were not increased. The prevalence of minor anomalies was similar for PHT, VPA and PRM monotherapies. Maternal polytherapy was associated with a higher frequency of minor anomalies in the offspring than was monotherapy. The type of maternal epilepsy and the presence of grand mal seizures during pregnancy were not related to the occurrence of minor anomalies.

Van Lang and colleagues (1984) identified 39 children exposed to AED in utero as part of a longitudinal study of major and minor anomalies in a large population of newborns. Seven children and two of their siblings had cephalometric radiographs at follow-up at age 4 to 10 years, and were found to have decreased craniofacial growth measurements including reduced bony interorbital distance, maxillary size, mandibular length, cranial base and skull size, and a reduction in nose dimensions, when compared to normal standards. These reductions were not evident on clinical examination. The children, exposed to PHT alone or in combination with PB, were not found to have decreased weight, length or head circumference, or major malformations at birth. The authors concluded that a subtle craniofacial change may occur in exposed children. Their findings were similar to the rat model of PHT embryopathy showing reduced craniofacial measurements (Lorente et al., 1981). In this study, selection bias may have played some role in the findings as only 18% of the cohort identified at birth were seen at follow-up.

Gaily and co-workers (1987, 1988a), in a prospective regional-based study in Finland, investigated the occurrence of minor anomalies in 121 children of epileptic mothers at age 5 1/2 years and controls born in the same area, taking into account minor anomalies in the parents. Eighty-two of the children had been exposed to PHT alone or combined with other AED in utero, 24 had been exposed to other AED, and 15 were unexposed. Examination was done blind to AED exposure. Minor anomalies considered to be typical of the fetal hydantoin syndrome (epicanthus, hypertelorism, small upturned nose, long philtrum, abnormal ears, low hairline, nail or distal phalangeal hypoplasia and three or more dermal arches) were found to be significantly more frequent in epileptic mothers and their offspring, as compared to control mothers and their offspring. Using multiple logistic regression analysis to separate the effects of PHT exposure and inheritance, the authors found that the presence of the same feature in the mother was significantly associated with epicanthus, low hairline, nail hypoplasia, and three or more dermal arches. Moderate to high maternal plasma PHT levels (greater than 10 µg/ml) in the first 20 weeks of pregnancy was significantly associated with hypertelorism, nail and phalangeal hypoplasia, and three or more dermal arches in the child. Surprisingly, mid-face hypoplasia, defined as hypertelorism, small upturned nose and long philtrum combined, was not found to be significantly associated with the presence of this combination of anomalies in the mother, or with high PHT exposure. The authors concluded that most of the typical anomalies previously attributed to PHT do not appear to be related to PHT exposure. In this study, 47.6% of mothers who took PHT during pregnancy had unknown PHT levels in the first 20 weeks of pregnancy.

It is not clear to what extent the loss of data may have influenced the lack of association between specific anomalies and PHT exposure.

Since the original report of Hanson et al. (1976) (see section II.K2 b(3)(a)(1),Type of Anticonvulsant, Hydantoins), several investigators have attempted to assess the occurrence of a pattern of anomalies consistent with the fetal hydantoin syndrome in offspring of epileptic women as compared to controls. These studies have shown variable frequencies, partly due to differences in the inclusion of characteristic anomalies. Differing frequencies may also reflect variation in the patient population with respect to the severity of the epilepsy, the type and number of AED, and differences in genetic background (see section II.K, Etiology of Congenital Anomalies in Offspring of Epileptic Patients).

Not all studies defined the syndrome as delineated by Hanson and colleagues which included craniofacial dysmorphic features and at least three of the four major characteristics - prenatal or postnatal onset growth retardation, microcephaly, and mental deficiency. In their study, 11% of the children were classified as clearly having the syndrome, and an additional 31% displayed some dysmorphic features compatible with the syndrome. Gaily et al. (1987,1988a) did not find any children with all of the main characteristics of the hydantoin syndrome among 106 children of treated mothers with epilepsy (82 of these were on PHT therapy with or without other AED). A total of 39% of the children of epileptic mothers had two or more typical minor features of the syndrome, as compared to 15% of control offspring. Hill et al. (1982) reported that 5 of 59 offspring (8%) exposed to AED in utero had intrauterine growth retardation and/or failure to thrive in the first

months of life; major malformations (in 4 of the 5); ≥ 9 minor anomalies; and intelligence quotients ≤ 90. All had been exposed to combinations of AED in utero.

Majewski et al. (1981) found 10 infants with some characteristics of the "hydantoin-barbiturate syndrome among 146 treated epileptic mothers, as compared to no affected infants among untreated epileptic mothers or epileptic fathers. All 10 had "typical facies" with or without growth retardation, microcephaly, mental retardation, nail hypoplasia, or major malformations. Polytherapy with hydantoins and barbiturates was associated with a higher frequency of the embryopathy than when these drugs were given alone. Dieterich et al (1980) found the combination of a short upturned nose, hypertelorism and phalangeal hypoplasia in 9 of 37 children (24%) exposed to AED in utero, while offspring of epileptic fathers were not affected. This combination of features occurred predominantly in children exposed to PHT. Rating et al (1987a,b) reported that a "typical face" consisting of hypertelorism, epicanthal folds, and a broad depressed nasal bridge occurred in 23.8% of offspring exposed to AED, particularly after exposure to PB/PRM, while only one child of a nonepileptic mother (0.9%) showed this combination of features. Only one offspring of 18 untreated epileptic mothers had a "typical face" and 19 offspring of epileptic fathers were not affected (Koch et al., 1983). Deblay et al. (1982) reported "typical facies" in 9 of 115 children (8%) exposed to AED, and atypical facies in 6 cases (5%).

K. Etiology of Congenital Anomalies in Offspring of Epileptic Patients

1. *Genetic Factors*

a. *Susceptibility to Malformations and Epilepsy*

Not all children exposed to AED in utero will have a birth defect or exhibit a pattern of altered growth and morphogenesis. For example, there have been a number of instances in the literature of sibs, fraternal twins, or triplets who were exposed to AED in utero and who exhibited differences in the type and extent of teratogenic effects (Bustamante and Stumpff, 1978; Chan and Poon, 1974; Goodman et al., 1976; Phelan et al., 1982). The clinical variability in the expression of abnormalities may be due in part to other environmental and genetic factors as depicted in Figure II.2. The genetic predisposition to abnormal development may involve susceptibility to errors in morphogenesis and/or differences in the metabolism of AED.

In an historical cohort study of pregnancy outcome in epileptic women, Dansky and colleagues (1982a) showed a higher prevalence of malformations in relatives of offspring with major malformations as compared to that for normal children. Other authors have also reported familial occurrence of congenital malformations in cases where an epileptic mother had been taking AED during pregnancy and subsequently had a malformed child (Annegers et al., 1974; Elshove and van Eck, 1971; Nakane et al., 1980; Pashayan et al., 1971; Starreveld-Zimmerman et al., 1974; Waziri et al., 1976).

In experimental studies, Finnell and Chernoff (1984a,b) found that several mouse strains showed an increased frequency of abnormalities in response to increasing dose and plasma levels of PHT, but the pattern

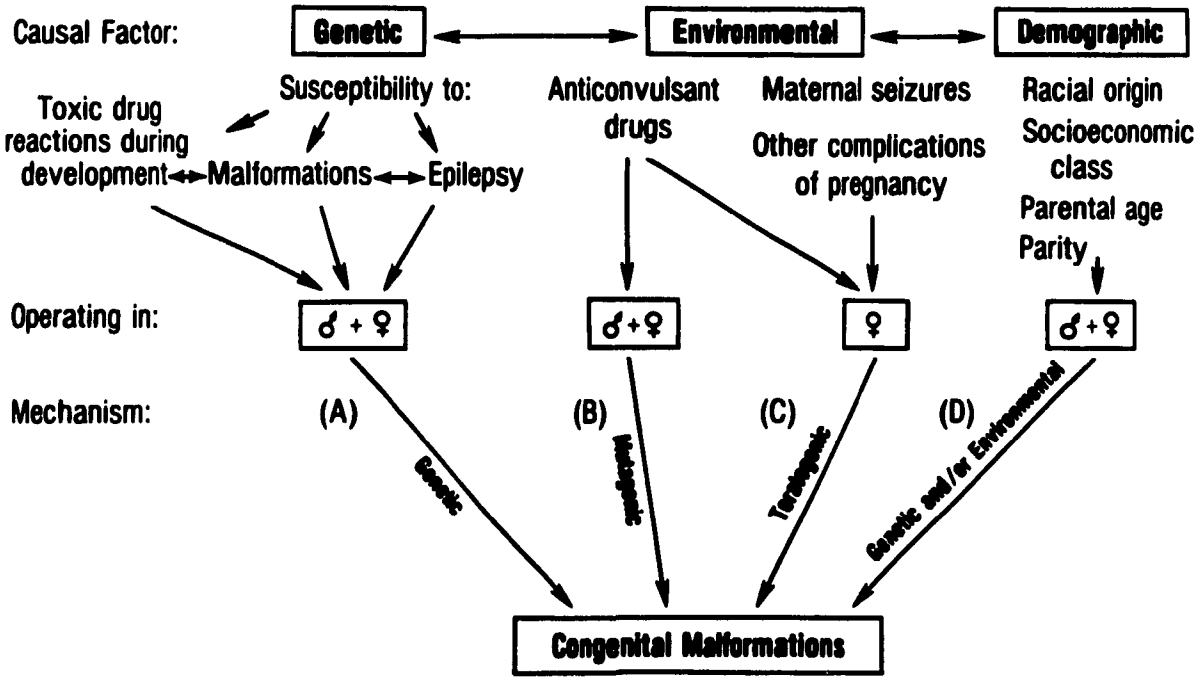

Figure II.2

Possible etiological factors for the increased risk of congenital anomalies and other abnormalities in the offspring of patients with epilepsy. If genetic factors related to epilepsy, mutagenic or demographic factors (A, B or D) play a role, then the offspring of males with epilepsy should also show an increased risk of the specific types of abnormalities observed in the offspring of women with epilepsy. Present evidence strongly points to the teratogenic effects of anticonvulsants, although other factors may also be contributing.

of abnormalities differed for the different strains. This variable response appeared to be related to genotypic differences in susceptibility to specific phenytoin-induced malformations. Finnell and Chernoff (1987) has postulated that the expression of isolated birth defects involves liability genes determining the susceptibility of a single organ system to maldevelopment, while the expression of a pattern of abnormalities involves liability genes for more than one organ system. A putative teratogen operates on a susceptible genotype which ultimately influences its phenotypic expression (Wilson, 1977a ; Finnell and Chernoff, 1984a).

An example of such liability genes are those which control the level of glucocorticoid receptors in the embryonic palate, which are thought to mediate the induction of cleft palate by glucocorticoids and PHT in the mouse (See section II.R3b, Glucocorticoids).

Since both the epilepsies and major congenital malformations are known to be inherited as multifactorial traits (Andermann, 1982a, Carter, 1965), involving interaction of many genes with environmental factors, these two groups of disorders may have some genes in common (Shapiro et al., 1976; Janz, 1982a,b,c).

Some studies have investigated the occurrence of congenital malformations in offspring of epileptic men as a means of evaluating a possible association between epilepsy and congenital malformations (Tables II. 7 and II. 8). Dansky and colleagues (1982a) and Annegers and co-workers (1978) found higher prevalences of major congenital malformations among offspring of treated epileptic women than among epileptic males. The prevalence of malformations in offspring of epileptic males was not higher than that in the general population.

Table II.7 : Occurrence of Congenital Anomalies in Offspring of Epileptic Fathers and Epileptic Mothers

		FATHERS						MOTHERS							
		TOTAL		AED		NO AED		TOTAL		AED		NO AED		CONTROLS	
Author(s), year	MA/MI[a]	N	% Malf	N	% Malf	N	% Malf	N	% Malf	N	% Malf	N	% Malf	N	%Malf
Meyer, 1973	MA,MI	270	11.1	160	16.9	110	2.7	323	17.3	199	18.6	124	15.3	-	-
Shapiro et al., 1976	MA	396	4.5	-	-	-	-	305	6.6	102	4.9	97[c]	8.2	49,590	2.7
	MI	396	3.8	-	-	-	-	305	3.9	102	6.9	97[c]	3.1	49,590	3.7
	MA,MI	396	8.3	-	-	-	-	305	10.5	102	11.8	97[c]	11.3	49,590	6.4
Annegers et al., 1978	MA	128	3.9	88	3.4	40	5.0	259	8.1	177	10.7	82	2.4	748	3.5
Majewski et al. 1981	MA[a]	71	21.1	42	19.0	29	24.1	184	17.9	138	16.8	46	15.2	-	-
	MA,MI[b]	71	0.0	42	0.0	29	0.0	192	5.2	146	6.8	46	0.0	-	-
Dansky et al., 1982a	MA	97	1.0	-	-	-	-	134	12.7	88	15.9	46	6.5	-	-
Beck-Mannagetta et al., 1982	MA,MI	371	11.1	-	-	-	-	397	15.4	268	19.8	129	6.2	-	-
Lindhout et al., 1985b	MA	29	10.3	28	10.7	1	0.0	186	7.5	172	7.6	14	7.1	-	-

Table II.7 cont'd

		FATHERS						MOTHERS							
		TOTAL		AED		NO AED		TOTAL		AED		NO AED		CONTROLS	
Author(s), year	MA/MI[a]	N	% Malf	N	% Malf	N	% Malf	N	% Malf	N	% Malf	N	% Malf	N	% Malf
Rating et al., 1987a	MA	32	12.5	-	-	-	-	150	8.7	116	8.6	34	8.8	116	6.0

[a] Major or minor anomalies

[b] Children with major congenital anomalies and no dysmorphic features; [c] Children with hydantoin-barbiturate syndrome with or without major congenital anomalies; [d] Births to women not taking phenytoin. The women may have been taking other anticonvulsants

Table II.8: Occurrence of Cleft Lip and / or Cleft Palate (CL± CP, CP) and Cardiovascular Malformations (CVM) in Offspring of Epileptic Fathers and Epileptic Mothers

	FATHERS									MOTHERS								
	TOTAL			AED			NO AED			TOTAL			AED			NO AED		
Author(s), year	N	CL ± CP (CP)	CVM	N	CL ± CP (CP)	CVM	N	CL ± CP (CP)	CVM	N	CL ± CP (CP)	CVM	N	CL ± CP (CP)	CVM	N	CL ±CP (CP)	CVM
Meyer et al., 1973	270	0	1	160	0	0	110	0	1	323	5[a]	5	199	5	5	124	0	0
Shapiro et al., et al., 1976	396	(3)	-	-	-	-	-	-	-	305	1	-	-	-	-	-	-	-
Annegers et al., et al., 1974, 1978	234	0	1	-	-	-	-	-	-	197	1 (2)	6	141	1	6	56	0	0
Majewski et al., 1981	71	0	0	42	0	0	29	0	0	194	2[a]	1	148	2	1	46	0	0
Beck-Mennagetta et al., 1982	371	0	4	-	-	-	-	-	-	397	5[a]	8	268	5	8	129	0	0
Dansky et al., 1982a	97	0	0	-	-	-	-	-	-	134	3	7	88	3	6	46	0	1
Friis and Hauge, 1985	1062	-	8	-	-	2[b]	-	-	6	1399	-	10	-	-	5[c]	-	-	5

[a] Type of orofacial cleft not specified

[b] 2 of 8 children with CVM were born to fathers who took AED prior to conception

[c] 5 of 10 children with CVM were exposed to AED in utero

Table II.8 cont'd

Occurrence of Cleft Lip and / or Cleft Palate (CL± CP, CP) and Cardiovascular Malformations (CVM) in Offspring of Epileptic Fathers and Epileptic Mothers

		FATHERS								MOTHERS							
		TOTAL			AED			NO AED			TOTAL			AED			NO AED	
Author(s), year	N	CL ± CP (CP)	CVM	N	CL ± CP (CP)	CVM	N	CL ± CP (CP)	CVM	N	CL ± CP (CP)	CVM	N	CL ± CP (CP)	CVM	N	CL ±CP (CP)	CVM
Friis et al., 1986	1300	(1)	-	-	-	-	-	-	-	789	4	-	477	3	-	312	1	-
Rating et al, 1987a	32	0	2	-	-	-	-	-	-	150	2	6	116	2	5	34	0	3

Occurrence of Specific Anomalies Over All Studies in Table II.8

Type of Anomaly	Total			AED			No AED		
	N	No.	%	N	No.	%	N	No.	%
Epileptic Fathers									
Cleft palate	2801	4	0.14	231	0	0.0	140	0	0.0
Cleft lip ± cleft palate	2801	1	0.04	231	1	0.43	140	0	0.0
Cardiovascular malformations	2167	16	0.74	231	0	0.0	140	1	0.71
Epileptic Mothers	**Total**			**AED**			**No AED**		
	N	No.	%	N	No.	%	N	No.	%
Cleft palate	1761	2	0.11	994	2	0.20	449	0	0.0
Cleft lip ± cleft palate	1761	12	0.68	994	19	1.01	449	1	0.13
Cardiovascular malformations	2980	46	1.54	1132	32	2.83	449	4	0.89

General population frequencies (Heinonen et al., 1977, Leck et al., 1968)- CL± CP: 0.10-0.14% ; CP: 0.06%; CVM 0.42-0.80%

Other studies found slightly higher frequencies of minor and/or major malformations in offspring of epileptic females (Meyer,1973 ; Shapiro et al., 1976; Beck-Mannagetta et al., 1982) or epileptic males (Lindhout et al., 1985; Majewski et al., 1981; Rating et al., 1987a). Shapiro and associates (1976) noted a higher prevalence of major congenital malformations among children of epileptic women than in nonepileptic parents, while offspring of epileptic males showed an intermediate frequency of malformation (Table II. 8). A higher prevalence of malformation was also observed by Rating and co-workers (1987a) among a small number of offspring of epileptic men than among controls. Majewski and colleagues (1981) observed a high prevalence of single malformations without dysmorphic features among offspring of both epileptic males and females, the majority of which were inguinal and umbilical hernias. In contrast, features of the hydantoin-barbiturate syndrome were only present in offspring of epileptic mothers.

When the data are combined over all studies which assessed the occurrence of congenital malformations in offspring of epileptic males and females, it can be seen that only the specific orofacial anomaly, cleft palate was increased in the offspring of epileptic males (Table II.8). The prevalence of this malformation (0.14%) is about 2-fold that found in the general population (0.06%) (Heinonen et al., 1977; Leck et al., 1968). The occurrence of cleft palate is confined to the studies of Shapiro et al. (1976) who employed the data of the U. S. Collaborative Perinatal Study, and Friis et al. (1986) who studied offspring of epileptic parents in a defined region of Denmark, by record linkage with a national cleft register (Table II.8). Congenital heart disease did not appear to be elevated among the offspring of epileptic males as

compared with the general population frequencies of 0.42 to 0.80% (Heinonen et al., 1977; Leck et al., 1968). In contrast to these findings, cleft lip with or without cleft palate, cleft palate and congenital heart disease were increased in offspring of treated epileptic women , as compared with the general population figures (Tables 11.6, 11.8).

In the study by Beck-Mannagetta and colleagues (1982), the prevalence of congenital heart disease in offspring of epileptic fathers may have been high (Table 11.8), but these data were collected in interviews with the parents. Rating and colleagues (1987a) also found a high prevalence of congenital heart defects in a small number of offspring of epileptic males, but these were two defects which closed spontaneously (ventricular septal defect, patent ductus arteriosus) in children of unknown gestational age. Friis and Hauge (1985) found similar prevalences of congenital heart disease in offspring of epileptic males and females which were not elevated relative to that in the general population and this is discussed in more detail in section II.J2a, Type of Major Congenital Anomaly).

Males with epilepsy are less likely to marry than women with epilepsy (Dansky et al., 1980). Therefore, males who reproduce may have less severe epilepsy than do women who have children. This might partially explain the differences observed in offspring of women and men with epilepsy, if constitutional factors associated with epilepsy contribute to the occurrence of malformations in the offspring (Annegers et al.,1978).

A second method of assessing the influence of constitutional factors associated with epilepsy in the occurrence of congenital malformations in the offspring of epileptic women, has been to evaluate the prevalence

of malformations in the offspring of untreated women with epilepsy. Most studies have not shown an increased risk of total or specific malformations in offspring of untreated women, and this was reviewed in more detail in section II. J2a, Type of Major Congenital Anomaly.

Another approach to the investigation of constitutional factors has been to study the familial aggregation of congenital anomalies and epilepsy. Dronamraju (1970) reported that 15 to 20% of his patients with cleft lip and/or palate had a first- or second-degree relative with epilepsy, and suggested that there may be a genetic relationship between epilepsy and oral clefts. The significance of this finding is difficult to evaluate, however, as the total number of relatives was not given, and possible teratogenic factors were not indicated.

In agreement with the hypothesis of Dronamraju, Friis (1979) in Denmark showed a 2-3 fold increased prevalence of epilepsy among fathers or mothers of 391 children with cleft lip and/or palate (ascertained in a national cleft register), as compared with the prevalence of epilepsy in fertile males and females age 15 years or older. Friis argued that these results provided evidence for a constitutional association between epilepsy and orofacial clefts. Several factors however, should be considered in evaluating this study. Age-specific prevalence rates of epilepsy for each sex were not used, and therefore the magnitude of the increased risk is questionable. Probands included in the study had either cleft lip with or without cleft palate or isolated cleft palate, which are considered to be etiologically distinct. If the data of Friis are reevaluated taking into account specific facial clefts, then a higher than expected prevalence of paternal epilepsy is found among cleft palate probands, but not among

probands with cleft lip and/or palate. The reverse is found for maternal epilepsy which is associated with cleft lip with or without cleft palate, but not cleft palate. In the study by Friis (1979), three times as many mothers as fathers of probands with facial clefts received AED therapy in the year prior to conception or in the first trimester of pregnancy, suggesting that maternal AED therapy may have contributed to the association of maternal epilepsy and facial clefts. No comparison was available, however, with the pattern of AED use among all epileptics in the general population.

A similar type of study was carried out by Kelly and associates (1984) who investigated the association of epilepsy and clefting in families of cleft lip and/or palate probands ascertained from a genetics counselling center. Eleven mothers (6.3%) and two fathers (1.1%) with epilepsy were found among 175 cleft probands. Six of the mothers and none of the fathers had taken AED therapy during pregnancy or at the time of conception. The expected prevalence of maternal epilepsy was given as 1 in 300 (0.3%). No figures were given for the expected prevalence of paternal epilepsy. A higher proportion of cleft probands whose parents had epilepsy showed a positive family history of epilepsy or orofacial clefts in other relatives, as compared to cleft probands without a history of parental epilepsy. Drawbacks of this study were similar to those of Dronamraju (1970) and Friis (1979).

In later studies, using record linkage to the national cleft register, Friis and associates (1981) found a 2-fold increased prevalence of cleft lip and/or palate in 3203 epileptic patients themselves, ascertained from defined regions. The distribution of different types of facial clefts and types of epilepsy did not differ from expected. In a

third study, Friis and associates (1986) compared the prevalence of orofacial clefts in sibs and children of 2,072 epileptic men and women, also ascertained in a defined region. The prevalence of cleft lip with or without cleft palate was about 4.7-fold higher than expected in children of epileptic mothers who took AED during pregnancy (upper limit of confidence interval nearly 14), and 2.4-fold higher in children of untreated women with epilepsy. The prevalence of this anomaly was not increased in children born prior to the onset of maternal epilepsy, in children of epileptic men or in sibs of epileptic males or females. Isolated cleft palate was not increased in any of the groups.

Fraser and coworkers (1978) did not find an increased prevalence of total or specific malformations among sibs of probands with epilepsy as compared with sibs of controls without epilepsy. However, an increase in relatively rare malformations could not be ruled out due to insufficient numbers. Annegers and Hauser (1982) attempted to separate the effects of constitutional factors from those of AED by investigating the risk of major congenital malformations in children and other relatives of women with epilepsy. The highest risks for total malformations, congenital heart disease and cleft lip and/or palate were found in the children exposed to AED in utero. A slight excess of congenital heart disease was noted in the parents of women with epilepsy but other relatives were not found to have elevated risks of congenital malformations.

When the results of studies in offspring of epileptic women taking AED are pooled (Table 11.6), the results show that cleft lip with or without cleft palate and cleft palate are elevated about 9 to 12-fold and 2.7-fold, respectively, relative to the general population. Present

evidence therefore suggests that constitutional factors associated with epilepsy may account for the increased risk of cleft palate in the offspring, but it does not entirely explain the increased risk of cleft lip with or without cleft palate in the offspring of epileptic women.

b. *Individual Differences in Drug Metabolism*

Genetic differences in drug metabolism may also be important in the occurrence of AED-induced congenital malformations. In the murine model, it has been shown that the susceptibility to birth defects caused by aromatic hydrocarbons such as benzo(a)pyrene is influenced by a single gene locus (*Ah* locus). Alleles at this locus regulate the induction of one form of cytochrome P-450 drug metabolizing enzymes (aryl hydrocarbon hydroxylase, P_1-450) which converts this compound to reactive metabolites. These metabolites have been correlated with increases in cancer, mutation, toxicity, and birth defects. Inducibility of cytochrome P_1-450 is inherited as an autosomal dominant trait, and has been shown to be correlated with the degree of teratogenesis induced by benzo(a)pyrene. In matings producing a mixture of inducible and noninducible embryos in a noninducible mother, the inducible embryos show a higher frequency of resorption, birth defects and binding of drug metabolites in response to maternal administration of benzo(a)pyrene. In contrast, such differences among the embryos are abolished in matings producing an admixture of embryos in an inducible mother, because of the influence of maternal metabolism. Thus the genotype of both the mother and the embryo are important in determining the outcome (Nebert, 1980; Shum et al., 1979). Although

the metabolism of AED such as PHT is not controlled by the Ah locus, similar genetic mechanisms may be involved in the metabolism of this drug to toxic products and the induction of birth defects (Nebert, 1980).

Phenytoin is metabolized by cytochrome P-450 monooxygenases to several oxidized products, primarily parahydroxylated, 5(*p*-hydroxyphenyl)-5-phenylhydantoin (*p*-HPPH) and dihydrodiol, 5-(3,4-dihydroxy-1,5-cyclohexadien-1-yl)-5-phenylhydantoin metabolites (Wells and Harbison, 1980). Indirect evidence suggests that a reactive arene oxide intermediate is produced during the biotransformation of PHT to these products. The arene oxide intermediate is converted to *p*-HPPH spontaneously, or to the dihydrodiol by the enzyme epoxide hydrolase . If not detoxified, such reactive metabolites can covalently bind to cell macromolecules, and have been implicated in toxic effects of PHT including gingival hyperplasia, hepatotoxicity, aplastic anemia, and drug-induced birth defects (Buehler, 1984, 1986; Dansky et al., 1987b; Martz and Blake,1977; Shear and Spielberg, 1988; Spielberg et al.,1981a; Strickler et al.,1985) (see section II. R1a, Toxic Metabolites of AED). The relative rates of production and detoxification of such intermediate metabolites determine the extent of toxic drug reactions (Wells and Harbison, 1980).

In vitro studies have suggested that the predisposition to phenytoin toxicity such as hepatotoxicity and aplastic anemia is mediated by an inherited defect in arene oxide detoxification (Shear and Spielberg, 1988; Spielberg et al., 1981a). Human lymphocytes were exposed to PHT metabolites generated by a murine hepatic microsomal drug -metabolizing system (Spielberg et al., 1981b). A dose-related increase in cell death was observed with increasing concentrations of PHT.

Toxicity was enhanced by inhibiting epoxide hydrolase and was diminished by adding epoxide hydrolase to the assay. In other studies, lymphocytes from patients with previous hepatotoxicity were tested in the assay (Spielberg et al., 1981a). The patients' dose-response curves resembled the response of control cells in which epoxide hydrolase was inhibited. Cells from the parents' of the patients showing an intermediate response, and the responses of sibs were consistent with an autosomal co-dominant pattern of inheritance. In vitro studies using this assay system have also implicated an inherited defect in the detoxification of arene oxide metabolites of PHT in the etiology of birth defects (Dansky et al., 1987b; Strickler et al., 1985) (see section II. R1a, Toxic Metabolites of AED).

Several members of some families have been shown to have hypometabolism of PHT showing insufficient hydroxylating capacity (Kutt, 1971; Vasko et al.,1980), and it has been suggested that this is inherited as an autosomal dominant trait. Individuals with this trait develop high plasma PHT levels and symptoms of drug toxicity with average dosages of the drug. There have also been reports of hypermetabolism of PHT (Lebrun and Villeneuve, 1983), in which unusually large doses of the drug are required to maintain plasma levels within the therapeutic range in order to control seizures. No studies have been done correlating the rate of metabolism of PHT with the occurrence of congenital malformations in humans.

Investigation of the plasma elimination-rates of PHT in 12 inbred strains of mice has led to the categorization of strains as fast or slow metabolizers of of PHT. Crosses of fast and slow metabolizers yield offspring with intermediate elimination rates, indicating additive

inheritance, presumably of the rate-limiting step involving cytochrome P-450 monooxygenation (Atlas et al., 1980). The rate of metabolism, however, does not necessarily correlate with strain susceptibility to PHT-induced malformations (Atlas, 1980; Finnell, 1987). (See section II. R1a, Toxic Metabolites of AED).

Matings of inbred strains of mice with two different phenotypes of microsomal epoxide hydrolase activity, based on heat sensitivity and the optimum activity pH , have provided evidence for two alleles at one gene locus with co-dominant expression in heterozygotes (Lyman et al., 1980). The epoxide hydrolase polymorphism, however, does not appear to explain differences in strain susceptibility to the teratogenic effects of PHT (Hartsfield, 1986). However, other studies in mice and humans indicate that genetic determination of differences in epoxide hydrolase activity may correlate with the teratogenic response to PHT (See section II. R 1a, Toxic Metabolites of AED).

It has been suggested that the two different enzyme systems, the monooxygenase and the epoxide hydrolase systems may be under separate genetic control. If both the parent compound and its arene oxide metabolite are teratogenic (see section II. R1a, Toxic Metabolites of AED), then an individual's susceptibility to PHT-induced malformations may depend on the genes controlling these two enzyme systems. For example, an individual who converts PHT slowly to its hydroxylated metabolites (slow metabolizer), and who has a relatively high epoxide hydrolase activity , may still have an affected offspring (Finnell and Chernoff, 1987).

2. *Anticonvulsant Medication*

a. *Mutagenic Effects*

In approximately 75% of epileptic patients, the onset of the disease occurs within the first two decades of life (Lennox, 1960). Adequate control of seizures usually requires longterm administration of AED, continuing well into the reproductive years. Thus, if AED cause malformations in the offspring of epileptic women, they may act as mutagenic and/or teratogenic agents. If the drugs are mutagenic, then the effect should also be noted in the offspring of epileptic males who are taking AED (Figure II.2).

Studies comparing the prevalence of congenital malformations in offspring of epileptic men according to whether or not they took AED at the time of conception have generally not shown evidence for mutagenic effects, but sample sizes were too small to detect a slight increase in malformations (Tables II.7, II.8). Meyer (1973), on the other hand, found a significantly increased prevalence of abnormalities in the offspring of treated epileptic males as compared with untreated males. In this study, data was collected by questionnaire and a broad definition of abnormality was used encompassing deafness, myopia, strabismus , etc. It is noteworthy that Meyer did not find that cleft anomalies and congenital heart disease occurred in relation to the use of AED at the time of conception (Table II.8).

Numerous case reports of children with multiple major and minor anomalies exposed to AED in utero have generally not shown any gross structural chromosomal abnormalities.

The results of in vivo and in vitro studies on the cytogenetic effects of AED therapy have been contradictory and difficult to interpret.

Several studies either did not include controls or did not indicate whether chosen controls were matched for factors such as age, sex and epilepsy; therefore, it is difficult to correlate a specific causative factor with changes in the chromosomes. Furthermore, in vivo studies have not always indicated whether cultures were derived from patients with therapeutic plasma levels of the drug.

A number of studies did not find evidence for mutagenic effects in cultured leukocytes, or fibroblasts of healthy subjects, incubated with any one of the commonly used AED at concentrations within or above therapeutic plasma levels (Bishun et al, 1975; Brøgger, 1970, Konigstein et al., 1984; Stenchever and Allen, 1973; Stenchever and Jarvis, 1970). However, other studies have shown an increased rate of chromatid exchanges and of structural chromosomal aberrations in human peripheral blood cultures (lymphocytes, mononuclear cells) of healthy subjects with therapeutic or toxic levels of PHT, PB, CBZ or ethosuximide (Margaretten et al., 1987, Maurya and Goyle, 1985; García Sagredo, 1988; Schaumann et al, 1985), as compared with controls.

Several in vivo studies have examined the chromosomes of patients taking only one anticonvulsant agent. Muniz et al. (1967) found no structural chromosomal abnormalities in patients with therapeutic plasma levels of PHT, whereas other authors reported a significantly increased frequency of structural and numerical changes and sister chromatid exchanges in male and female epileptic patients with average doses or therapeutic plasma levels of PHT, PB, CBZ or VPA as compared with controls (Curatolo et al., 1986; Habedank et al, 1982; Herha and Obe, 1976; Kulkarni et al., 1984 ; Márquez-Monter et al., 1970; Satish and Kalyanaraman, 1984).

Other studies have described tetraploidy and heteroploidy (Detoni at al.,1967) and chromosome breaks and rearrangements (Ayraud et al., 1968; Matsushima et al., 1981; Neuhauser et al., 1970) in blood cells of children whose mothers had been on a combination of AED throughout pregnancy. Grosse et al. (1972) showed a highly significant increase of chromosomal aberrations in 32 epileptic mothers treated with a combination of AED and their children who had been exposed to the drugs during pregnancy, compared to untreated healthy controls. The aberration rate of the mothers and their respective children were significantly correlated. Details concerning the health of the children were not given, however.

Several in vitro studies have found a positive correlation between the incidence of chromosomal aberrations or sister chromatid exchanges and the concentration of AED in culture (Maurya and Goyle, 1985; Garcia Sagredo, 1988; Schaumann et al., 1985). In vivo studies, on the other hand, have generally not shown a correlation between the occurrence of chromosomal abnormalities and dose, number or plasma concentrations of AED, or the duration of AED therapy (Curatolo et al.,1986; Habedank et al., 1982; Kulkarni et al., 1984; Matsushima et al., 1981), with some exceptions (Matsushima et al., 1984, Satish and Kalyanaraman, 1984). This may reflect the fact that in vivo, the metabolism of AED to arene oxide metabolites may be important in their mutagenic effects.

Barcellona et al. (1987) administered PHT (100 mg/kg) in the pregnant mouse and various inducers and inhibitors of PHT metabolism, and found that the occurrence of micronucleated polychromatic erythrocytes (a measure of genotoxic effects) in the fetus appeared to be due to the formation of the arene oxide.

It has also been hypothesized that folic acid deficiency induced by AED may be one of the factors predisposing to chromosomal abnormalities, as folate plays a fundamental role in DNA synthesis (Roman and Caratzali, 1971). However, a correlation between serum folate levels and the frequency of chromosomal abnormalities in epileptic patients treated with CBZ (Schaumann et al.,1985) or PHT (Satish and Kalyanaraman,1984) has not been found.

There is also some question of the occurrence of chromosomal abnormalities in relation to epilepsy itself. In the few studies which compared treated and untreated epileptics, two studies found a higher incidence of chromosomal aberrations in lymphocytes of treated epileptics than in untreated epileptics (Curatolo et al., 1986; Kulkarni et al., 1984), although in one study some untreated epileptics had high frequencies of chromosomal aberrations (Curatolo et al., 1986). Goyle et al. (1987) found an increased rate of sister chromatid exchanges in lymphocytes of both treated and untreated male and female epileptic patients, as compared with controls.

Lutwak-Mann et al. (1967) have suggested that drugs excreted in semen may induce adverse reproductive effects, although the mechanism is not clear. These authors detected thalidomide in the semen of rabbits following the administration of this drug to male rabbits. Pretreatment of male rabbits with the drug yielded an increase in fetal loss. Although PHT and VPA have also been measured in rabbit semen (Swanson et al., 1978 a,b), their influence on the conceptus was not determined. Swanson et al. (1978 a,b) also detected PHT and VPA in human semen following acute or chronic administration of the drug.

Drugs in semen may exert adverse effects on reproduction by

impairing sperm motility or inducing mutation. The drug or metabolite could also be absorbed by the uterine mucosa and disturb normal implantation and growth of the early conceptus (Finnell and Baer, 1986).

It has been shown that PB at doses of 42 to 126 mg/kg administered by intubation induced chromosomal aberrations, such as univalents, polyploids and translocations in male germ cells of Swiss mice (Devaki Nandan and Sanjeeva Rao, 1982). A decreased number of implantations and an increase in dead implantations were found in females mated to the treated males (Devaki Nandan and Sanjeeva Rao,1983). Evidence for mutagenic effects in somatic and germ cells of mice was also found for PRM administered orally at doses of 1/4 to 3/4 LD_{50} (Rao et al.,1986). Finnell and Baer (1986) did not find that treatment of male mice with PHT influenced reproductive outcome including number of implantations, resorptions, fetal weights or the occurrence of congenital malformations.

b. *Teratogenic Effects*

(1). Pharmacokinetics of anticonvulsants during pregnancy

A number of studies have reported that plasma levels of AED usually decrease during pregnancy, returning to prepregnancy values at varying intervals postpartum. The decline in plasma levels may be one of the critical factors contributing to increased seizure frequency observed in some patients during pregnancy. The extent and time-course of the effects of pregnancy on AED disposition differ according to the type of AED, and may show large inter- and intra-individual variation. In general, the changes appear to be greatest for PHT and VPA, less so for PB, and least for CBZ and PRM. The topic has been reviewed by Eadie et al. (1977), Levy and Yerby (1985), Philbert and Dam (1982), and Nau et al.(1982a).

Not all studies investigated patients on monotherapy and therefore in these cases, it is difficult to assess the effects of pregnancy on plasma AED levels. Studies of the pharmacokinetics of AED during pregnancy have investigated trends in the plasma levels of AED with dosages unchanged during pregnancy, or have shown the increased need for increased drug dosages to maintain therapeutic plasma AED levels, in view of falling levels during pregnancy. Other studies have calculated apparent plasma clearances (daily dose in mg/ plasma concentration in mg/L), in order to control for changes in dose during pregnancy. In some cases, calculation of clearance has also taken into account weight gain during pregnancy. A disadvantage with the calculation of plasma clearance is that it assumes a linear relationship between dose and serum level, which is not the case with PHT once concentrations approach the therapeutic range [the capacity of the liver to metabolize

PHT is a saturable process, with saturation usually occurring within the therapeutic range. As dosage is increased a point is reached where small changes in dose result in major changes in the plasma level (Eadie and Tyrer, 1980)]. Some studies have looked at free pharmacologically active (unbound) concentrations of AED and have calculated intrinsic clearances.

Bardy and colleagues (1987), in a study of 111 pregnancies, showed an increase in apparent plasma clearance (daily dose/concentration) of PHT in all patients. The mean PHT clearance increased gradually over the first 32 weeks of pregnancy and reached twice the prepregnancy value during the last 8 weeks of pregnancy. Dansky and associates (1982c), in a study of 48 patients, showed that the mean level-dose ratio (µg/ml/mg/kg) during pregnancy decreased by 34% of the value outside of pregnancy. The data suggested that the decrease may occur as early as the first 12 weeks of pregnancy. In 45% of pregnancies with some decrease in the level-dose ratio, the frequency of major seizures increased over that in the 9 months prior to pregnancy.

A progressive decrease in plasma PB levels (or increase in plasma clearance) during pregnancy has been observed in a number of studies (Bardy, 1982; Bardy et al., 1982; Battino et al., 1982a, 1984; Bossi et al., 1980; Chiba et al., 1982; Dam et al., 1979; Kan et al., 1984; Lander et al., 1977; Mygind et al., 1976). Bardy and co-workers (1982) observed a decrease in plasma PB levels in about one-half of the patients taking this drug. The changes in plasma levels are smaller and show more inter-individual variation than those observed for PHT. In some patients the apparent clearance (taking into account the weight of the individual) is unaltered (Bossi et al., 1980). Both total and free concentrations of

PB have been observed to decrease during pregnancy (Chen et al., 1982).

Changes in PRM levels are variable. In some patients, PRM clearance increased after the onset of pregnancy , persisted at high levels during pregnancy, but returned to prepregnancy values after delivery (Nau et al., 1982d). Other investigators report no significant changes or a decrease in plasma levels late in pregnancy or the puerperium (Bardy, 1982; Bardy et al., 1982 ; Battino et al., 1982a;1984; Kaneko et al., 1984b; Otani et al., 1984). Plasma PB levels derived from PRM show a clear decrease during pregnancy which appears to be larger than that seen with PB given directly (Battino et al., 1982a; Otani, 1985). Nau and colleagues (1982d) and Rating and associates (1982b) reported that the metabolite to parent compound plasma concentration ratios for PB and phenylethylmalonic acid diamide (PEMA) were decreased during pregnancy as compared to nonpregnant values. The decrease in plasma concentrations of PB and PEMA occurred early in pregnancy and were observed throughout pregnancy. Levels of the metabolites rose to pre-gestational values following birth. Co-medication influenced the metabolite/PRM ratios as in non-pregnant subjects. For example, during pregnancy, the PB/PRM ratio was 0.84 when PRM was given alone (lower than in nonpregnant subjects), and this increased to 2.5 (similar to the value in nonpregnant patients) when PHT was given wih PRM. Renal clearance of PRM and its metabolites was increased in one patient in whom these were measured.

Some studies have reported a decrease in plasma levels or increased plasma clearance of CBZ during pregnancy (Battino et al.,1982a; Dam et al., 1979; Eadie et al., 1977; Kan et al., 1984; Yerby et al., 1985) while others have noted no significant alteration (Bardy, 1982; Bardy et al.,

1982; Kuhnz et al., 1983; Niebyl et al., 1979; Otani, 1985). Plasma clearance of CBZ has been shown to increase to a maximum in late pregnancy or early postpartum (Battino et al., 1982a; Dam et al., 1979). Higher CBZ epoxide/CBZ ratios have been reported (Battino et al., 1985; Dam et al., 1979; Yerby et al., 1985). Yerby et al. (1985) has presented evidence that this may be due to inhibition of epoxide hydrolase which metabolizes the epoxide to dihydrodiol (Yerby et al., 1985), rather than to increased conversion of CBZ to the epoxide.

Plasma VPA levels have also been reported to fall during pregnancy with maximum decrease occurring in late pregnancy (Chiba et al., 1982; Kan et al., 1984; Otani,1985; Philbert and Dam, 1982; Philbert et al., 1985). Detailed pharmacokinetic studies carried out by Nau and colleagues (1982a) showed that plasma VPA levels decreased by more than one-half from the first to third trimesters. The results indicated that the low blood levels of VPA observed during the third trimester could not be explained by a single mechanism, and that they were caused at least in part by increased hepatic clearance and an increased apparent volume of distribution.

Ethosuximide has been the least studied AED. A requirement for increased dosages during pregnancy, with increasing plasma concentrations postpartum has been reported in a few cases (Eadie et al., 1977; Koup et al., 1978; Rane and Tunell, 1981). Kuhnz et al. (1984) and Otani (1985) reported only minor fluctuations in plasma clearance during pregnancy and after delivery.

A number of physiological changes during pregnancy may contribute to the decline in plasma AED levels. There may be different mechanisms involved for the different drugs, and their relative importance may

differ from patient to patient.

During pregnancy, there are changes in gastrointestinal function with altered absorption (Krauer and Krauer, 1977). Ramsay et al. (1978) initially reported that decreased PHT absorption was responsible for the decrease in plasma levels of the drug during pregnancy, but this has not been confirmed by other investigators. Kochenour et al. (1980) found reduced excretion of HPPH in some pregnant patients with low plasma levels of PHT and this was taken as possible evidence for reduced absorption of PHT or altered metabolism.

Increased body fluid and tissue volume during pregnancy may play a role in the decline of plasma AED levels by increasing the volume of distribution (Eadie et al., 1977).

The concentration of plasma protcins falls during pregnancy and this results in a higher free fraction of highly protein bound drugs. A reduction in protein binding changes the relationship between total serum level and pharmacological response, as a greater proportion of the drug is unbound and pharmacologically active (Perucca and Crema, 1982). Because of the decrease in protein binding, total plasma concentrations may underestimate the concentration of free drug. Changes in free drug levels during pregnancy may well be important in terms of possible effects on the embryo and fetus.

In a study measuring plasma free and saliva concentrations during pregnancy, Knott and associates (1986) found a significant elevation of the free PHT fraction in mid to late pregnancy. Both total and free PHT concentrations have been observed to decrease during pregnancy, suggesting that there are other mechanisms apart from reduced protein binding which result in lower total PHT concentations (Chen et al.,

1982; Knott et al., 1986). Significant increases in the free fraction of PB in the second and third trimesters (Chen et al., 1982) have also been reported. A significant increase in the free fraction of VPA has been observed in all three trimesters, with maximum elevation in the third trimester (Perucca and Crema, 1982). No significant changes have been found in the percent free fraction of CBZ or its epoxide (Dam et al., 1979; Yerby,1987)

Hepatic metabolism may be increased during pregnancy due to such factors as increased levels of progesterone which stimulates hepatic microsomal enzyme activity (Krauer and Krauer, 1977). Although increased metabolism has been suggested as an explanation for the decreased plasma levels of AED, the data are conflicting (Chiba et al., 1982; Lander et al., 1984; Philbert et al., 1984). For PHT, an increase in the excretion of the main metabolite (HPPH) has not been observed, and it has been suggested that the apparent increase in clearance of PHT may be due to an increase in alternate pathways of metabolism (Philbert and Dam,1982). Chiba et al. (1982), using antipyrine disposition as a measure of hepatic metabolism in epileptic women, found evidence that hepatic drug metabolic rate is not enhanced but rather reduced during late pregnancy.

During pregnancy, there are changes in renal function such as increased glomerular filtration. Since renal excretion plays only a minor role for most AED, these changes are probably not significant (Krauer and Krauer, 1977; Philbert and Dam, 1982).

Psychological factors such as fear of the effects of AED on the unborn fetus may result in reduced compliance, and decreased dose and plasma levels of AED. Nausea and vomiting particularly in the first

trimester may result in reduced absorption of AED. The effects of vitamin supplementation should also be considered as folate intake may alter plasma AED levels (Baylis et al., 1971; Eadie et al., 1977).

(2). Placental transfer and distribution of anticonvulsant drugs to fetal tissues

Nau and colleagues (1982a) have reviewed the literature on the placental transfer of AED and their distribution to fetal tissues. This has been shown for PHT (Baughman, 1970; Mirkin, 1971; Rane et al., 1974; Shah and Miller, 1985); PB (Kuhnz et al., 1988; Melchior et al., 1967; Ploman and Persson, 1957); PRM (Kuhnz et al., 1988; Martinez and Snyder, 1973; Nau et al., 1980;1982b); CBZ (Kuhnz et al., 1983; Niebyl et al., 1979; Pynnönen et al., 1977; Rane et al.,1975); VPA (Alexander, 1979; Dickinson et al., 1979; Kaneko et al., 1983b; Kondo et al., 1987; Nau et al., 1981a, 1982c; Philbert et al., 1985; Tsuru et al., 1988); ethosuximide (Koup et al., 1978; Kuhnz et al., 1984) and diazepam (Erkkola et al., 1974). The embryo and fetus are exposed to these drugs throughout gestation.

Concentrations of AED (PHT, PB, CBZ) in umbilical cord plasma of fetuses age 12 to 16 weeks have ranged from 40 to 80% of those measured in maternal plasma (Mirkin, 1971; Nau et al., 1982a; Ploman and Persson, 1957; Pynnönen et al., 1977).

At this period of gestation, the highest concentrations of PHT in fetal tissues occurred in kidney, liver, adrenal gland and heart, while concentrations in brain, lung, gastrointestinal tract, cerebrospinal fluid and amniotic fluid were very low (Mirkin, 1971). Metabolites of PHT such as the dihydrodiol have been found in fetal tissues including fetal

brain (age of fetuses investigated: 9 to 14 weeks) and these have varied from 10 to 20% of the unconjugated fraction in maternal plasma (Nau et al., 1982 a). Phenobarbital and PRM have been shown to accumulate in fetal brain and/or liver. Levels of the metabolites PEMA and *p*-hydroxy-phenobarbitone were much higher in fetal tissues than in maternal serum (Nau et al., 1982a; Ploman and Persson, 1957). The highest concentrations of CBZ were found in fetal liver and kidney. Notably, the cerebral cortex in a neonate at autopsy contained higher concentrations of CBZ than did the early fetal brain suggesting that the accumulation of the drug differs according to the stage of development (Nau et al., 1982a, Pynnönen et al., 1977).

For most AED at term, the ratio of total concentration of the drug in cord blood relative to maternal blood is equal to or slightly lower than one (Bossi, 1982; Kuhnz et al., 1983,1984; Mirkin, 1971; Nau et al., 1980, 1982a; Suzuki et al., 1984; Yerby et al., 1985), while for VPA (Alexander, 1979; Dickinson et al., 1979; Kaneko et al., 1983b; Kondo et al., 1987; Nau et al., 1981a; 1982c; Philbert et al., 1985; Suzuki et al., 1984; Tsuru et al., 1988), it may be higher (1.0 to 3.8 in these studies). The gradient between maternal and fetal plasma protein binding may be a determinant of the extent of placental transfer. For example, protein binding of VPA in the fetus is higher than that in the mother at term, while the converse is true early in gestation. Accumulation of total concentrations of the drug in the fetus correlates with the protein binding gradient. However, free concentrations which equilibrate across the placenta are similar in the fetus and mother, both in early and late gestation (Nau and Krauer, 1986).

(3) Epidemiological studies

Although the results of epidemiological studies show an increased prevalence of congenital malformations in the offspring of epileptic women as compared to nonepileptic controls (Tables II.3 to II.6), it is difficult to prove that this is caused by AED treatment during the first trimester of pregnancy. Basically, two methodological approaches have been employed to evaluate the teratogenicity of AED. The first method compares reproductive outcome among epileptic women taking AED during pregnancy and women without epilepsy or the general population. This approach, however, does not separate the possible effects of maternal epilepsy (seizures, genetic factors) from those of drug exposure. Alternately, reproductive outcome is compared between epileptic women receiving AED during pregnancy and epileptic women not taking such medication. Because the latter group may include women who are in remission, those who are non-compliant, or those in whom AED were ineffective or had toxic side effects, the two epileptic groups may differ with respect to other factors such as age, parity, and type and severity of the seizure problem. The relatively small number of epileptic patients who are not treated present further difficulties in this type of analysis (Annegers et al., 1974).

Using either approach, epidemiological studies have provided consistent evidence of an association between AED therapy during pregnancy and an increased risk of congenital anomalies in the offspring of epileptic women (Tables II.3 to II.6). Although consistent findings suggest a causal relationship, the findings may merely reflect the consistency of confounding factors which are related to both AED

treatment and pregnancy outcome, such as the severity of maternal epilepsy.

(a) Type of anticonvulsant associated with teratogenic effects

A number of studies have found that, in general , AED polytherapy during pregnancy is associated with a higher prevalence of congenital malformations in the offspring of epileptic women than is monotherapy (A Collaborative Study of the French Chapter of I.L.A.E., 1984; Fedrick, 1973; Kallen, 1986; Lindhout et al., 1982,1984,1985b; Miyakoski and Seino, 1984; Nakane et al., 1980).

The majority of malformations in the older literature have been associated with hydantoins (most commonly, PHT) and barbiturates (PB, PRM), but these have also been the most frequently prescribed drugs. Newer drugs associated with dysmorphogenesis have been VPA (Robert and Gibaud, 1982) and recently CBZ (Jones et al., 1989).

Although there has been a trend towards monotherapy in recent years, many of the subjects evaluated in prospective and historical cohort studies were receiving polytherapy and therefore, it has been difficult to evaluate the teratogenic potential of any one drug, as well as possible additive or synergistic effects of specific drug combinations.

It does not appear that specific single malformations or patterns of malformations are associated with specific drugs among the commonly taken AED (PHT, PB, PRM or CBZ), as there is much overlap in the type(s) of malformations associated with these drugs.

(i) Hydantoins

Monson et al. (1973), in a report from the Collaborative Perinatal Project, found that the prevalence of selected malformations identified during the first year of life (chosen because of previous reports in the literature) among children of epileptic women was highest for those exposed regularly to PHT early in gestation, intermediate for those exposed sporadically or late in gestation, and least for those not exposed to PHT. Cleft anomalies only occurred among infants of mothers who were early, regular users of PHT. These results were maintained when allowance was made for potentially confounding factors such as maternal age, socioeconomic class and pregnancy complications. The findings suggested that PHT is most harmful during the critical period of embryonic development. However, the authors concluded that the association between early regular use of PHT and congenital malformations may reflect "the teratogenic effects of the drug, of epilepsy itself or of both factors".

A second report from the Collaborative Perinatal Project considered all malformations identified during the first year of life and at death before the age of four years (Shapiro et al.,1976), and reported contrary results. Although children of epileptic mothers had a significantly higher prevalence of total malformations (major and minor) than did children of women without a convulsive disorder (relative risk 1.6), the occurrence of total, major or minor malformations did not differ significantly according to PHT use. In particular, the prevalence of malformations was not higher in children exposed regularly to PHT early in gestation than in those not exposed to this drug at any time during pregnancy. Nakane et al. (1980), in a report from the collaborative

historical and prospective cohort studies in Japan, also did not find that PHT was significantly associated with the occurrence of malformations, when the relative frequency of anomalies was compared according to whether or not PHT was taken during pregnancy. In both of these studies (Nakane et al., 1980; Shapiro et al., 1976), failure to find an association with PHT use may have been due to the fact that women who were not taking PHT may have been taking other AED, and therefore an association between PHT and the occurrence of malformations may have been obscured by the effects of other AED.

Hanson et al. (1975,1976) investigated a specific relationship between hydantoin use and a pattern of malformation or syndrome, and coined the term the fetal hydantoin syndrome. Their study was carried out in two parts. In the first part, 35 children born to 23 mothers were ascertained retrospectively because their mothers had been taking hydantoins with or without barbiturates during pregnancy. The children were personally examined by one of the authors. The first part of this study had obvious drawbacks with respect to selection of patients, inclusion of more than one child to the same mother, knowledge of exposure, and lack of a control group. In the second part of the study, 104 children exposed to hydantoins in utero and matched controls were ascertained from the records of the Collaborative Perinatal Project. In both parts of the study, the authors found that 11% of children exposed to hydantoins with or without other AED exhibited a recognizable pattern of abnormalities of which prenatal growth deficiency, postnatal growth deficiency, microcephaly and mental deficiency were the most consistent features. Associated abnormalites included craniofacial anomalies, nail and/or phalangeal hypoplasia and, less frequently major

anomalies such as congenital heart and cleft anomalies. None of the controls exhibited this particular pattern of abnormalities.

Despite concerns about whether or not AED and hydantoins, in particular, were responsible for the syndrome (Janz, 1982a,b,c), a number of case reports followed which expanded on the phenotype of the "hydantoin syndrome". Other abnormalities reported have included urogenital malformations (Hirschberger and Kleinberg, 1975; Michalodimitrakis et al., 1981; Pinto et al., 1977); cerebellar abnormalities (Mallow et al., 1980); subcutaneous vascular abnormalities (Kousseff, 1982); transplacental carcinogenesis [neuroblastoma (Pendergrass and Hanson, 1976); malignant mesenchymoma (Blattner et al.,1977); melanotic neuroectodermal tumor (Jimenez et al., 1981)]; and ocular manifestations such as nasolacrimal duct deformity and agenesis, congenital glaucoma, colobomas, retinoschisis, and optic nerve hypoplasia (Hampton and Krepostman, 1981). Most of these reported cases were also exposed to other AED, and therefore the specificity of hydantoins in the etiology of these defects is uncertain.

(ii) Barbiturates

Despite early studies singling out hydantoins as the group of drugs associated with harmful effects in the fetus (Monson et al., 1973; Hanson et al., 1976), other studies have shown that the occurrence of congenital malformations is also associated with the prenatal use of barbiturates such as PB and PRM (Annegers et al., 1974, 1978; Dansky et al., 1981, 1982a,b; Giraud et al., 1982; Hill et al., 1974, 1982; Lindhout et al., 1982; Majewski et al., 1981; Nakane et al., 1980; Rating et al.,

1982b). For example, in an historical cohort study, Annegers et al. (1974, 1978) found that either PHT or barbiturates taken alone were associated with the occurrence of cleft anomalies and congenital heart disease. Similar results were found by Hill et al. (1988) in England, in a case-control study of orofacial clefts, although in this study, it was not specified whether PHT or PB were taken alone or in polytherapy with other AED. On the other hand, Lakos and Czeizel (1977) did not find an association between maternal PB and orofacial clefts in a case-control study utilizing the Hungarian malformation register.

In an uncontrolled historical cohort study, Lindhout and colleagues (1982) found that mothers who took PHT or PB during pregnancy had similar proportions of children with congenital malformations, which were significantly higher than those of mothers who took other AED. In this study, however, it is not clear how many patients were on PHT or PB alone. In the collaborative Japanese study, Nakane and associates (1980) reported that mothers who took PB or PRM, taken alone or in combination with other AED, had significantly higher risks of congenital malformations in their offspring than did mothers who did not take these drugs.

A number of studies have indicated that a combination of PHT and barbiturates appears even more likely to be associated with congenital malformations than when one or the other drug is taken alone (Hill et al., 1974, 1982; Lowe, 1973; Majewski et al., 1981; Starreveld-Zimmerman et al., 1974); however, other studies reported a lower prevalence of defects with a combination of these drugs (Annegers et al., 1974, Dansky et al., 1981, 1982b; Koppe et al., 1973). This may in part reflect differences in AED dose and/or individual

differences in the disposition of AED administered alone or in combination.

Dansky and colleagues (1981, 1982b) in a prospective study of 53 pregnancies showed that the prevalence of malformations was lower with a combination of PHT and PB (6%) than with either drug alone (28% and 25% for PHT and PB, respectively), and the highest malformation prevalence was seen in patients receiving PHT in combination with PB and PRM (60%).

Of particular interest are studies concerned with evaluating the dysmorhogenic potential of barbiturates when they are administered for indications other than epilepsy. In a large historical cohort study comparing drugs prescribed to mothers of normal and malformed children, Nelson and Forfar (1971) found that PB taken at anytime during pregnancy was significantly associated with major birth defects. A similar trend was noted for PB use in the first trimester, but the difference was not statistically significant. A prospective study from the Birmingham Research Unit of the Royal College of General Practitioners (1975) (Crombie et al., 1970) found an excess of barbiturate prescriptions among mothers of children with congenital anomalies, but this was only evident for barbiturates prescribed after the 9th week, and many of the anomalies were minor skin malformations. Shapiro and associates (1976), on the other hand, did not find an increased risk of malformation in the children of women without a convulsive disorder who were taking PB during pregnancy. The lack of association between PB exposure in utero and congenital anomalies in the general population does not exclude the possibility of such an association among epileptic women, as doses of the drug

prescribed to nonepileptic mothers may be less than those prescribed to epileptic women.

Although a pattern of anomalies has been observed among children exposed prenatally to hydantoins alone, similar features have been noted in relation to PB or PRM taken as the only AED during pregnancy (Berkowitz, 1979; Béthenod and Frédérich, 1975; Goodman et al., 1976; Krauss et al.,1984; Majewski et al., 1981; Myrhe and Williams,1981; Rating et al., 1982b, 1987a,b; Rudd and Freedom, 1979; Seip, 1976), thus suggesting that the drugs may act through a common biochemical pathway (Smith, 1977a). However, only two of these studies (Majewski et al., 1981; Rating et al., 1982b, 1987a,b) are controlled studies, while the others are case reports or case series. Smith (1977a) suggested that the term hydandantoin-barbiturate syndrome be used.

(iii) Carbamazepine

The possible teratogenic effects of CBZ are unclear. A number of studies have not shown evidence that CBZ taken alone or in combination with other AED is associated with an increased teratogenic risk with respect to major or minor congenital anomalies (Bertollini et al., 1987; Kuhnz et al., 1983; Nakane et al., 1980; Starreveld-Zimmerman et al., 1974). Some of these studies have been reviewed by Dansky (1978) and Niebyl et al., (1979). On the other hand, maternal use of this drug has been associated with reduced head circumference, weight and length (Bertollini et al., 1987; Hiilesmaa et al., 1981, 1982b; Jones et al., 1989) (see section II. M, Somatic Parameters at Birth). There have been isolated case reports of abnormalities (Hicks, 1979 ; Robertson et al.,1983) such as cranial nerve agenesis. The Finnish prospective study

(Granström and Hiilesmaa, 1982a), which followed a group of 20 infants exposed to CBZ monotherapy in utero, noted two children with congenital dislocation of the hip, one with inguinal hernia, and two children with mild facial dysmorhism (hypertelorism and depressed nasal bridges).

Recently, Jones and associates (1989) reported the results of a prospective study of 54 liveborn children, of whom 37 children were exposed to CBZ monotherapy in utero. The women were ascertained from a teratogen registry which they had voluntarily contacted early in pregnancy, because they were concerned about the possible teratogenic risks of CBZ. The women were then followed through pregnancy, and the outcomes documented. Women who had also contacted the registry about the possible effects of minimal amounts of alcohol, which they had taken during the first trimester of pregnancy, were chosen as controls. Physical examinations of 48 children exposed to CBZ were carried out blind to the prenatal history of the women. Six women dropped out of the study prior to delivery and their children were examined by their own physicians. Five of the 48 offspring (10.4%) who were personally examined had major congenital malformations as compared to 5 of 70 control (7.1%) children. A significantly higher proportion of the children exposed to CBZ with or without other AED had three or more minor anomalies (38%) as compared to controls (6%). Two of the 6 children who were evaluated elsewhere had major malformations (minor anomalies were not evaluated). Of 35 children personally examined by the investigators who were exposed to CBZ alone, 9% had major malformations (lumbosacralmeningomyelocele; ventricular septal defect; and inguinal hernia), 6% had prenatal and/or

postnatal growth deficiency, 11% showed craniofacial abnormalities, and 20% exhibited developmental delay.

These data were taken as evidence that CBZ is itself teratogenic. However, comparison frequencies for developmental delay , craniofacial abnormalities and growth deficiency were not given for the control group. The frequency of major congenital malformations in the control group may have been high, suggesting that women who contact a teratogen registry may be a selected group. Furthermore, the possible role of maternal and paternal characteristics and family history was also not addressed.

Lindhout (Lindhout, 1988; Lindhout and Meinardi,1985) presented data on the type of AED exposure identified among 20 spina bifida cases ascertained from several sources (e.g, case reports, spina bifida teams, etc.) and found 3 exposures to CBZ monotherapy. Rosa, quoted by Lindhout (1988), found a 1% prevalence of spina bifida among 600 exposures to CBZ in prospective cohort studies considered collectively, as compared with a general population figure of 0.05 to 0.09%.

In two other reports, the specific drug combination CBZ, PB, and VPA with or without PHT has been shown to be associated with a high risk of congenital malformations. It has been postulated that this may be due to high levels of CBZ epoxide metabolites (Lindhout et al., 1984, 1987) or to reactive metabolites of VPA formed as a result of metabolic interactions (Kaneko et al., 1988) (See section II.R1b, Toxic Metabolites of AED).

(iv). Valproic acid

The association of VPA with congenital malformations, particularly

spina bifida has been discussed previously under case-control studies (section II.J1d).

The numbers of pregnancies in cohort studies have been too small to adequately assess the risk of major congenital malformations, particularly spina bifida. When the results of published historical and prospective cohort studies are taken together, it is found that among 546 infants exposed to VPA with or without other AED, there were 76 malformed children (13.9%). The most frequent types of anomalies were facial dysmorphism (n=13); congenital heart disease (n=12); congenital dislocation of the hip (n=8); other postural deformities (n=5); hypospadias (n=7); neural tube defects (n=7); skeletal aplasa/dysplasia (n=6); cleft lip and/or cleft palate (n=5); inguinal hernia (n=4); and digestive canal stenosis (n=3) (A Collaborative Study of the French Chapter of the I.L.A.E., 1984; Biale and Lewenthal, 1984; Gaily et al., 1988a; Hiilesmaa et al., 1980; Jäger-Roman et al., 1986; Kaneko et al., 1988; Kelly, 1984b; Lindhout et al., 1985b; Martin, 1978; Miyakoshi and Seino, 1984; Nakane, 1982; Nakane et al., 1980; Oguni, 1989; Rating et al., 1987a; Robert et al., 1986; West et al., 1985). In the combined data, the relative frequency of malformations did not appear to differ among children exposed to VPA monotherapy or polytherapy with VPA (A Collaborative Study of the French Chapter of the I.L.A.E., 1984; Biale and Lewenthal, 1984; Gaily et al., 1988a; Hiilesmaa et al., 1980; Jäger-Roman et al., 1986; Kaneko et al., 1988; Kelly, 1984b; Martin, 1978; Miyakoshi and Seino, 1984; Oguni, 1989; Robert et al., 1986).

In a combined report of 13 prospective cohort studies around the world totally 2111 infants exposed to any AED (Lindhout and Schmidt, 1986), 12 infants had neural tube defects (11 spina bifida and 1

anencephaly). The prevalence of spina bifida in infants exposed to VPA monotherapy was 2.5%, and 1.1% in infants exposed to VPA polytherapy, as compared to 0.35% for other AED exposures, and 0.05-0.3% for the general population. The actual prevalence of neural tube defects in association with VPA may be lower than 1.5% as the cohort studies were not done on a population basis, and only 13 of 18 prospective studies from which data were requested responded. This may have led to reporting bias in which more groups aware of spina bifida cases than those who were not may have provided data.

Valproic acid has also recently been associated with a pattern of anomalies in various case series and small cohort studies (Ardinger et al., 1988; Chessa and Ianetti, 1986; DiLiberti et al., 1984; Huot et al.,1987; Jäger-Roman et al.,1986; Massa et al.,1987; Tein and MacGregor, 1985; Winter et al.,1987). Although certain features such as mid-face hypoplasia overlap with those found in relation to other AED, other features found in infants exposed to VPA monotherapy appear to be quite different (Jäger-Roman et al., 1986; Rating et al.,1987a,b). Such features include infraorbital crease , small mouth and downturned lips with thin vermilion border, arachnodactyly, hyperconvex nails, and respiratory tract abnormalities such as tracheomalacia.

(v) Oxazolidinediones

Present evidence indicates that the oxazolidinediones (trimethadione and paramethadione) used infrequently during the reproductive years for the control of petit mal seizures are potent human teratogens. German and colleagues (1970a,b) were the first to report a possible association between prenatal use of trimethadione or paramethadione and abnormal

pregnancy outcome. Feldman and associates (1977) summarized the literature and showed that, among 53 pregnancies reported in case reports and case series in which the mother took trimethadione or paramethadione, 13 resulted in spontaneous abortion (25%), and 40 were live births. Among the latter, 33 (83%) had at least one major anomaly. In historical cohort studies, Nakane and associates (1980) found that women who took trimethadione during pregnancy had a significantly higher frequency of congenital malformations in the offspring as compared to epileptic women who did not take this drug , and Dansky and colleagues (1982a) found that maternal use of this drug was associated with a high prevalence of congenital heart disease in the offspring. Zakai and co-workers (1975) delineated the "fetal trimethadione syndrome" consisting of growth deficiency, developmental delay, speech difficulty, V-shaped eyebrows, other craniofacial abnormalities, and major malformations such as orofacial clefts and congenital heart disease.

(vi) Other anticonvulsants

With regard to other, less commonly used AED -succinimides, benzodiazepines, acetylureas-prenatal use of all of these drugs has been reported in association with the birth of a child with a congenital abnormality. However, in most cases these drugs were taken together with the more commonly prescribed AED. Nakane et al. (1980), from the collaborative Japanese historical and prospective cohort studies, reported that mothers who took trimethadione, pheneturide or acetazolamide alone or in combination with other AED had significantly higher risks of congenital malformations in their offspring than did

mothers who did not take these drugs.

In the past, conflicting reports have appeared in the literature about the relationship between diazepam and orofacial clefts (see section II. J1b(2), Case-control studies). A recent prospective study has reported an embryofetopathy associated with excessive benzodiazepine use in pregnancy (30 mg diazepam or 7 mg oxazepam or more daily) consisting of prenatal and postnatal growth retardation, dysmorphic features, microcephaly, major malformations and central nervous system dysfunction (Laegrid et al., 1989). The patients had high plasma levels of the drug in early pregnancy.

(vii) Comparative studies

Several historical and prospective cohort studies have compared the prevalence of major malformations among offspring exposed to one or more AED in utero (Dansky et al., 1982a; Lindhout et al., 1982,1985b; Speidel and Meadow, 1972). There are difficulties with this type of comparison, as the groups are not mutually exclusive. Dansky et al. (1982a) found similar prevalences of major congenital malformations for PHT, PB and PRM used alone or in combination (16.0%, 17.7%, and 12.5%, respectively), whereas trimethadione yielded a much higher percentage of major congenital malformations, particularly congenital heart disease (44.4%). Speidel and Meadow (1972) found the same trend, although the prevalences of malformations were lower for each drug , except trimethadione (PHT, 5.3%; PB, 4.5%; PRM, 5.6%; trimethadione, 50%).

Lindhout and associates (1985b) reported the outcome of pregnancies in the Netherlands, in which the prevalence of major

congenital malformations in 323 liveborn offspring of patients on any AED was 8.7%, including mono - and polytherapies. The relative frequencies of malformations for specific drugs with or without other AED were 13% for PB; 12% for PRM; 11% for VPA; 10% for PHT; and 10% for CBZ. Ethosuximide was associated with a lower percentage of malformations (4%), in keeping with the results of animal studies (see section II.L.8).

More recent studies have combined data from historical cohort and prospective studies in an effort to compare the outcome of pregnancy among specific monotherapies or polytherapies, utilizing larger numbers of exposed pregnancies. Data from these studies must be viewed with caution as the data are combined from different areas with different background frequencies of malformations. Furthermore, as with individual cohort studies, there may be unknown confounders associated with different AED and abnormal pregnancy outcomes. For example, generalized seizures are treated more often with VPA than are partial seizures. If hereditary factors associated with generalized epilepsy predispose to malformations in the offspring, then this might disfavor VPA-exposed pregnancies (A Collaborative Study of the French Chapter of I.L.A.E., 1984).

A collaborative study from Sweden, Italy and France (Bertollini et al. 1987), which amalgamated data on pregnancy outcome in relation to specific AED monotherapies (total number of pregnancies=577), showed that the relative frequencies of congenital malformations did not differ significantly among different AED monotherapies: these were for VPA (8.2%); PHT (4.6%); PB (2.8%); CBZ (1.4%); and other AED (2.3%). The prevalence of malformations among controls ranged from 1.3% to 1.8%.

The data suggested, however, an increased risk for all drugs except CBZ, with the highest risk associated with VPA. Due to the nature of the data collection which was partly based on malformation registries, the authors pointed out that the higher risk associated with VPA may have been due to current interest in that drug (i.e. biased ascertainment). There were also possible unidentified confounders in this study. As opposed to other investigations of epileptic women who took monotherapy and/or polytherapy during pregnancy, an increased risk of orofacial clefts and cardiac anomalies was not noted in this study, thus implicating polytherapy in the elevated risk of these anomalies.

In a collaborative report from the French Chapter of the International League Against Epilepsy (1984) based on historical cohort studies, 56 major malformations were identified among 1010 live births. The prevalence of malformations was 1.8% for untreated epileptic women, (n=112); 2.1% in children exposed to VPA monotherapy (n=47), 3.6% for other AED monotherapies (n=359); 6.2% for VPA polytherapy (n=144) and 8.9% for other AED polytherapies (n=348). Monotherapies without VPA were most commonly PB. The prevalence of neural tube defects was 0.3% for other AED taken alone or in polytherapy while the prevalence of neural tube defects in relation to polytherapy with VPA was 0.7%. Neural tube defects did not occur in children exposed to VPA monotherapy.

(b) Dose of anticonvulsant

If the occurrence of malformations in relation to AED use during pregnancy were dose-dependent, then this would provide evidence for

the teratogenicity of AED (keeping in mind that higher dosages of AED will probably also be associated with more severe epilepsy). Only a few studies have assessed a dose-response relationship and some studies have shown evidence of a dose-response. As higher doses of individual drugs may correlate with polytherapy, it is generally not clear in these studies whether the number of drugs or the individual dose of drug(s) is the important factor. Only some studies have reported the occurrence of major congenital malformations (Dansky et al., 1981, 1982b; Hiilesmaa et al., 1983; Lindhout et al., 1985b; Oguni, 1989, Rating et al., 1987a,b) or minor anomalies (Gaily et al., 1988a; Rating et al., 1987a,b) in relation to plasma AED levels

Nakane and associates (1980) and Kaneko and colleagues (1988) from the collaborative studies in Japan, noted that the frequency of congenital malformations increased with the total amount of AED (as measured by the drug "score" taking into account both the number and doses of AED) ingested during the first or second trimester of pregnancy. In the latter study, doses of individual drugs were not related to the occurrence of malformations, and therefore the correlation of the total drug score with malformations in the offspring was probably due to polypharmacy.

Dansky et al. (1981,1982b) in a preliminary study of 53 pregnancies followed prospectively showed that 10 mothers bearing malformed children had significantly higher mean dosages (mg/kg) and /or mean plasma levels of PHT, PB and PRM over all trimesters combined than did mothers bearing normal children. Furthermore, there was a significant positive correlation between the risk of malformation and mean maternal plasma PHT levels. The highest plasma PB levels were found

in women who took a combination of PHT, PB and PRM. This combination results in more extensive metabolism of PRM to PB in the presence of the other drugs as compared with PRM given alone (Fincham and Schottelius, 1982). None of the mothers had plasma AED levels within the toxic range. In contrast to these findings, (Hiilesmaa et al., 1983) did not find a dose response relationship between plasma AED levels and major congenital anomalies. In this study, it is not clear how plasma levels were assessed (e.g., maximum level or mean level).

In other studies, dosage effects have been noted with PB (Nakane et al., 1980; Nelson and Forfar, 1971; Fedrick, 1973), mephophenobarbital and PRM (Nakane et al., 1980). One small prospective study found no association between the plasma concentration of PB and the occurrence of malformations (Melchior et al., 1967). Seip (1976) reported high plasma PB levels in a mother who took a combination of PB and PRM during pregnancy and who had two children with the "barbiturate syndrome" . He raised the question of whether high levels of this drug might induce teratogenic effects. Béthenod and Frédérich (1975) noted a positive correlation between the dose of PB or polytherapy and the occurrence of marked facial dysmorphism.

Rating et al (1987a,b) showed that the dose (mg/kg/day) of PRM among women taking monotherapy was significantly positively correlated with the number of minor anomalies in the offspring. Among 14 children of mothers who took PRM during pregnancy, four children with marked facial dysmorphism had the highest plasma PB / PRM and PEMA /PRM ratios during pregnancy (Rating et al., 1982b). In a later report however (Rating et al., 1987a,b), the authors stated that they did not find a significant relationship between plasma PRM or PB levels and

the occurrence of "typical dysmorphic facies" (epicanthal folds, widely spaced eyes, and broad depressed nasal bridge) or with the number of minor anomalies per child.

In the same study, the dose of VPA (mg/kg/day) taken in monotherapy was significantly positively correlated with the number of minor anomalies per child (Rating et al, 1987a,b). The data also suggested a relationship between VPA concentrations and the occurrence of major and minor anomalies (Rating et al., 1987a,b). Similar results were found by Lindhout and colleagues (1985b). Among 8 mothers who took VPA monotherapy during pregnancy, Oguni (1989) found significantly higher VPA concentrations over all trimesters combined in 3 mothers of malformed children (86.6µg/ml) than in 5 mothers of normal children (42.6µg/ml).

There was some suggestion in the data of Lindhout and colleagues (1985b) that total serum concentrations of CBZ were higher in pregnancies resulting in children with malformations than in children with normal outcomes, but there did not appear to be a relationship between serum metabolite levels of the CBZ-10, 11-epoxide or CBZ-10,11-diol and the occurrence of malformations

In a prospective study in Finland, Gaily and associates (1988a) found that moderate to high levels of PHT (> 40 µmol/L) in the first 20 weeks of pregnancy were significantly associated with hypertelorism, phalangeal and nail hypoplasia, and 3 or more dermal arches in the child. In contrast, Rating and associates (1985, 1987a,b) did not find a relationship between dose or plasma levels of PHT and the number of minor anomalies per child, the occurrence of "typical facial dysmorphia", or of digital hypoplasia.

Six other studies found no evidence that congenital malformations were more common in children of epileptic mothers taking higher doses of PHT (Fedrick, 1973; Hanson et al., 1976; Kaneko et al., 1988; Monson et al., 1973; Nakane et al., 1980; Van Dyke et al., 1988), while another report found two mothers with symptoms of phenytoin toxicity (in one the blood level exceeded the therapeutic range) among 7 mothers of malformed infants (Loughnan et al., 1973). Other authors have also noted no difference in the dosage of individual AED between mothers of normal and malformed children (Biale et al., 1975; Cvetko, 1969; Kaneko et al., 1988; Koch et al., 1983; Martin, 1978; Miyakoshi and Seino, 1984, Speidel and Meadow, 1972).

In these studies, maternal plasma AED levels were generally not monitored, and there may be several reasons why a dose-response was not found, particularly for PHT These include small numbers of women taking low or high doses of the drug; interindividual variation in weight; and large interindividual variation in plasma AED levels with particular dosages or combinations of drugs.

3. *Type of Maternal Epilepsy and Seizures*

The effects of type of maternal epilepsy, and type and frequency of clinical seizures, must also be considered in the etiology of congenital malformations. Lactic acidosis (Orringer et al., 1977), increased prolactin and cortisol levels (Abbot et al., 1980), and hypoxic signs in fetal heart rate (Teramo et al., 1979) are some of the sequelae of maternal seizures. Furthermore, status epilepticus has a high maternal and perinatal mortality (Teramo and Hiilesmaa, 1982).

Most studies have shown that epileptic women may have an increase in seizure frequency during pregnancy and the puerperium. The likelihood of such an increase has varied greatly among different studies, ranging from 4% to as high as 75%, with an average risk of 25% (Schmidt, 1982). The variation in findings among studies may be explained by differences in the type of study (historical versus prospective cohort); differences in the patient groups, such as the type of seizure and efficacy of drug therapy; and in the definition of altered seizure frequency.

Rémillard, Dansky and co-workers (1982) found that the likelihood of increased frequency of major seizures was highest in the first and third trimesters. Huhmar and Järvinen (1961) and Bardy (1987) found an increased risk of seizures in the third trimester or puerperium, while other studies have found that the increased risk is usually evident in the first trimester or the beginning of the second trimester (Canger et al., 1982; Knight and Rhind, 1975).

As reviewed by Newmark and Penry (1980) and Schmidt (1982), various factors have been proposed to explain the increased frequency of seizures observed during pregnancy and the puerperium. These

include altered hormonal concentrations, water retention, decrease in electrolyte concentration, a decrease in plasma AED levels, type and severity of the epilepsy, poor compliance, psychological problems and fatigue.

Rémillard and associates (1982) found that the prepregnancy frequency of major seizures, duration of epilepsy and course of epilepsy in previous pregnancies were correlated with seizure prognosis during pregnancy. The likelihood of increased seizure frequency was also correlated with type of epilepsy , it being highest for patients who had secondary generalized epilepsy with focalization or complex partial seizures; however, these patients also had relatively frequent attacks outside of pregnancy.

Although it has been difficult to assess maternal seizure history during pregnancy, particularly in historical cohort studies, most studies do not indicate that congenital malformations in the offspring are related to the type and / or severity of maternal epilepsy (Annegers et al., 1974, 1978, 1983; Beck-Mannagetta et al., 1982; Béthenod et Frédérich, 1975; Cvetko, 1969; Dansky et al., 1982b; Fedrick, 1973; Friis, 1979; Friis et al., 1981; Hill et al., 1974; Koch et al., 1982b; Lindhout et al., 1982; Martin, 1978; Nakane et al., 1980; Shapiro et al., 1976; Speidel and Meadow, 1972).

Some studies, however, reported positive results. Seino and Miyakoshi (1979) found that secondary generalized epilepsy and complex partial seizures in the mother were associated with a higher frequency of abnormal pregnancy outcomes (congenital malformations, miscarriages and stillbirths) than was primary generalized epilepsy. Nakane and colleagues (1980) reported a higher prevalence of congenital

malformations associated with partial epilepsy as compared with generalized epilepsy. Kaneko and co-workers (1984a) also found an association between partial seizures and the occurrence of congenital malformations. Majewski and co-workers (1981), on the other hand, observed a slightly higher prevalence of major malformations without dysmorphia in children of mothers or fathers with genuine epilepsy as compared to symptomatic epilepsy.

Starreveld-Zimmerman and associates (1974) found that tonic-clonic convulsions occurred more often in the case of malformed babies than when a normal baby was born. Miyakoshi and Seino (1984) and Lindhout and associates (1985b) found similar results when the occurrence of convulsive and non-convulsive seizures were considered together. Nakane et al., (1980) showed that the prevalence of fetal malformations was lowest in mothers free from seizures and not medicated during pregnancy (1.8%), followed by those having seizures but not medicated (2.6%), those free from seizures but medicated (11.5%), and those having seizures and medicated (12.7%). Differences in the prevalence of congenital malformations between the offspring of patients with or without seizures were not significantly different in either the medicated or not medicated groups. Majewski et al. (1981) reported that women who took AED and who had seizures had an increased relative frequency of children with major malformations without dysmorphia as compared to treated women who did not have seizures during pregnancy. In contrast, the relative frequency of the hydantoin-barbiturate embryopathy was similar in the two groups.

In these studies, the number and/or dose of AED were also associated with abnormal pregnancy outcome. As type and frequency of maternal

seizures and type, number, and dose of AED therapy are highly correlated (Miyakoshi and Seino, 1984; Seino and Miyakoshi, 1979), the possible separate effects of maternal seizures and drugs could not be determined from these studies. Kaneko and colleagues (1984a) found that partial seizures in the mother was one of the variables along with CBZ and VPA which maintained a significant association with the occurrence of congenital malformations by multivariable analyses (see section II.K7, Multivariable Studies). But this was not corroborated in a later study of another sample of pregnancies (Kaneko et al., 1988).

4. *Pregnancy Complications*

Certain pregnancy complications must also be considered (Figure II.2). Such factors may adversely affect pregnancy outcome with respect to intrauterine growth, the development of congenital malformations, and survival of the fetus and infant.

The findings concerning pregnancy complications in epileptic women are controversial with some studies showing no increased risk of complications (Battino et al., 1982b; Hiilesmaaa et al., 1982a,1985; Martin, 1978; Robertson et al.,1986; Teramo and Hiilesmaa, 1982; Watson and Spellacy, 1971; Weber et al., 1977), while others have noted an increase in certain complications such as vaginal bleeding (Bjerkedal and Bahna,1973; Egenaes, 1982; Nelson and Ellenberg,1982); placental abruption (Egenaes, 1982; Hill et al., 1974; Monson et al., 1973); toxemia (Bjerkedal and Bahna,1973; Huhmar and Järvinen, 1961; Janz and Beck-Mannagetta, 1982a; Nelson and Ellenberg,1982; Ogawa et al.,1982;1984; Pritchard et al., 1971; Vert et al., 1979; Yerby, et al., 1985); polyhydramnios (Monson et al., 1973); threatened abortion

(premature contractons and/or bleeding) (Koch et al., 1982a, 1983); premature rupture of the membranes (Svigos, 1984); anemia (Gatenby, 1960; Svigos, 1984); and various maternal illnesses, such as maternal diabetes (Kallen, 1986), urinary tract infection (Nelson and Ellenberg, 1982) and infectious diseases (Nelson and Ellenberg, 1982; Yerby et al., 1985).

Attempts to correlate pregnancy complications with AED or seizures have not yielded any significant associations (Cvetko, 1969; Hiilesmaa et al., 1982a,1985; Koch et al., 1982a,1983; Nelson and Ellenberg, 1982), although Janz and Beck-Mannagetta (1982a) noted a higher occurrence of toxemia in treated epileptic women, compared to untreated women. Nakane and colleagues (1980) found a significant association between pregnancy complications and the occurrence of congenital malformations in the offspring of epileptic women (treated and untreated combined) as well as a trend towards an association between pregnancy complications and AED treatment during pregnancy.

As reviewed by Hill and Tennyson (1982a), complications of delivery such as forceps or vacuum extraction, abnormal presentation, and caesarean section may also be increased in epileptic women.

Some of the reports of pregnancy and obstetric complications are based on pregnancies ascertained over a decade ago and may not reflect newer developments in prenatal and obstetric care (Hiilesmaa, 1982). As well, it is not always clear whether the data for the nonepileptic comparison groups were obtained in the same manner as that for the epileptic group (Bjerkedal and Bahna, 1973), and whether the groups were matched for such factors as socioeconomic class.

5. *Demographic Factors*

Demographic variables such as those in Figure II.2 may also be potential confounding factors. For instance, women with epilepsy may be of older maternal age (Beck-Mannagetta et al., 1982; Monson, 1973) and of lower socioeconomic class (Fedrick, 1973, Higgins and Comerford, 1974; Hiilesmaa et al., 1982a; Kelly, 1984b; Monson, 1973; Nelson and Ellenberg, 1982) than mothers without epilepsy, and these characteristics are in themselves related to an elevated risk of malformation in the general population.

6. *Pregnancy Complications and Demographic Factors versus Pregnancy Outcome*

Andermann, Dansky and Kinch (1982b) reported that the increased occurrence of malformations in 52 children of treated epileptic mothers followed prospectively was not explained by maternal age, pregnancy or birth order, pre-eclampsia, fever or bleeding during pregnancy, polyhydramnios, gestational age or sex of the offspring. Similar negative results were found by Cvetko (1969).

A number of studies have shown an increased risk of congenital malformations associated with treated maternal epilepsy, once one or more potential confounders (pregnancy and /or demographic factors) are taken into account either by matching of controls, statistical analysis showing no differences between the epileptic group and controls (Beck-Mannagetta et al., 1982; Bertollini et al., 1985; Bjerkedal, 1982b; Fedrick, 1973; Hill et al., 1974; Kallen, 1986; Monson et al., 1973; Speidel and Meadow, 1972; Svigos, 1984) or multivariable analyses (Monson et al., 1973). Many other studies, however, have not controlled

for such factors.

7. *Epidemiological Multivariable Studies*

A few studies have investigated the role of AED and maternal epilepsy in the occurrence of congenital malformations after having controlled for the possible confounding effects of other possible risk factors.

Monson et al. (1973) found an increased risk of congenital malformations associated with early regular use of PHT (see section II.K2b(3)(a)(i), Type of Anticonvulsant, Hydantoins. This association was maintained when other risk factors associated both with maternal epilepsy and the occurrence of congenital malformation were taken into account, by stratification on the confounding factors (Slone et al., 1973). These variables included race, low placenta weight, low birth weight, elevated maternal age, and low socioeconomic class.

The Japanese collaborative studies also studied the association of multiple risk factors with the occurrence of congenital malformations in the offspring of epileptic women. In one study of 458 livebirths (54 with congenital malformations) to epileptic women taking AED ascertained historically or prospectively (Nakane and Nakamura, 1982; Nakane et al., 1984), step-wise multiple discriminant function analysis and logistic regression analysis were employed to assess the independent association of various epileptic and nonepileptic risk factors with the occurrence of congenital malformations. The risk factors assessed were those which had been previously significantly associated with the occurrence of congenital malformations by univariate analysis (Nakane et al., 1980). Discriminant function

analysis showed that maternal age at birth of the child; the presence of malformations in the mother; parity; outcome of previous pregnancies; frequency of seizures; use of metharbital, trimethadione, phenacemide, ethylphenacemide, sultiame, or primidone (but not phenytoin); number of AED; maximum daily "drug score" and total dose of AED taken during the first trimester of pregnancy (based on the number and doses of specific drugs); and sex of the child, were the best linear combination of variables to discriminate between congenital malformations and normal pregnancy outcomes. Multiple logistic regression analysis of the same data yielded somewhat different results: the nonepileptic variables, presence of maternal malformation and previous pregnancy outcome (miscarriage, stillbirth), and the epileptic variables, absence of organic cause of maternal epilepsy; frequency of maternal seizures; maximum daily total "drug score"; and the use of primidone, trimethadione, acetylpheneturide, or sulthiame, were the combination of variables which correlated with the occurrence of congenital malformations.

In another report by the same authors, only the pregnancies followed prospectively were studied (151 livebirths, 21 with malformations) (Nakane, 1982; Nakane et al., 1984) by step-wise multiple discriminant analysis. It was found that the anticonvulsants trimethadione, acetylpheneturide or phenytoin as well as the maximum daily total "drug score", and previous pregnancy outcome (artificial abortion, stillbirth, miscarriage, livebirth), were the combination of variables which best discriminated between congenital malformations and normal pregnancy outcomes.

Different time periods of the studies involving different sets of

patients probably contributed to the variable results of the studies. As well, multiple discriminant analysis is not as suitable a method as multiple logistic regression for the analysis of a dichotomous outcome such as the presence or absence of congenital malformations, and thus could give different results.

In these studies, prediction of the occurrence of malformations was poor (Nakane et al., 1984). The impact of presence or absence of the drug and the total drug score were studied and not plasma AED levels, and this may have been one reason for the poor prediction. As well, both major and minor anomalies were included in one group, and risk factors for major anomalies may not have been the same as those for minor anomalies.

In a later preliminary study by the same Japanese group of data collected prospectively on 112 live births of epileptic women, Kaneko and colleagues (1984a) analyzed the relationship of 15 possible risk factors with the occurrence of congenital malformations in the offspring, employing discriminant function analyses. It was found that partial seizures, the combination of CBZ and VPA , and number of pregnancies were the critical risk factors for the occurrence of congenital malformation in the offspring. A subsequent prospective study by the same workers (Kaneko et al., 1988), of 172 infants born to epileptic mothers, investigated the relationship between congenital malformations in the offspring and maternal age at delivery; parity; outcome of previous pregnancy; etiology and type of maternal epilepsy; seizure frequency during pregnancy; and maternal AED combinations. They found that polypharmacy, particularly the combination of CBZ and VPA were the risk factors significantly associated with the occurrence

of congenital malformations, while the type and frequency of seizures and other risk factors were not significantly associated with malformations.

In conclusion, the results of multivariable analyses showed a significant association between AED and the occurrence of congenital malformations with or without other risk factors, although the exact type of AED varied for the different studies. This may have been due to differences in the patient groups, such as AED regimens, and in methodology.

Gaily and colleagues (1988a), in Finland, investigated the occurrence of minor anomalies in the offspring of epileptic women in relation to AED exposure, maternal seizures, and minor anomalies in the parents by multiple logistic regression analysis. The results are discussed in section J.2b.

L. Experimental Animal Studies of Congenital Malformations

1. *Phenytoin*

Phenytoin has been the most extensively investigated anticonvulsant, and it has consistently been shown to induce teratogenic effects in a number of species including mouse, rat, and rabbit (Finnell, 1980,1981; Fritz et al., 1976; Hansen and Hodes, 1983a,b; Harbison and Becker, 1969, 1970; 1972; Lorente et al., 1981; Martz et al., 1977; McClain and Langhoff, 1980; McDevitt et al., 1981; Mercier-Parot and Tuchmann-Duplessis, 1974; Paulson et al., 1979; Sullivan and McElhatton, 1975, 1977). The drug has been found to be teratogenic in doses ranging from 20 to 250 mg/kg, given subcutaneously, intraperitoneally or orally. Daily human therapeutic doses are 3-10 mg/kg. A wide spectrum of defects is produced which depends on the species, day, dose, route and frequency of administration. These malformations include cleft lip and/or palate, ocular, skeletal, neural, cardiac, renal, genitourinary, and gastrointestinal anomalies. A pattern of multiple anomalies (including hypoplasia of the distal phalanx) consistent with that described in some children exposed to PHT in utero has been produced in the mouse (Finnell, 1980,1981).

In a mouse model of tonic-clonic convulsions that could be controlled by chronic oral treatment, the frequency of defects in the offspring correlated positively wih maternal plasma PHT levels within the mouse therapeutic range (2-10 µg/ml). An increased incidence of defects was evident even at the lower limit of this range (Finnell, 1981). Presence of a seizure disorder (untreated) in either the sire or dam did not adversely affect pregnancy outcome. As well, treatment of sires with or without a seizure disorder did not result in an increase of

adverse pregnancy outcomes. Thus genetic factors associated with the seizure disorder, the occurrence of seizures and possible mutagenic effects of PHT were not critical factors for the induction of adverse pregnancy outcome in this model (Finnell, 1982; Finnell and Baer, 1986).

Phenytoin has been found to have a low level of teratogenicity in primates. In rhesus monkeys (Wilson, 1973), abortion occurred in two offspring, and a minor urinary tract anomaly was observed in 3 of 14 fetuses.

Phenytoin has been shown to cross the placenta in the rat, mouse and hamster, with some variation according to gestational stage (Stevens and Harbison, 1974). Fetal tissue concentrations of PHT were highest in early organogenesis, decreased in mid-gestation and increased near parturition. Highest fetal concentrations were found in mice, and lowest in rats which correlated with the teratogenic response of the different species [teratogenic response in mice occurs at lower dose (50 mg/kg) than in rats (150 mg/kg)]. In the mouse, Tanaka et al. (1984) found a good correlation between fetal concentration of PHT in different tissues and maternal plasma concentrations. The concentration of PHT was distributed equally among the various tissues except for some predominance in fetal liver.

2. ***Phenobarbital***

Phenobarbital has been shown to produce teratogenic effects in the mouse, rat and rabbit, when administered for extended periods during embryogenesis (A Collaborative Study by Sixteen Laboratories , 1966 ; Fritz et al., 1976; McColl et al.1963, 1967; Somers, 1969; Sullivan and McElhatton 1975,1977; Vorhees, 1983). Skeletal, orofacial, cardiac

and/or neural defects were produced at doses (orally or parentally) ≥ 10 times the human therapeutic dose of 1-5 mg/kg. The higher doses required to produce such malformations in these animals may not be excessive in view of the high metabolic activity of rodents. Murine studies have shown a dose-response effect and a lower degree of teratogenicity than with PHT.

Finnell and co-workers (1987a,b) assessed the influence of genotype on the sensitivity to PB-induced malformations in three inbred strains of mice receiving chronic oral doses of the drug. Phenobarbital was administered in doses of 60 to 240 mg/kg and plasma PB levels ranged from 1 to 85 µg/ml. The most consistent feature induced by PB was delay in ossification of the cranial and axial skeleton bones. Other defects were hydronephrosis, dilated cerebral ventricles, cleft palate, undescended testes, and cardiac defects. In all strains, the defects were produced in a concentration-dependent fashion, but the strains differed in the type and frequency of specific PB-induced malformations.

The patterns of malformation induced by PB and PHT in the mouse were compared (Finnell et al., 1987a), and were found to differ. Phenytoin induced more neural, renal and cardiac defects than did PB, whereas PB induced a higher frequency of urogenital and cleft palate defects.

3. *Primidone*

In the mouse, PRM produces teratogenic effects similar to those of PB (Sullivan and McElhatton,1977). Malformations such as cleft palate are produced at levels comparable to human therapeutic plasma levels

of PRM (McElhatton et al., 1977). However, dose-response effects have not been consistently demonstrated, and this may be due to the fact that the drug is rapidly metabolized in the mouse.

4. *Carbamazepine*

Carbamazepine has not consistently produced teratogenic effects, when administered orally in the mouse (Eluma et al., 1981; Finnell et al., 1986; Fritz et al., 1976; Paulson et al., 1979; Sullivan and McElhatton, 1977; Wray et al., 1982). In the first study investigating the effects of CBZ, Fritz et al. (1976) reported no significant increase in the incidence of malformations in NMRI-1 derived mice when up to 240 mg/kg of CBZ was administered by gastric intubation on gestational days 6-15. Sullivan and McElhatton (1977) administered the drug at doses of 40 to 240 mg/kg by gastric intubation to pregnant CD-1 mice throughout organogenesis. Dilated cerebral ventricles and clubfoot occurred in 4.3% of the fetuses (2.7 times higher than controls). In the CD-1 mouse, Paulson et al. (1979) investigated the effects of CBZ mixed in the diet (100 to 1600 mg/kg) on gestational days 8 to 13. Plasma levels were in or below the low human therapeutic range. There was some increase in neural defects and cleft palate as the dosage increased (10 to 17%). In this study, however, blood was taken from pregnant dams for the determination of plasma CBZ levels and it is not clear whether controls were treated in the same manner. As well, toxic effects in the mother increased with the dose of the drug. In this model, the incidence of anomalies following CBZ treatment was much less than that seen with PHT.

Eluma et al. (1981) tested CBZ administered by gastric intubation

(375 mg/kg to 938 mg/kg) in the CD-1 mouse for its ability to induce craniofacial defects, and found that there was a dose-related increase in the incidence of malformations. Defects observed were submucous cleft palate, exencephaly, and open eye. Wray et al. (1982) administered CBZ, up to 6 times the human oral dose, to pregnant A/J mice (susceptible mouse strain) on gestational day 10 , the critical time for cleft lip and palate induction. Because CBZ was cleared rapidly from from the blood, multiple oral gavages were used in order to increase the length of exposure to human therapeutic plasma levels. However, actual plasma levels were measured in nonpregnant mice, and therefore may not have given an accurate indication of plasma levels during pregnancy. There was some increase in the frequency of cleft lip and/or palate but the differences were not significant.

Finnell et al. (1986) found that administration of CBZ in the diet prior to pregnancy and throughout gestation (yielding human therapeutic plasma levels) resulted in only a moderate increase in malformations in two inbred strains of mice. The most frequent defects observed were dilated cerebral ventricles and hydronephrosis in one strain, and undescended testes in the second strain. However, no correlation was found with dose administered, or maternal plasma levels of the parent compound, the level of the epoxide metabolite, or the total drug concentration. Carbamazepine was less teratogenic than PB or PHT in this mouse model (Finnell et al., 1986; Finnell, 1981; Finnell et al., 1987a,b)

El-Sayed et al. (1983) found that CBZ was teratogenic in the rat when given throughout organogenesis at doses of 85 to 170 mg/kg by gastric intubation. Skeletal defects (e.g. unossified vertebra and

decrease in the number of ribs) and brain defects (e.g. hydrocephalus) were produced. Histological examination revealed extensive hemorrhage in the dams, incomplete formation of the placenta, and degeneration of trophoblast and decidual cells.

5. *Valproic Acid*

Valproic acid has definitely been shown to be teratogenic in experimental animals (mouse, rat, rabbit, hamster, monkey), when administered in sufficiently high doses (75 to 600 mg/kg). Therapeutic doses in humans are 15 to 30 mg/kg/day. The teratogenic effects were dose-related and differed from those found for PHT. Several overviews of teratological studies of VPA are published (Andermann, 1979; Hendrickx et al., 1988; Nau and Hendrickx, 1987).

An increased occurrence of skeletal defects (rib, vertebral, digital, craniofacial) were the most consistent findings, at doses of 150 to 200 mg/kg in all animal models tested (Consultox Laboratories, 1973; Hendrickx et al., 1988; Kao et al., 1981; Mast et al.,1986; Ong et al., 1983; Petrere et al., 1986; Ritter et al., 1987; Vorhees, 1987b; Whittle, 1976). Other malformations produced by VPA were kidney and neural tube defects, and a low level of cardiac defects (Hendrickx et al., 1988).

Valproic acid was found to induce neural tube defects in the mouse and hamster (200 to 300 mg/kg intraperitoneally, subcutaneously, orally, or via implanted minipumps) (Eluma et al., 1984; Finnell et al., 1988; Nau and Hendrickx, 1987; Nau and Spielmann, 1983; Nau et al., 1981b; Nau, 1985, 1986b; Paulson et al., 1985) but not in the rat, rabbit or monkey.

In the NMR-1 mouse, Nau and colleagues (Nau et al., 1981b; Nau 1985) showed that a particular threshold level of VPA must be reached to induce exencephaly (230 to 250 µg/ml), and that it made little difference whether the drug was administered after repeated injections yielding large fluctuations in plasma levels or by infusion resulting in steady-state levels. As the half-life of VPA is longer in humans than in experimental animals, lower fluctuations are observed in humans. However, single daily dosing regimens of VPA in humans may result in higher total and free plasma concentrations, and may therefore result in a greater teratogenic risk than with multiple dosing regimens (Nau and Hendrickx, 1987). This is particularly important in view of the fact that protein binding of VPA is concentration dependent in man, with protein binding decreasing (and free concentrations increasing) with increasing plasma concentrations (Cloyd and Brundage, 1987).

Differences in the susceptibility of mouse strains to VPA-induced exencephaly have been demonstrated in vivo (Finnell et al., 1988) and in vitro (Naruse et al., 1988). In vitro studies suggest that the basis of the strain difference is due to the genotype of the fetus rather than that of the mother (Naruse et al., 1988).

Hendrickx et al. (1988) characterized the teratogenic potential of VPA in the Rhesus monkey. At doses of 20 to 75 mg/kg, minor craniofacial defects were observed in a minority of fetuses; at 100 to 150 mg/kg, craniofacial and skeletal defects were encountered as well as a decrease in fetal weight, length and head circumference , and an increase in embryolethality. At 200 mg/kg, cardiac defects were observed and a decrease in other organ weights, in addition to the abnormalities observed at the lower doses. There was a direct

relationship between the concentration of unbound or free VPA and the induction of malformations. Peak free concentrations of > 200 µg/ml (13-20 times human free concentrations) resulted in 100 % malformed offspring.

Dickinson et al. (1980) reported that the plasma concentration of VPA in the rhesus monkey embryo relative to maternal plasma concentrations is 1.3 at gestational day 131 (total gestation 165 days). Differences in the percent of drug bound to plasma proteins in the mother and fetus may account for this ratio. VPA concentrations in the fetus were highest in the blood; moderate in the heart, liver, lung, spleen, and kidney, and skeletal muscle; and low in brain.

Nau (1986a) and Hendrickx et al. (1988) reviewed possible explanations for interspecies differences in the teratogenic effects of VPA. These include differences in genetic susceptibility to malformation, placental transfer of the drug, and maternal pharmacokinetics which will result in variation in the extent of embryonic exposure and the degree of teratogenic response. For example, plasma protein binding may be a major determinant of placental transfer of highly protein bound drugs, as it is the free drug which is available for placental transfer and equilibration with the embryo. Because of lower protein binding of VPA in the mouse, as compared to the monkey or rat, the exposure of the mouse embryo is greater than that of the rat or monkey embryo, and this correlates with the greater sensitivity of the mouse embryo to teratogenic effects, particularly the induction of neural tube defects. Free concentrations of VPA are also relatively low in man, and this would be expected to result in lower embryonic exposure than in the mouse (Hendrickx et al, 1988).

In vitro studies have shown that VPA administered to rat or mouse embryos in concentrations of ≥ 86 µg/ml, produces neural tube defects, abnormal curvature, irregular somite formation and/or craniofacial defects in a dose-dependent fashion (Bruckner et al., 1983; Kao et al., 1981; Lewandowski et al., 1986). Administration of the metabolite 4-en-VPA additionally developed cardiac defects (Klug et al., 1985; Neubert et al., 1986).

6. *Ethosuximide*

In mice, ethosuximide appears to be a much less potent teratogen than PHT or barbiturates, requiring higher dosages to achieve similar teratogenic effects (Brown et al., 1980; Kao et al., 1979).

7. *Oxazolidinediones*

Teratogenic studies of the oxazolidinediones have shown varying results depending on the species tested. Brown et al. (1979) showed that trimethadione was highly embryotoxic and teratogenic in the CD-1 mouse. The effects observed were time, route and dose-dependent and occurred at doses which did not cause overt maternal toxicity, except for slight sedation at the highest dose (1045 mg/kg). Adverse effects were most marked when the drug was administered early in embryogenesis. A wide variety of developmental anomalies were produced, including abnormalities of the skeletal, cardiovascular, renal, genital, and respiratory systems. The most common defects were those of the vertebral column and the aortic arch. In late embryogenesis, cleft palate and defects of the great vessels of the heart were the most

frequent defects observed. The cardiovascular defects produced by trimethadione experimentally were similar to those reported in children exposed to the drug in utero (See section II. K2b(3)(a)(v), Teratogenic Effects, Oxazolidinediones).

Trimethadione has been shown to produce skeletal defects in rats (Buttar et al, 1976) and monkeys (Wilson, 1974). Wilson (1974) reported 2 spontaneous abortions and one case of colonic atresia among 6 rhesus fetuses.

Paramethadione has also been found to be teratogenic in the CD-1 mouse model (Kao et al., 1979), but not in rats (Buttar et al.,1976) or monkeys (Poswillo, 1972), although in the latter model, the drug was embryolethal at doses which induced maternal sedation.

8. ***Comparative Studies***

A number of studies have evaluated the relative teratogenicity of different AED in the mouse or rat. The first study (Sullivan and McElhatton, 1977) evaluated six AED at 3, 9 and 18 times the human therapeutic dosages. The drugs were ranked in order of teratogenicity with respect to the occurrence of all types of malformations. Three categories of drugs emerged: phenytoin > carbamazepine, phenobarbital, primidone > clonazepam; ethosuximide. The occurrence of malformations in offspring exposed to the latter two drugs was two-fold that of controls.

Later four studies were carried out in the CD-1 mouse by a group of investigators from the National Institute of Environmental Health Sciences (Kao et al., 1979; Brown et al, 1980, 1982; Fabro et al., 1982). In the first study from this group (Kao et al., 1979), three classes of

AED were tested. The relative teratogenic potential of the different classes was based on the percentage LD_{50} (dose producing a 50% lethality rate) which resulted in a significant increase in malformations. The classes were ranked as follows: oxazolidinediones > hydantoins > succinimides.

The second study (Brown et al., 1980) utilized a relative teratogenic index (RTI) which was based on the ratio of the minimal lethal dose to that which induces a 5% malformation rate in liveborn offspring. This index is a measure of the extent to which an agent can exert developmental toxicity at dose levels lower than adult toxic doses. According to this method of evaluation, valproic acid had the greatest teratogenic potential, followed by trimethadione, phenytoin, and ethosuximide, in that order.

The third study (Brown et al., 1982) assessed different hydantoins according to the RTI. The teratogenic potential of these were ranked as follows: phenacemide > phenytoin > ethotoin > hydantoin > mephenytoin. Malformation profiles were similar for the different drugs. This study also suggested a common mechanism for lethal and teratagenic potencies associated with these compounds: these appeared to be related to the degree of lipid solubility, possibly reflecting the rate of passage of the compounds through biological membranes.

The final study from this group (Fabro et al., 1982) evaluated a number of different types of AED, again using the RTI, and found that they ranked as follows: sodium valproate > trimethadione > phenacemide > phenytoin > ethosuximide > phensuximide.

Eluma et al. (1984) evaluated the relative ability of PHT, VPA and

CBZ to produce craniofacial abnormalities in the CD-1 mouse, when administered in doses of 375 to 938 mg/kg for varying periods during organogenesis. Carbamazepine was the least teratogenic of the three drugs. The types of malformation were dose, time and drug dependent. For example, exencephaly and cleft lip and/or palate were induced by PHT or VPA, but not CBZ.

In the rat, it was found that phenytoin had the greatest teratogenic potential, followed in decreasing order by ethosuximide and carbamazepine (El-Sayed et al., 1983).

In contrast to the results in vivo, in vitro studies of the development of embryos exposed to AED indicated that PHT is more teratogenic than VPA. Both VPA and PHT produced a similar pattern of malformation involving neural tube defects, craniofacial abnormalities, abnormal body curvature and yolk sac defects (Bruckner et al., 1983).

Although an increased incidence of major and minor congenital malformations in offspring of epileptic women, particularly in those taking antconvulsant medication, is now recognized, other abnormalities of pregnancy outcome are less well documented. In the following sections, the current literature is reviewed concerning somatic parameters at birth, postnatal growth and mental development, spontaneous abortions, perinatal deaths, infant deaths, prematurity, and the condition of the newborn.

The following findings are derived from studies conducted in the same manner as those described for major congenital anomalies, and therefore are subject to similar methodological strengths and weaknesses.

M. Studies of Somatic Parameters at Birth in Offspring of Epileptic Patients

1. *Case Reports and Case Series*

Prenatal growth deficiency has been noted as one of the features of malformation syndromes associated with maternal AED therapy in published case reports. These reports have focused attention on the occurrence of a general disturbance in growth and morphogenesis in children exposed to AED in utero. A decrease in head circumference (HC), birth weight (WT) and/or birth length (LT) have been reported in relation to a variety of AED including multiple drug therapy (Gómez and Bolaños, 1981; Naruto et al., 1984; Wallar et al., 1978); hydantoins ± barbiturates (Desmond et al., 1972; Hanson et al., 1976; Krauss et al., 1984; Page et al., 1982; Wood and Young, 1979); trimethadione ± other AED (Feldman et al.,1977; German et al., 1970a,b; Zakai et al., 1975); PB

and/or PRM (Béthenod and Frédérich, 1975; Myhre and Williams, 1981; Rudd and Freedom, 1979; Seip, 1976); CBZ monotherapy (Robertson et al., 1983); VPA ± other AED (Ardinger et al., 1988; Dalens et al.,1980; DiLiberti et al.,1984; Huot et al., 1987; Tein and MacGregor, 1985); and diazepam monotherapy (Laegreid et al., 1989). These studies, however, do not provide any testing of hypotheses concerning the causes of decreased growth parameters.

2. Cohort Studies

Historical and prospective cohort studies of somatic parameters at birth have generally compared offspring of epileptic women who did or did not take anticonvulsant medication during pregnancy with a control group of nonepileptic women, who may or may not have been matched for variables such as socioeconomic class, parity and smoking history. Only three studies also assessed growth parameters in newborns of epileptic fathers (total n=104) (Dieterich et al., 1980; Koch et al., 1983; Majewski and Steger,1984). In some studies, growth parameters were standardized for gestational age, sex and/ or parity , whereas in other studies these were reported without such adjustments. Therefore differences in the following findings might be explained, at least in part, by differences in methodology.

Table II.9 outlines the general approach and results of cohort studies which have assessed growth parameters in newborns of epileptic women (n ranging from 28 to 3873). The majority of these studies have been carried out historically, and have evaluated WT at birth, while fewer studies have assessed newborn LT and HC. The results of these studies will be discussed in detail in the following sections.

TABLE II.9

Cohort Studies of Growth Parameters in Newborns of Women with Epilepsy

AUTHOR, YEAR	PLACE	TYPE COHORT STUDY	ASCERTAINMENT	TOTAL NO.OFFSPRING OF EPILEPTIC WOMEN (NO. EXPOSED TO AED)	FINDINGS[a] WT	LI	HC
Pritchard, J.A., et al., 1971	Dallas, Texas	Historical	All inpatient records, Parkland Memorial Hosp: 1958-1970	69 (69)	N[b]	-	-
Speidel, B.D & Meadow S.R., 1972	Leeds, England	Historical	Neurology records of 2 general hosps. & records of 3 maternity units: 1948-1972	388 (329)	N	·	-
Fedrick, J., 1973	Oxfordshire & Berkshire, England	Historical	Hosp. and home births, Oxford Record Linkage Files: 1966-1970	217 (?)	N	-	-
Monson, R.,R., et al., 1973	U.S.A.	Prospective	Collaborative Perinatal Study, 12 U.S. hosps: 1959-1965	306 (?)	↓[c]	-	..
Higgins, T.A. & Comerford, J.B., 1974	Dublin, Ireland	Historical	Consecutive deliveries, National Maternity Hosp: 1959-1973	115 (115)	N	-	-
Hanson, J.W., et al. 1976	U.S.A.	Prospective	Collaborative Perinatal Study, 12 U.S. hosps: 1959-1965	87 (87)	-	↓[d]	↓

TABLE II.9 cont'd

Cohort Studies of Growth Parameters in Newborns of Women with Epilepsy

AUTHOR, YEAR	PLACE	TYPE COHORT STUDY	ASCERTAINMENT	TOTAL NO. OFFSPRING OF EPILEPTIC WOMEN (NO. EXPOSED TO AED)	FINDINGS[a] WT	LT	HC
Weber, M., et al., 1977	Nancy, France	Historical	Neurologic consultations	547 (?)	N	-	-
Martin, F., 1978	Switzerland	?	?	109 (78)	N	-	-
Nakane, Y., 1979	Japan	Historical	Records of 11 epilepsy facilities	580 (?)	N	-	-
Dieterich, E. et al., 1980	Mainz, West Ger.	Historical	?	28	N	N	-
Bjerkedal, T., 1982	Norway	Historical	Birth registry: 1967-1978	3873 (?)	I	-	-
Debray, M.F., et al., 1982 Vert, P., et al. 1979,1982.	Nancy, France	Historical	Consecutive admissions neonatal ward, University of Nancy, Regional Maternity Unit: 1973-1980	115 (?)	N	N	I
Hill, R.M. et al., 1974, 1982 Hill, R.M.& Tennyson, L., 1982a	Houston, Texas	Prospective	Admissions for delivery to private hosps., Baylor Medical College: 1969-1979	59 (59)	N[b]	N[b]	I

TABLE II.9 cont'd

Cohort Studies of Growth Parameters in Newborns of Women with Epilepsy

AUTHOR, YEAR	PLACE	TYPE COHORT STUDY	ASCERTAINMENT	TOTAL NO. OFFSPRING OF EPILEPTIC WOMEN (NO. EXPOSED TO AED)	FINDINGS[a] WT	LT	HC
Nelson, K. B. & Ellenberg, J. H., 1982	U. S. A.	Prospective	Collaborative Perinatal Study, 12 U.S. hosps: 1959-1965	410 (215)	N	-	-
Hiilesmaa, V.K., 1983, Hiilesmaa et al., 1981,1982b	Finland	Prospective	Women seen at, regional maternity units and outpatient clinic of Helsinki Univ. Central Hosp: 1976-1979	143 (126)	N	N	I
Göpfert-Geyer,I. et al., 1982, Koch, S., et al.,1983	West Berlin	Prospective	Women seen prenatally at 5 obstetric clinics & women referred by neurologists:1976-1982	88 (70)	I	I	I
Neri, A. et al. 1983	Petah Tiqva, Israel	Historical	Obstetric records, Bielinson Medical Centre: 1971-1980	86 (86)	N	N	I
Majewski, F. & Stoger, M., 1984	Tübingen & Düsseldorf, West Germany	Historical	Outpatient records of Neurologic University Hosps.	144 (115)	N	N	I
Ogawa, Y., et al., 1982, 1984	Hirosaki, Japan	Prospective	Referrals from Hirosaki University Hosps.	116 (116)	I[f]	I	I

Table II.9 cont'd

Cohort Studies of Growth Parameters in Newborns of Women with Epilepsy

AUTHOR, YEAR	PLACE	TYPE COHORT STUDY	ASCERTAINMENT	TOTAL NO. OFFSPRING OF EPILEPTIC WOMEN (NO. EXPOSED TO AED)	FINDINGS[a] WT	LT	HC
Svigos, J.M., 1984	Adelaide, Australia	Historical	Consecutive deliveries, Queen Elizabeth Hosp: 1968-1979	76 (59)	N	-	-
Andermann, E., et al., 1982b, 1985	Montreal, Canada	Prospective	Referrals from private neurologists and Seizure Clinic of Montreal Neurological Hospital: 1972-1980	52 (52)	↓	N	↓
Yerby, M., et al., 1985	Seattle, Washington	Historical	Birth certificate records, Washington State: 1980-1981	204 (?)	↓	-	-
Kallen, B., 1986	Sweden	Historical	Swedish Medical Birth Register: 1973-1981	644 (551)	↓ monotherapy ↓↓[f] polytherapy	-	-
Kliepel, H. et al., 1986	Magdeburg, East Germany	Prospective	Women seen at Neurology clinic, Medical Academy of Magdeburg	85 (67)	↓	N	↓

Table II.9 cont'd

Cohort Studies of Growth Parameters in Newborns of Women with Epilepsy

AUTHOR, YEAR	PLACE	TYPE COHORT STUDY	ASCERTAINMENT	TOTAL NO. OFFSPRING OF EPILEPTIC WOMEN (NO. EXPOSED TO AED)	FINDINGS[a] WT	LT	HC
Robert, E. et al., 1986	Lyon, France	Historical	Records of EEG service and 3 maternity units:	147 (112)	N	-	-
Robertson, L.D. et al., 1986	Marshfield, Wisconsin	Historical	Records of regional multispecialty clinic	89 (89)	N	N	↓
Akhtar, N. & Millac, P., 1987	Leicester, England	Historical	Records, Leicester Roy. Infirm. Matern. Hosp: 1977-1981	188 (152)	↓	-	-
Bertollini, R. et al., 1987 (Collective report on AED monotherapy)	France	Historical	Records of French Chapter of Intern. League Against Epilepsy: 1973-1983;	224 (224)			
			Records of EEG service and 3 maternity units, Lyon: 1976-1984.	50 (50)			
	Italy	Prospective	Italian Multicentric Register on Congen. Malfs: 1983-1985	49 (49)			
	Sweden	Historical	Swedish Medical Birth Registry: 1973-1981	254 (254)			
	Total No. Studied by Bertollini et al.:			577 (577)	↓	↓	↓

Legend Table II.9

a Weight, length, and head circumference in newborns of all epileptic women as compared to those of nonepileptic controls, or birth measurements in newborns of epileptic women taking AED during pregnancy as compared with those of untreated epileptic women, epileptic fathers, or nonepileptic controls.

b N = not decreased relative to comparison group.

c ↓ = growth parameter was found to be decreased relative to the comparison group , expressed as either a decrease in mean growth measurements or an increase in the proportion of children with small growth measurements

d Although overall distribution of birth length in children of epileptic women was not significantly different from controls, the proportion of children with short birth lengths (< 2 SD below the mean) tended to be higher among children of epileptic women Prenatal linear growth deficiency was also a feature among children with the "fetal hydantoin syndrome".

e The proportion of children with small head circumferences was higher among offspring of epileptic women than in controls, while the proportion of children with small birth weight or short length did not differ from controls. However, children with small head circumferences also had decreased length and/or weights, suggesting proportionate growth retardation.

Legend Table 11.9 cont'd

[f] ↓↑ = Findings were both a decrease in birth weight, expressed as an increase in the proportion of low birth weight infants (<2500 grams), and an increase in the proportion of infants with heavy birth weights (> 4000 grams).

a. Birth Weight

Conflicting results have been reported about the occurrence of decreased birth weight (WT) among offspring of epileptic women. The risk of small-for-gestational-age infants has ranged from 3 to 23%, in various controlled and uncontrolled studies. This has usually been defined as less than the 10th or 5th percentiles; < 2 standard deviations below the mean for gestational age and sex; or the term has not been defined (Bossi et al. 1980, 1982a; Dieterich et al., 1980; Göpfert-Geyer et al., 1982; Hiilesmaa, 1983; Hill et al., 1974; Hill and Tennyson, 1982a; Rating et al., 1982a; Robertson et al., 1986; Yerby et al., 1985; Vert et al., 1979, 1982).

As presented in Table II.9, several studies involving cohorts of 52 to 3873 newborns of epileptic women have provided evidence of a decrease in birth weight.

The largest historical cohort study involving over 3000 births in epileptic women, registered over 11 years in the Norwegian Birth Registry (Bjerkedal, 1982a; Bjerkedal and Bahna, 1973), showed a small but statistically significant decrease of mean WT (uncorrected for gestational age) among offspring of epileptic women (mean ± SD grams: 3386 ± 660) as compared to the nonepileptic population (3438± 622). A significant increase in the proportion of newborns with WT less than 2500 grams (6.6% versus 4.7%) was also found. These differences were maintained when birth order was taken into account. Prematurity (≤ 37 weeks) was also increased in the epileptic group, and this partially but not entirely explained the difference in mean WT between the epileptic and nonepileptic groups. In this study, the epileptic group also showed a higher frequency of pregnancy complications such as toxemia and

uterine bleeding and it is not clear to what extent these and other factors (such as socioeconomic class) may have contributed to decreased WT.

Similar findings to those of Bjerkedal were reported by Akhtar and Millac (1987) in England, in their historical cohort study of 188 pregnancies in 92 epileptic women, ascertained in a maternity hospital over a 4 year period. Babies of epileptic women showed significantly lower mean WT (3178 ± 594 gm) than did nonepileptic women who delivered in the same period (mean WT: 3268 ± 537 gm). The proportion of babies with WTs less than 2500 gm were 10.6% and 8.5%, respectively The proportion of pre-term babies did not differ between the groups. Epileptic women were more often multigravidas than were controls, but this was not taken into account in assessing the WT difference. No information was given on other possible confounding factors in this study.

Yerby and colleagues (1985), in their historical cohort study of 204 births to epileptic women derived from all birth records over a two year period in Washington state, found that epileptic mothers were 2.8 times more likely to have babies with low birth weight (< 2500 grams) (6.4%) than nonepileptic controls (2.3%). This difference could not be explained by a variety of potential confounding factors, such as gestational age, marital status, parity , previous fetal loss, and maternal age. However, preeclampsia and maternal herpes were also significantly elevated in the epileptic group as compared with nonepileptic controls, (odds ratios 2.5, 6.0, respectively) and these were not evaluated as potential confounders with respect to the occurrence of low WT. As well, the sample of epileptic women ascertained from

birth records may not have been representative of the population at large, as there was a lower than expected prevalence of epilepsy on birth certificates. Thus, it is not known to what extent selection bias involving more severe epilepsies, or unknown confounders associated with the reporting of epilepsy on birth records and low birth weight, may have contributed to the observed difference in WT between the epileptic group and controls.

Andermann, Dansky and Kinch (1982b) in Montreal, in a prospective study of 52 livebirths of epileptic women who took AED during pregnancy, reported that there was an increase of WTs below the 25th percentile for gestational age, especially in female offspring, suggesting intrauterine growth retardation. However, the use of standards based on a different population (Britain) in this study may have biased the results in some unknown direction. The data were then reanalyzed (Andermann et al.,1985), using the Montreal standards of Usher and McLean (1969). It was found that offspring exposed to AED in utero showed a significant decrease in mean WT corrected for gestational age, as compared with the standard population.

In West-Germany, a prospective study of 70 children of 63 epileptic women taking AED medication during pregnancy, who had 70 offspring, (Göpfert-Geyer et al.,1982; Koch et al., 1983) found a significantly decreased mean WT and an increased risk of low WT (corrected for gestational age) among offspring exposed to AED, as compared with children of epileptic fathers or non-epileptic parents (matched on a number of potential confounding variables, such as maternal age, parity, social status, and cigarette smoking). The risk of small-for gestational-age children (≤ 10th percentile) was 18% for epileptic

women as compared with 2.4% for non-epileptic parents (Göpfert-Geyer et al., 1982). There was no difference in the frequency of small-for-gestational age infants of treated and untreated epileptic mothers. However, the number of infants in the latter group was small (n=16) (Göpfert-Geyer et al., 1982). Both treated and untreated epileptic women had a higher frequency of bleeding during pregnancy than did the controls, and it is unclear how this complication may have contributed to the findings.

A prospective study by Ogawa and co-workers (1982, 1984) in Japan, of 116 infants of epileptic women who took AED during pregnancy, showed a higher frequency of infants with either low (< 2500 grams) (4.3% versus 0.6%) or heavy (> 4000 grams) WTs (5.2% versus 2.5%), as compared with nonepileptic controls of the same socioeconomic class. However, epileptic mothers also had more frequently pre-term and post-term infants and toxemia, and were more often primiparas (Ogawa et al.,1982, 1984), and this may have explained at least in part the differences in birth weight.

Two studies of offspring of epileptic mothers, both based on the data of the large U.S. Collaborative Perinatal Project, but with varying methodology, reported somewhat different results. Monson and colleagues (1973) showed that epileptic mothers (n = 306) tended to have a higher frequency of infants with WT < 2500 grams (14.1%) as compared with mothers without a convulsive disorder (11.3%). Epileptic mothers were also more commonly of lower socioeconomic class and of older maternal age, and it is not known to what extent these differences may have explained the excess of low WT infants. The second study by Nelson and Ellenberg (1982) used a different cohort of women with

convulsive disorders (n = 410), in that the author reviewed the neurological records and recoded the presence or absence of a convulsive disorder, defined as at least one non-eclamptic seizure within 5 years of delivery (current seizure disorder). Low WT (< 2501 grams) was not found to occur more frequently in offspring of women with current seizure disorders (11.3%) than among women without such disorders (10.3%).

Other historical and prospective cohort studies involving cohorts of 28 to 547 offspring of epileptic women (Deblay et al., 1982; Dieterich et al., 1980; Fedrick, 1973; Hiilesmaa et al.,1981; Hiilesmaa, 1983; Higgins and Comerford, 1974; Hill et al.,1974; Hill and Tennyson, 1982a; Majewski and Steger, 1984; Neri et al., 1983; Pritchard et al., 1971; Robert et al., 1986; Robertson et al.,1986; Speidel and Meadow, 1972; Svigos,1984; Vert et al., 1982; Weber et al.,1977), did not find that mean WT of the offspring, or the proportion of newborns with low WT (uncorrected or corrected for gestational age) differed significantly between epileptic women, treated and/ or untreated and nonepileptic controls. In about one-half of these studies, the control groups were matched or were shown not to differ from epileptic women with respect to maternal age, sex of offspring, parity, and/ or socioeconomic class. Only two of these studies (Hiilesmaa et al.,1981; Hiilesmaa, 1983; Robertson et al.,1986) studied a number of other important variables, such as cigarette smoking, alcohol consumption, pregnancy complications, in addition to the above variables.

Several studies did not find that WT in the offspring of epileptic women was significantly correlated with AED treatment during pregnancy, the number of AED, duration of therapy, etiology and

duration of maternal epilepsy, or number and type of seizures during pregnancy (Göpfert-Geyer et al., 1982; Hiilesmaa et al.,1981; Hiilesmaa, 1983; Martin, 1978; Monson et al., 1973; Nakane et al., 1980; Nelson and Ellenberg, 1982; Ogawa et al., 1984; Robert et al., 1986, Vinci and Di Dio, 1986). In two studies, WT was not found to be associated with the total "drug score" based on the number and dosage of anticonvulsants (Ogawa et al., 1984), or with plasma AED levels late in pregnancy (Hiilesmaa, 1983).

On the other hand, a small retrospective study in Italy (Vinci and Di Dio 1986) (n=25) noted low WTs (<2500 gm) only among babies born to mothers who had seizures during pregnancy (3 of 9 babies had low WTs). In the West Berlin prospective study (Göpfert-Geyer et al., 1982; Koch et al., 1983; Rating et al., 1982a), children of mothers on polytherapy were found to be more often small-for-gestational-age than children of women on monotherapy.

In a study of 644 births to epileptic women ascertained from the Swedish Birth Registry over an 8 year period, Kallén (1986) compared the weight distribution of children of epileptic women to that expected after standardization for maternal age, parity, sex, and year of birth. Infants exposed to monotherapy showed a shift towards lower WTs, while those exposed to polytherapy showed an increased risk of either low or heavy WTs. In the monotherapy group, CBZ (n=49) was associated with a 2.5-fold increased risk of low WT babies (2.5-3 kg) relative to that expected, while this was not noted for PHT (n=147) (Bertollini et al., 1987). This finding was not due to a decrease in gestational age among babies exposed to CBZ. It should be pointed out that in this study, there was a potential for selection bias as there was

an underascertainment of births to mothers with epilepsy. How this might have affected the differences in pregnancy outcomes among different drug regimens is uncertain.

Additional studies have also shown some indication of an association between other specific maternal AED regimens during pregnancy and decreased WT of newborns of epileptic women (Andermann et al., 1985; Klepel et al., 1986; Majewski and Steger, 1984). In the Montreal prospective study, Andermann, Dansky and colleagues (1985) found that the combination of primidone with other AED, but not other drug regimens, was associated with a reduction in birth weight. Similarly, Majewski and Steger (1984), in an historical cohort study of 115 infants of mothers taking AED during pregnancy, showed a trend toward lower WTs (corrected for gestational age and sex) for women who were taking PHT together with PRM and/or PB during pregnancy (n=22), as compared with epileptic women on other drug regimens, epileptic women not taking AED, or children of epileptic fathers. Klepel and co-workers (1986), in a prospective study of 67 infants exposed to AED in utero, noted a trend towards lower mean WT but not gestational age among infants of mothers who took PRM alone during pregnancy (n=28), than in infants of other treated or untreated epileptic mothers, or non-epileptic controls. In the latter two studies, WT in a small number of children of untreated epileptic mothers (n=46 in both studies combined), or of epileptic fathers (n=63), did not differ significantly from those of nonepileptic controls, suggesting that epilepsy per se was not associated with decreased WT.

Two studies have not found an association between PB use during pregnancy and decreased WT in the offspring of epileptic women. Birth

weight was not found to be significantly decreased among infants exposed to PB with or without other AED in a French historical cohort study of 115 infants of epileptic mothers (Deblay et al., 1982; Vert et al., 1982). In a collaborative report combining data on maternal AED monotherapy and pregnancy outcome from France, Italy and Sweden (Bertollini et al. 1987), PB (n=250) was not found to be significantly associated with decreased weight in infants of epileptic mothers.

In summary, there appears to be decreased mean WT or an increased risk of of low WT (<2500 grams) among offspring of epileptic women, at least in some studies. The increased risk has ranged from 1.2 to 7.2 times that of nonepileptic women. The relative role of anticonvulsant medication; type of epilepsy; seizure frequency; socioeconomic class; pregnancy complications such as toxemia, and other maternal factors in the etiology of low WT remains unclear.

b. Birth Length

Intrauterine growth retardation with reference to birth length (LT), has been reported in 0% to 19% of children exposed to AED in utero (Bossi et al., 1982a; Dieterich et al., 1980; Hanson et al., 1976; Hill and Tennyson, 1982a; Robertson et al., 1986; Vert et al., 1979, 1982). Reduced birth length has been defined as less than or equal to the 10th, 5th or 3rd percentile, or < 2 SD below the mean for gestational age ± sex.

In a study using the data from the Collaborative Perinatal Project, Hanson et al. (1976) showed that the distribution of LTs did not differ significantly between children of women who took hydantoins with or without barbiturates ± other AED (n=87) and nonepileptic controls,

although there was a trend toward a higher proportion of infants with short LTs < 2 SD below the mean (16 versus 8%), and with very short LTs (< 3 SD below the mean) (10% versus 3%), among treated epileptic women. This study also found that prenatal growth deficiency (length ≤ 3rd percentile) was a consistent feature among 11 of 104 children exposed to AED, who showed multiple features of the fetal hydantoin syndrome.

In the West-German prospective study (Koch et al., 1983), significantly decreased LT (corrected for gestational age) was noted in 70 infants of mothers who took AED during pregnancy, as compared with infants of epileptic fathers or of non-epileptic parents (matched controls). Maternal AED polytherapy tended to be associated with a greater reduction in LT than was monotherapy.

Surprisingly, Ogawa et al. (1982) found a significant increase in crown-heel length but not gestational age in 42 offspring of epileptic women exposed to AED in utero as compared with nonepileptic controls, and this was particularly noted for male offspring. In this study, a negative correlation was also noted between the total AED dosage and the crown-heel length.

In a collective report of historical and prospective cohort studies from France, Italy and Sweden involving growth parameters of 577 newborns of epileptic women who took different AED monotherapies during pregnancy, only CBZ (n= 54) was associated with a decrease in LT with no decrease in gestational age (Bertollini et al., 1987).

In contrast to the above findings, most studies did not find a statistically significant difference in mean LT or the proportion of children with short LTs (adjusted or unadjusted for gestational age and

sex), between offspring of treated epileptic mothers on the one hand , and children of untreated epileptic mothers, epileptic fathers, or of non-epileptic parents (Andermann et al., 1985; Deblay et al., 1982; Dieterich et al., 1980; Hiilesmaa et al.,1981; Hill et al., 1974; Hill and Tennyson, 1982a; Klepel et al., 1986; Majewski and Steger, 1984; Neri et al., 1983; Nomura et al., 1984; Robertson et al., 1986; Vert et al., 1982). There was also no difference between children of untreated epileptic mothers or epileptic fathers and nonepileptic controls (Dieterich et al., 1980; Hiilesmaa et al., 1981; Klepel et al., 1986; Majewski and Steger, 1984).

c. Head Circumference at Birth

A number of studies have reported decreased head circumference (HC) at birth in offspring of epileptic women, and frequently this has been called microcephaly (Andermann et al., 1985; Bertollini et al., 1987; Bossi et al.,1980, 1982a; Deblay et al., 1982; Göpfert-Geyer et al., 1982; Hanson et al., 1976; Hiilesmaa et al., 1981, 1982b; Hill et al.,1974, 1982; Hill and Tennyson, 1982a; Kelly, 1984b; Klepel et al., 1986; Koch et al., 1983; Majewski and Steger, 1984; Neri et al., 1983; Ogawa et al., 1982, 1984; Robertson et al., 1986; Speidel and Meadow,1972; Vert et al., 1979, 1982). The frequency of microcephaly defined as < 2 SD below the mean for gestational age ± sex in infants exposed to AED has ranged from 8 to 27.3% (Bossi et al.,1982a; Deblay et al., 1982; Göpfert-Geyer et al., 1982; Hanson et al., 1976; Koch et al., 1983).

Hanson et al. (1976), in their study based on the U.S. Collaborative Perinatal Project, found a significantly decreased mean HC (mean ± SD

cm. : 33.8 ± 1.9) among 84 offspring exposed to hydantoins (with or without barbiturates ± other AED) in utero as compared with offspring of nonepileptic controls matched with respect to socioeconomic status, maternal age, race and institution of birth (34.4 ± 1.4). About 8% of the newborns of epileptic mothers were microcephalic (< 2 SD below the mean for age and sex) as compared with 3% of the control infants.

In Wisconsin, a population-based historical cohort study of growth parameters in 89 newborns of epileptic women, who took AED during pregnancy, was carried out by Robertson and colleagues (1986). They found that male but not female offspring (term babies) of epileptic women showed significantly lower mean HC as compared to controls, matched for sex and date of delivery. Eighteen percent of exposed children showed HCs at birth < 5th percentile as compared with 8.6% of control children. As two thirds of the women in this study were on polytherapy, most frequently PHT and PB, the association between specific AED and growth parameters was not studied.

In the prospective study by Andermann, Dansky and colleagues in Montreal (1985), mean HC at birth corrected for gestational age was significantly decreased in babies born to mothers who took polytherapy during pregnancy. Monotherapy was not associated with a reduction in head circumference.

In the West German prospective study by Koch and colleagues (1983), mean HC was significantly decreased and the proportion of microcephalic children (< 2 SD below the mean for gestation) (22.4%) was significantly increased in 67 children of epileptic mothers who took AED during pregnancy, as compared with children of untreated mothers, or of nonepileptic parents. Microcephaly was seen more

frequently following maternal AED polytherapy than with monotherapy.

Ogawa and colleagues (1982) in their prospective study from Japan, extensively assessed various head growth parameters at birth in 42 newborns of epileptic mothers who received AED during pregnancy. They reported a significant decrease of the vertex circumference, as well as the fronto-occipital , biparietal , and bitemporal diameters in term babies of epileptic mothers, particularly female children, as compared with those measured in children of nonepileptic parents. These measurements were also found to be inversely correlated with the total "drug score", and were particularly decreased in infants of women on high doses of AED. This, however, was a crude measure of the impact of AED and it is not known which specific AED and in what amounts may have been associated with decreased HC.

Majewski and Steger (1984), in West-Germany, noted a significant decrease in mean HC, either uncorrected or standardized for gestational age and sex, among 115 infants exposed to AED in utero as compared with infants of untreated epileptic mothers or of epileptic fathers. Significant differences were noted for mothers who were on monotherapy with PHT (n=17); monotherapy with PB or PRM (n=12); and on polytherapy with PHT and PB or PRM (n=16). The latter combination was associated with the smallest mean HC. HC did not differ between children of epileptic fathers (n=39) or children of untreated epileptic mothers (n=17) and non-epileptic controls, suggesting that epilepsy per se was not the explanation for the decreased HC observed in offspring of treated epileptic women.

Klepel and co-workers (1986), in their prospective study in East Germany, noted that HC was smaller in offspring of epileptic women

who took PRM alone during pregnancy (n=28), than in children of untreated epileptic mothers or non-epileptic mothers.

Neri and colleagues (1983) in Israel, in an historical cohort study of 86 pregnancies of treated epileptic women, reported lower mean HC in children exposed to PRM alone or in combination with other AED than in controls matched for age, parity and date of delivery, but not gestational age. However, this study also showed a higher frequency of twin pregnancies in the epileptic group, and these were included in the comparison of growth parameters.

In a study in France of 115 infants born to 94 epileptic mothers, who mostly took phenobarbital with or without other AED during pregnancy, Deblay and colleagues (1982) noted that 19.1% of the infants had HCs < 2 SD below the mean for gestational age and 31.3% had measurements ≤ 2 SD. Small cranial volumes were confirmed on x-ray. Mean plasma PB levels in cord blood were found to be significantly higher among infants with HC < 2 SD below the mean (21.8 ± 16.7 µg/ml) than among those with larger heads (15.9 ± 11.0 µg/ml). Withdrawal symptoms were also noted to occur more frequently in infants with small HCs and these may have been related to the higher plasma PB levels.

Hiilesmaa and colleagues (1981, 1982b) also assessed newborn growth parameters in a prospective population-based study, involving about 75% of children born to epileptic mothers in a defined area of Finland, in the period 1976 to 1979 (Gaily et al., 1988a,b). They compared HC (standardized for gestational age, sex, WT and LT) in 143 newborns of epileptic mothers with a control group of nonepileptic parents, matched for maternal age, parity, social class and fetal sex.

The authors found a significantly decreased mean HC in offspring of women who had been on CBZ monotherapy during pregnancy (n=20), as well as in offspring of women who had been on combination therapy that included PRM or PB (n=26). It is of interest that a decrease in biparietal diameter of exposed fetuses was not noted on ultrasound at 16 weeks of gestation, but was present from 20 weeks onward (Hiilesmaa, 1982). Mean HC among a small number of infants of mothers who had not taken AED during pregnancy (n =17) was not decreased. However, HC was not found to correlate with maternal plasma AED levels. It is not clear, however, how plasma AED levels were analyzed in relation to HC. The etiology or duration of maternal epilepsy, and the type and number of seizures during pregnancy was also not found to be related to HC (Hiilesmaa et al., 1981, Hiilesmaa, 1982).

Women with epilepsy and controls did not differ significantly with respect to a number of potential confounding factors including maternal HC, pregnancy complications, weight gain during pregnancy, smoking, and alcohol consumption. Although the authors in this report attributed their findings to the effects of CBZ monotherapy and of barbiturates taken in combination, it now appears that the fathers of the children exposed to these medications had smaller HCs than those of controls (Granström, personal communication). This illustrates the pitfalls encountered in studying the potential teratogenic effects of AED, and emphasizes the need to control carefully for possible confounding factors in order to assess the independent effects of AED.

d. Head Circumference in Relation to Length and Weight

In a recent paper (Bertollini et al., 1987), data was combined from

historical and prospective cohort studies and birth registries in France, Italy and Sweden in order to assess the association between newborn growth parameters and monotherapies in a large series of 577 epileptic women. CBZ monotherapy was associated with a decrease in mean measurements of all three growth parameters -HC, WT and LT- with no decrease in gestational age, suggesting proportionate growth retardation. However, the significance of these findings is open to question as no data were available concerning unknown potential confounders in this collective study.

Koch and colleagues (1983), in West Germany, also found a reduction in all three growth parameters in offspring of epileptic women taking AED, as compared with matched nonepileptic controls. Polytherapy was associated with a greater reduction than monotherapy, particularly with respect to HC. The same study showed that specific drug regimens PRM or CBZ ± other AED were associated with decreased HC, WT, and LT corrected for gestational age (Rating et al. 1982b, Kuhnz et al., 1983). A dose-response relationship was established between PRM exposure and decreased birth measurements (Jacob et al., 1985). VPA polytherapy but not monotherapy was also significantly associated with decreased HC and WT (Jäger Roman et al., 1986). However, another small prospective study in West Germany (Majewski et al., 1985) did not find a significant association between VPA or CBZ ± other AED and prenatal growth retardation.

Andermann, Dansky and colleagues (1985) noted a reduction in mean HC and WT corrected for gestational age, but not in birth length. All polytherapies were associated with a reduction in HC. However, the types of drug regimens were not mutually exclusive, because a woman

who may have been taking , for example, PRM with CBZ was included in the CBZ polytherapy and PRM polytherapy groups. Only PRM taken in combination with other AEDs was associated with decreased birth weight.

Hanson et al. (1976) and Hill et al. (1982) also noted that infants exposed to AED in utero showed prenatal growth retardation involving HC and length and/or weight measurements, as well as a characteristic pattern of major and minor anomalies.

The above findings are in contrast to those of other studies which found that HC was reduced but not other body measurements in children exposed to AED therapy (Robertson et al., 1986), CBZ monotherapy (Hiilesmaa et al., 1981, 1982b), or barbiturates with or without other AED (Deblay et al., 1982; Hiilesmaa et al., 1981,1982b; Majewski and Steger, 1984; Neri et al., 1983; Verl et al., 1979, 1982). Only one of these studies actually assessed head circumference in relation to body length and weight for each individual child, and found HC to be small for WT and LT (Hiilesmaa et al., 1981).

e. Summary

In summary, despite different geographical areas and different methodologies, retrospective and prospective cohort studies of growth parameters in newborns of epileptic women consistently report a decrease in mean HC or an increased frequency of microcephaly associated with AED therapy. In contrast, WT and/or LT may or may not be decreased.

The conflicting results with some studies showing decreased WT and LT and others not may be due to a number of reasons. In some

studies, selection bias may have led to the inclusion of a disproportionate number of women with severe epilepsy and thus to the observed differences between epileptic women and nonepileptic controls. Small sample sizes may also have led to false negative results. There may be genetic differences as well as differences in anticonvulsant regimens among different samples of epileptic women. Lack of an adequate control group could have biased the differences towards false positive or negative results. Measurement error may also have contributed to differences among studies, particularly with respect to LT which is difficult to measure. In some of the negative studies of WT, only the proportion of WTs below a certain cut-off point (e.g. 3rd percentile) was compared between groups (Higgins and Comerford, 1974; Hill and Tennyson,1982a; Nakane et al., 1980; Nelson and Ellenberg,1982; Robert et al., 1986; Speidel and Meadow, 1972; Vinci and Di Dio, 1986). Thus differences in the total distribution of WTs or mean WTs may have been missed.

The study of growth parameters in relation to AED therapy has been hampered by the small numbers of women taking specific AED during pregnancy in individual studies, and it is only recently that efforts have been made to combine data from different studies in order to evaluate pregnancy outcomes such as newborn growth parameters, in relation to specific AED therapy (Bertollini et al., 1987). However, the comparability of data from different areas may be a problem in these studies. In various studies, the maternal AED regimens which have been associated with decreased growth parameters have been monotherapies with PHT, PB or PRM, and CBZ, as well as polytherapy, particularly barbiturates taken in combination with other AED. There is still

insufficient data regarding maternal VPA therapy in relation to newborn growth parameters.

Very few studies have investigated the relationship between newborn growth parameters and dose and plasma levels of AED. These have either used a crude measure of dose (total drug score), or have looked at plasma AED levels only in late pregnancy or at term.

The association between other potential confounding factors and decreased growth parameters must be explored before conclusions can be made about the influence of the number, type, and dose of specific AED. Only a few studies have examined the association of one or more factors other than AED with newborn growth parameters. For example, some studies have assessed newborn somatic parameters in children of untreated epileptic women and of epileptic fathers in order to evaluate the influence of epilepsy per se, and these groups have generally not shown a decrease in newborn growth parameters relative to nonepileptic controls. However, the numbers of subjects have been small, and the non-random treatment of epileptic women (i.e. women with more severe epilepsy requiring AED therapy) has not been addressed. Further detailed analysis of the relative influence of AED, type and severity of epilepsy , maternal seizures, socioeconomic status, growth measurements of the mothers and fathers, cigarette smoking and other possible adverse factors during pregnancy, on newborn growth parameters is needed. A multivariable analysis, which simultaneously controls for many of these variables, would be the most desirable method to analyze the relative role of these factors, in the etiology of decreased growth parameters in offspring of epileptic women.

In the following sections, the literature on postnatal growth and development are discussed. These have been initially reviewed by Helge (1982) and Andermann (1982b). Several studies have had longitudinal follow-up of children of epileptic mothers (Granström, 1987, 1988; Granström and Hiilesmaa, 1982b; Hiilesmaa et al., 1981; Hill et al., 1982; Jäger-Roman et al., 1982; Vert et al., 1982), while others have been cross-sectional in design. Data on the postnatal growth of children of epileptic mothers who did not take AED during pregnancy are lacking. Most studies did not take into account the growth measurements or intelligence of the parents and not all studies used matched control groups. The follow-up period was also short in many studies. Furthermore, the contribution of postnatal nutrition and other environmental variables such as maternal care to the findings concerning postnatal growth and development in offspring of epileptic women is largely unknown. All of these factors make it difficult to evaluate the role of intrauterine exposure to AED therapy in postnatal growth and development.

N. Postnatal Growth in Offspring of Epileptic Patients

Kaneko et al. (1982b) studied neonates of treated mothers with epilepsy from a middle socioeconomic class, and observed poor weight gain in the early postnatal period, particularly in those on a mixed feed schedule as compared with controls. These infants also showed poor sucking and drowsiness, the duration of which was correlated with the maternal "drug score". The ingestion of AED via breast milk as well as the slow clearance of transplacentally acquired AED may have accounted for the poor weight gain in the infants.

Vert et al. (1982) found an increased frequency of decreased weight, height and head circumference in children exposed to AED in utero who were followed for 6 months to 6 years. Most of the children had only decreased head circumference at birth. Height was less frequently affected than weight or head circumference. About one-half of those with microcephaly at birth continued to have a similar reduction in head circumference with general somatic and mental retardation (Deblay et al., 1982).

Granström (Granström and Hiilesmaa, 1982b; Granström, 1987; Granström, 1988) from the Finnish prospective study, has reported on the postnatal growth of children up to the age of 5 years, of whom the majority were exposed to AED in utero. Various unmatched control groups have been used at different stages of the study, making interpretation of the longitudinal trends in postnatal growth difficult. Children exposed to AED showed poorer weight and length gains in the first few postnatal months than did control children (Granström, 1987). This was particularly evident in those exposed to PHT monotherapy or barbiturate combinations. Sedative effects resulting in feeding difficulties might have explained the delay in weight gain in barbiturate exposed children. Height, but not weight of the epileptic children tended to be somewhat lower at the age of one year. At age 5 years (Granström, 1988), six of the children exposed to AED in utero (5.1%) had heights below two standard deviations from the mean measurement of the control group. The mid-parent height of these children also tended to be low.

The reduced head circumference noted at birth in the Finnish study was noted to persist at age 18 to 24 months (Granström and Hiilesmaa,

1982b; Hiilesmaa et al , 1981). Seven of 93 children examined at this age were found to be microcephalic; two of these had small head circumference at birth, while the others showed postnatal onset of growth delay (Granström and Hiilesmaa, 1982b). The decrease in head circumference was significant for those exposed to CBZ with or without other AED and those exposed to barbiturate combination therapy. Mean head circumference of children exposed to PHT monotherapy did not differ from controls (Hiilesmaa et al., 1981).

At age 5 years (Granström, 1988), mean head circumference was significantly reduced in children exposed to barbiturates in utero, but the father's head circumference was also significantly reduced as compared with controls. There were 3 microcephalic children exposed to AED in utero (2.6%) (PHT and/or CBZ). In one case the father was microcephalic, and in another case both the mother and father had head circumferences one standard deviation below the mean for controls. Only one of the three showed borderline intelligence, while the other two were of normal intelligence. Granström concluded that there is no evidence that prenatal exposure to AED causes persistent growth deficiency.

Hill et al. (1982) noted poor growth of head circumference (less than two standard deviations from the mean) and weight (less than 3rd percentile) in 6 of 47 infants (13%) exposed to AED in utero during the first two years of life. By four years, all but two of the children had heights and weights between the 3rd and tenth percentiles. None of the 252 infants of nonepileptic parents showed this aberrant pattern of growth.

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In a follow-up period of 5 months to 3 years after birth, Jäger-Roman et al. (1982) found an increased prevalence of poor somatic development with respect to weight, height and head circumference among offspring exposed to AED in utero. This was not evident for a small number of unexposed children. Children exposed to polytherapy were more affected than those exposed to monotherapy. Primidone therapy was associated with the highest prevalence of microcephaly. Children of treated epileptic mothers who were small for gestational age had a significantly increased risk of microcephaly and of weights below the third percentile, as compared to children of normal birth weight. In children who had reduced measurements at birth, there was some catch up growth in height and weight, but not in head circumference (Koch et al., 1983). Postnatal somatic development was not related to the type of epilepsy (Jäger-Roman et al., 1982)

In a report from the Collaborative Perinatal Study (Nelson and Ellenberg, 1982), children of epileptic mothers were found to have a 2-fold increased risk of microcephaly at one year of age as compared to children of nonepileptic women.

0. Experimental Animal Studies of Anticonvulsants and Growth

A number of experimental studies have been carried out which have assessed the effect of specific AED on somatic parameters either in vivo, by administering AED to pregnant dams at various stages of gestation or to neonates, or in vitro, by exposing whole embryos or specific cell types to AED in the culture medium. Studies in vivo were usually primarily designed to assess the dysmorphogenetic potential of AED during embryogenesis, and therefore have not fully evaluated critical periods for reduced growth. Neonatal studies in rodents have been done because this period corresponds to midgestation through later postnatal development in humans. As there is an inverse relationship between fetal weight and litter size, it is important to control for drug effects on litter size; however, not all studies have taken this into account (e.g. Ching and Tang, 1986; Khera, 1979; McCLain and Langhoff, 1980).

1. *Hydantoins*

A number of in vivo studies in various strains of mice have shown that hydantoins, including PHT, administered during pregnancy alter body weight, crown-rump distance, and/or transumbilical distance at birth (Barcellona et al., 1987; Brown et al., 1982; El-Sayed et al.,1983; Eluma et al., 1984; Finnell, 1980,1981; Gabler, 1968; Gibson and Becker, 1968a ; Hansen and Hodes, 1983a,b; Harbison and Becker, 1969,1970, 1974; Lum and Wells, 1986; McDevitt et al., 1981; Paulson et al., 1979; Ross, 1973; Sucheston et al., 1986; Sullivan and McElhatton, 1977; Tanaka et al., 1984). The effect appears to be dose-related, strain and stage dependent (Eluma et al., 1984), and has been produced using a

variety of modes of administration including the oral route (Finnell, 1981). For example, Gibson and Becker (1968a) noted that in different strains, fetal weight was reduced following PHT treatment in late embryogenesis, while crown-rump distance and trans-umbilical distances were reduced or increased with treatment in early and/or late embryogenesis, depending on the strain used. Shortening of the long bones also occurred in a strain, route, dose and day dependent fashion, with maximal effects occurring early in gestation, or with treatment on consecutive days (Gibson and Becker, 1968a; Harbison and Becker, 1969). Retarded ossification of the skeleton such as the sternebrae, hands and feet has also been shown to occur in a dose-related manner, with PHT treatment during embryogenesis (Finnell, 1980, 1981; Harbison and Becker, 1969, 1970; McDevitt et al., 1981; Miller and Becker, 1975; Sullivan and McElhatton, 1977).

Although some studies have shown a decrease in one or more of these parameters only at high doses which produce toxic effects in the mother, such as reduced weight gain during pregnancy or ataxia (Elshove 1969; Fritz et al., 1976; Gabler, 1968; McDevitt et al., 1981; Paulson et al., 1979; Ross, 1973; Sullivan and McElhatton, 1977), other studies have shown that reduced growth parameters in the offspring occur at doses which do not produce toxic effects in the mother (Finnell, 1980 ; Hansen and Hodes, 1983a; Tanaka et al., 1984) and/ or which yield plasma AED levels close to or within the mouse or human therapeutic range of plasma levels (Finnell, 1980, 1981; Paulson et al., 1979; Tanaka et al.,1984). In one study, a dose dependent decrease in fetal weights was seen at dosages which yielded plasma PHT levels ranging from the lower to the upper human therapeutic range, but toxic effects

in the mother were also dose-dependent (Paulson et al., 1979).

Tanaka et al. (1984) showed that PHT, at doses yielding human therapeutic plasma levels, produced a dose-related decrease in brain weight, along with a reduction in fetal weight and length. Levels of PHT in fetal serum, carcass, brain , liver, and kidney correlated with maternal serum PHT levels.

In a mouse model of spontaneous seizure disorder, the presence of seizures or of genetic factors associated with a seizure disorder in the sire or dam, did not adversely affect growth of the fetus. Treatment of the sire with PHT also did not adversely affect fetal growth (Finnell, 1982; Finnell and Baer, 1986).

In different rat strains, PHT in doses of 30 to 1000 mg/kg, administered orally or intraperitoneally for varying periods during gestation (which did or did not result in observable malformations), reduced fetal or newborn weight in a dose-related manner (El-Sayed et al., 1983; Harbison and Becker, 1972; Lorente, et al., 1981; Shapiro et al., 1987). Weight was also found to be depressed postnatally (Elmazar and Sullivan, 1981; Lorente et al. 1981; Mullenix et al., 1983; Shapiro et al., 1987; Vorhees, 1983, 1985). Treatment for as little as a few days during early to mid embryogenesis (1000 mg/kg, orally) reduced fetal or neonatal weight and/or postnatal weight (Lorente et al., 1981; Mullenix et al., 1983). Doses used resulted in plasma PHT levels in the mid (Lorente et al., 1981) or slightly above the human therapeutic range Vorhees, 1983, 1985). In one study, maternal weight gain during pregnancy was reduced relative to controls (Vorhees 1983), but in other studies, pair-fed controls were used (Elmazar and Sullivan, 1981; Lorente et al., 1981; Mullenix et al., 1983).

Doses of 50 to 100 mg/kg PHT, injected intraperitoneally from gestational day 17 through postpartum day 7, reduced birth weight in male offspring, and both male and female offspring showed a reduction in body weight gain throughout life (Shapiro et al.,1987). Seminal vesicle weights were also reduced, but ovarian and uterine weights and serum concentrations of sex steroids were normal.

In the rat, reduced crown-rump and transumbilical distances occurred in a dose-related fashion, as in the mouse (El-Sayed et al., 1983; Harbison and Becker 1972). Skeletal defects such as fetal long bone shortening (Harbison and Becker, 1972; Lorente et al., 1981) and incomplete ossification of the cranial bones, ribs, pelvic girdle, and phalanges also occurred (Elmazar and Sullivan, 1981; El-Sayed et al., 1983) in a dose related manner (El-Sayed et al., 1983).

Craniofacial measurements, brain and cerebellar weights were noted to be reduced in the fetus, neonate, and adult rat but there was no change in the brain/body weight ratio (Elmazar and Sullivan, 1981; Lorente et al., 1981). Vorhees (1985) and Seth et al. (1986), however, noted no significant difference in postnatal brain weights, but did find a reduction in brain protein (Vorhees, 1985). Ultrastructural changes in Purkinje cells occurred following oral administration of PHT throughout gestation and/or lactation in a dose-dependent manner (Biggiogera et al., 1983; Origuchi, et al. 1975). These changes occurred following PHT doses of 5-7 mg/kg. Pathological findings in the Purkinje cells were the same as those found in patients who receive PHT therapy (Origuchi et al., 1975). In one of these studies, no difference in birth weight was observed with up to 100-150 mg/kg (Origuchi, et al. 1975).

In contrast to the findings in rat and mouse, fetal weight and crown-rump distance was not reduced with teratogenic doses of PHT in the rabbit (McCLain and Langhoff, 1980) or cat (Khera, 1979), although shortening of the long bones was produced in the rabbit (McCLain and Langhoff, 1980). In the monkey (Phillips and Lockard, 1985), no correlation was found between maternal plasma PHT levels in the third trimester (within the human therapeutic range) and birth weight.

Using the whole embryo culture technique, Bruckner et al. (1983) showed that growth and development were retarded in mouse embryos exposed to PHT, as indicated by a dose-dependent decrease in protein and DNA content at concentrations of ≥15 µg/ml, and a reduction in somite number, crown-rump length and yolk-sac diameter at concentrations of at least 35 µg/ml. Extended exposure to PHT concentrations of 25 to 50 µg/ml resulted in significant depression of DNA synthesis in human fibroblast cells in culture (Moy et al., 1985). In vitro, the administration of PHT has been shown to inhibit neuronal process formation in dorsal root ganglia cultures of chick embryos in a dose-dependent fashion; the concentration at which PHT inhibited process formation by 50% of control values was 25 µg/ml (Dow and Riopelle, 1988). PHT was shown to inhibit differentiation of rat embryo midbrain cells in a concentration-dependent manner above 25 µg/ml (Flint and Orton, 1984). Addition of a metabolizing enzyme system did not potentiate the inhibition of differentiation, suggesting that in vitro bioactivation to toxic metabolites was not involved in PHT-induced inhibition of differentiation. Phenytoin at concentrations of 15 to 50 µg/ml has been shown to decrease the number of mouse cortical neurons in culture (Swaiman et al., 1983).

2. *Phenobarbital*

In experimental in vivo studies, it has been difficult to attribute adverse effects of PB solely to the direct effects of the drug because of possible effects of lethargy, increased sleeping time, and sedation-induced undernutrition which may accompany PB administration in pregnant dams and/or their offspring (Middaugh, 1986; Yanai and Iser, 1981).

In rats, PB has been shown to decrease fetal weights, when administered in the diet, subcutaneously, or by gavage during mid-embryogenesis or all of gestation, at doses (80 mg/kg) which did not interfere with maternal weight gain (McBride and Rosman, 1984; Seth et al., 1986; Vorhees, 1983). Brain weight was not decreased (Seth at al.,1986). Phenobarbital concentrations measured in one study were within the human therapeutic range (McBride and Rosman, 1984). In contrast, other studies (Ching and Tang,1986; McColl, 1966) did not find that 30 to 80 mg/kg of PB given subcutaneously or in the diet throughout gestation reduced fetal weight, but in these studies a decrease in litter size may have obscured a relationship between PB and decreased fetal weight. However, rat pups showed delayed ossification of the skull, manifested by abnormally wide sutures between the cranial bones (McColl 1966).

In rats, Vorhees (1983) found that administration of PB (80 mg/kg) by gavage in mid-embryogenesis depressed birth weight, but there was catch up growth by postnatal day 7. Gupta et al. (1980) showed that subcutaneous injections of PB (40 mg/kg) on days 12 to 19 resulted in normal newborn weights, but reduced postnatal body weight gain, disorders of reproductive function, and altered concentrations of sex

steroids, gonadotropic hormones and estrogen receptors. Sobrian and Nandedkar (1986) also observed normal newborn weights followed by a depression of postnatal weight gain, with 2 mg/kg of PB (median human therapeutic dose), subcutaneously injected on gestational days 15 to 20. Auroux (1973) found that PB (5 mg/kg), administered by intramuscular injection late in gestation and in the first few days of life, resulted in increased birth weight as compared with controls, and that this increase persisted postnatally. Neonates of treated mothers were less active than controls.

In some studies of inbred mice, PB was not found to reduce fetal weights (Fritz et al.,1976; Gibson and Becker, 1968b; Harbison and Becker, 1970; Sullivan and McElhatton, 1975,1977).

In contrast, Finnell and colleagues (1987b) showed that there was a strain difference in PB-induced reduction of fetal weight in the mouse. Fetal weight was reduced in the C57BL/6J strain, but not in two other inbred strains, following administration of PB in drinking water (60 to 240 mg/kg) from 20 days before mating through all of gestation. Abel et al. (1987) also showed a reduction in fetal weight when pregnant Swiss-Webster mice were subcutaneously injected with PB (25 mg/kg/day) throughout gestation. In these studies, mean maternal plasma PB levels ranged from below to within the human therapeutic range (8 to 26 µg/ml). Fetal weight was negatively correlated with plasma PB levels greater than or equal to 10 µg/ml (Finnell et al., 1987b). A dose-related increase in the number of skeletal defects, most commonly incomplete ossification of the craniofacial bones, was also found (Finnell et al., 1987b). There was no evidence of maternal toxicity even at the highest drug concentrations (Finnell et al. 1987b;

Abel et al., 1987). Kuprys and Tabakoff (1981) also found that PB in the diet (up to 3 mg/g chow) reduced birth weight as compared with offspring of pair-fed control dams.

In the gerbil, administration of PB (60 mg/kg) in drinking fluid throughout gestation and lactation resulted in a reduction in birth weight, and later in life, a reduction in body weight more marked in males than in females, and a decrease in brain weight in males only (Chapman and Cutler, 1988).

Fetal weight was not found to be decreased in the rabbit with teratogenic doses of 50 mg/kg administered by oral intubation on days 8 to 16 (McColl et al., 1967), but litter size was decreased. Phenobarbital, however, was shown to affect the skeletal system: abnormal wide sutures and missing, fused and small sternebrae were seen. In a collaborative laboratory study, there was some reduction in fetal weight in the rabbit with oral doses (10 to 30 mg/kg) on days 8 to 16, which resulted in some maternal deaths (Somers, 1969).

Experiments in rodents have shown that PB can adversely affect brain development, when administered at various periods in the last two trimesters of pregnancy and/or the neonatal period. Injections of PB (80 mg/kg) from mid-embryogenesis to parturition resulted in reductions of nucleic acid and protein in brains of offspring (Zemp and Middaugh, 1975). Many articles have been published by Yanai and colleagues showing a reduction in brain weight and neuronal loss in various parts of the brain including cerebellum, hippocampus, and cerebral cortex (Bergman et al., 1980; Yanai and Bergman, 1981; Yanai and Iser, 1981; Yanai et al., 1982b,c; Yanai et al., 1983). Body weight was found to be decreased at birth and decreased weight was also

evident in adulthood (Middaugh, 1986; Yanai and Iser, 1981). Plasma PB levels in mothers and neonates were high, ranging from 40 to 200 µg/ml (Bergman et al., 1980). In these studies, prenatal administration of PB was via the diet , and increasing doses of PB in the diet were negatively correlated with maternal weight gain during pregnancy (Middaugh, 1986).

In the rat, Hannah et al. (1982, 1988) showed that a small dose of PB (10 mg/kg), given intramuscularly late in gestation to 21 days postnatally, resulted in a reduction in the number of Purkinje cells and in abnormal growth and morphology of these cells. Body weight was not decreased. They hypothesized that the reduced activity in PB-treated animals retarded Purkinje cell growth. Retarded development of neuropil and synaptic junctions of cerebral and brain stem neurons were found in rabbit neonates, following maternal treatment with 18.5 mg/kg of PB in the last 10 days of gestation. These changes were not evident at one-half this dosage (Dydyk and Rutczynski, 1980). Diaz et al. (1977) showed that the administration of PB via subcutaneous injections to rat neonates during the period of the brain growth spurt (days 5 to 18) resulted in decreased brain weight, DNA, RNA, and cholesterol, without any reduction in body weight. Peak levels of PB were within the human therapeutic range. The corresponding developmental period in humans begins at mid-gestation, peaks just after birth, and continues for several years postnatally (Dobbing,1970; Dobbing and Sands, 1973).

In vitro studies circumvent possible confounding effects of undernutrition in in vivo studies of PB. In vitro (Daval et al., 1988), administration of PB in pharmacologically active concentrations to rat brain neurons interfered with the uptake of energy substrate

(2-deoxy-D-glucose), protein and RNA metabolism, and growth of neuronal processes. Bergey et al. (1981) found a dose-dependent decrease of neuron counts in fetal mouse spinal cord culture with PB concentrations of 30 to 120 µg/ml, which returned to normal when PB was washed from the culture, suggesting that PB interfered with neuronal growth. There appeared to be a threshold for inhibition of protein synthesis in immature rat brain, with concentrations of at least 1.0 mM (232 µg/ml) producing this effect, much higher than those found in brain after the administration of PB. Phenobarbital was not as effective as PHT in the inhibition of protein synthesis (Swaimann and Stright, 1973).

3. *Primidone*

In the mouse, Sullivan and McElhatton (1975,1977) found no significant reduction in fetal weights with PRM treatment on days 6 to 16 at doses of 25 to 2500 mg/kg, administered by gastric intubation or in the diet. Plasma PRM levels were within the human therapeutic range at the lowest dosage administered by gastric intubation, but the drug was more rapidly cleared from the plasma than in humans (Sullivan and McElhatton, 1975).

4. *Carbamazepine*

In the CD-1 mouse, fetal weights were reduced only with high doses of CBZ (1600 mg/ kg), administered in the diet on days 8 to 13 of gestation. This dose produced sedation, ataxia, and some reduction of appetite (Paulson et al.,1979). At this dosage, plasma CBZ levels were within the human therapeutic range (4µg/ml). Sullivan and McElhatton

(1977) observed no decrease in fetal weights when CBZ was administered, in doses up to 240 mg/kg by gastric intubation, to pregnant CD-1 mice during embryogenesis. In the CD-1 mouse, Eluma et al. (1984) found that the decrease in fetal weights, following administration of CBZ (375 to 938 mg/kg) by gastric intubation at varying periods during embryogenesis, was time and dose-dependent. The greatest reduction occurred following treatment during mid to late embryogenesis.

Although Wray et al. (1982) did not find that fetal weight was significantly reduced in offspring of A/J dams treated with CBZ (100 to 300 mg/kg, administered in multiple doses per day by gavage) as compared with controls, offspring with cleft palates who were exposed to CBZ in utero weighed less than their noncleft littermates. These authors showed that multiple dosing increased the time that plasma CBZ levels were within the human therapeutic range. Peak levels were in the highly toxic range. In NMRI-derived mice, Fritz et al. (1976) found a delay in ossification, but fetal weights were unaffected when high doses of CBZ (250 mg/kg), producing toxic effects in the dams, were given by gavage during embryogenesis.

Finnell et al. (1986) administered up to 2000 mg/kg of CBZ in the diet, from 2 weeks before mating to day 18 of gestation, to two inbred mouse strains (SWR/J and LM/Bc), which differed in their capacity to convert the major metabolite of CBZ (10, 11 epoxide metabolite) to less toxic products. The authors found a slight reduction of fetal weights in both strains with the highest dose, as compared with pair-fed controls. Total maternal plasma CBZ levels were within the human therapeutic range, and there was no apparent maternal toxicity. The occurrence of

reduced fetal weight did not correlate with any difference in the levels of the parent compound or the epoxide metabolite formed in the two strains.

In the rat (El-Sayed, et al., 1983), CBZ at 85 mg/kg, administered by gastric intubation on days 7 to 19, significantly decreased fetal crown-rump length, but this was not observed at twice this dose. Incomplete ossification of the skeleton and other skeletal defects were also observed.

5. *Valproic Acid*

In vivo, VPA has been shown to decrease fetal weight in a dose-related manner in the rat, mouse and rhesus monkey, when administered for varying periods of time during embryogenesis (Eluma et al., 1984; Finnell et al., 1988; Hendrickx et al., 1988; Kao et al., 1981; Löscher et al., 1984; Mast et al., 1986; Nau, 1986b; Nau and Loscher, 1986; Ong et al., 1983; Paulson et al., 1985; Ritter et al., 1987; Vorhees, 1987b). Doses shown to reduce fetal size have ranged from 5 to 15 times the human therapeutic dose of 20 mg/kg, similar to those producing structural malformations. The higher doses used in these studies induced some degree of maternal toxicity in the dams (Mast et al., 1986; Nau and Loscher, 1986; Ong et al., 1983; Vorhees 1987b), but weight reduction was also observed at lower doses which did not affect the dams (Kao et al., 1981; Nau and Löscher, 1986; Vorhees, 1987b). Fetal weight was not reduced in the rabbit following daily oral doses of 50 to 350 mg/kg on days 6 to 18 (Petrere et al., 1986).

The extent of reduction in fetal weight has been shown to be positively correlated with dose (Nau et al., 1981b; Nau, 1985) and to be

dependent on the time, mode and frequency of administration (Eluma et al., 1984; Nau et al., 1981b; Nau and Spielmann, 1983; Nau, 1985, 1986b). In the mouse, Eluma et al. (1984) showed that the administration of 225 to 563 mg/kg/day of VPA by gastric intubation resulted in the greatest reduction of fetal weight following treatment in late embryogenesis (days 14 to 16), or when given throughout embryogenesis (days 8 to 16). Nau and Spielmann (1983) reported that, with comparable doses of VPA (300 mg/kg), daily subcutaneous injection during organogenesis resulted in increased embryolethality, fetal growth retardation, and an increased incidence of exencephaly (peak levels 450 µg/ml), while constant rate application, yielding therapeutic steady-state concentrations, also produced embryolethality and fetal weight retardation, but to a lower degree than with injection. In addition, there was no increased incidence of exencephaly with the latter mode of administration. In a comparison of the embryotoxicities of the parent compound and its metabolites, the 4-en -VPA metabolite was shown to induce fetal weight reduction to the same extent as the parent compound (Nau and Löscher, 1984).

In outbred mice, Chapman and Cutler (1984) noted that 160 to 180 mg/kg of VPA, given in drinking fluid throughout gestation and postnatally, had no effect on birth weight or postnatal weight gain. Mice exposed to VPA prenatally and postnatally tended to weigh more than control mice. Vorhees (1987b) noted no significant effects on postnatal weight gain, following VPA administration (150 to 200 mg/kg) to rats on days 7 to 18 of gestation.

In the monkey, Hendrickx et al. (1988) observed that the extent of intrauterine growth retardation was dose-dependent, when animals

were treated during all or part of organogenesis. At the lowest doses (20 to 75 mg/kg), no growth retardation was evident; at 100 to 150 mg/kg, a decrease in fetal weight, head circumference and brain weight were observed; and at 200 mg/kg, there was a decrease in head circumference, weight and length, with a reduction in brain and other organ weights.

Diaz and Shields (1981) showed that VPA (200 mg/kg) administered to neonatal rats resulted in a reduction of total brain and cerebellar weights, without a reduction in body weight, when caloric intake of the pups was controlled for.

Valproic acid has also been shown to induce dose-dependent growth retardation, including reduction of protein and DNA content, crown-rump and head lengths, when early rodent embryos were exposed to increasing concentrations of the drug, ranging from within to above the human therapeutic range (Bruckner et al., 1983; Kao et al. 1981.; Lewandowski et al., 1986; Naruse et al., 1988). Naruse et al. (1988) showed that the dose at which growth retardation occurred was dependent on the strain from which the embryos were derived. Kao et al. (1981) observed a diminution in growth at concentrations which did not affect the development of the yolk sac.

6. ***Ethosuximide***

Nau and Löscher (1986) found that ethosuximide in high doses (600 mg/kg), administered in the NMRI mouse subcutaneously on day 8, resulted in reduced fetal weights in the mouse. Sullivan and McElhatton (1977) did not observe a reduction in fetal weight in the CD-1 mouse, with doses of 60 to 360 mg/kg administered on days 6 to 16 of

pregnancy.

7. *Oxazolidinediones*

Fetal weights were found to be reduced following maternal intraperitoneal administration of trimethadione in the CD-1 mouse. The most sensitive embryonic period were days 8 to 10, with daily doses as little as 35/mg/kg resulting in reduced fetal size. In contrast, oral administration of the drug was not effective in reducing fetal weights (Brown et al., 1979).

In the rat, trimethadione reduced fetal weight and postnatal body weight, when administered in doses of 250 to 500 mg/kg by gavage, on gestational days 7 to 18 (Vorhees, 1983, 1985).

Fetal weight was not shown to be decreased in a small group of monkeys treated with paramethadione or trimethadione during embryogenesis, at doses one-third to two-thirds the maternal or embryolethal dose (Poswillo, 1972).

8. *Comparative Studies of Anticonvulsants*

In vivo, Sucheston et al. (1986) examined the relationsip between the degree of ossification of the skeleton and weight at birth in the CD-1 mouse, both of which have been used as parameters of growth. They found that 59 to 66% of the variability in weight is predicted by ossification measurements of the long bones. Carbamazepine had the least toxic effect on fetal body weight and ossification of long bones, followed by VPA, whereas PHT had the most severe effect.

Bruckner et al. (1983) found that PHT appeared to be more potent than VPA with respect to a dose-dependent decrease in protein and

DNA, observed in mouse embryos exposed to these drugs in vitro during early organogenesis. The reductions were noted at minimal concentrations of 15 and 35 µg/ml for each drug, respectively (within the human therapeutic ranges). At these concentrations, 10 to 14 % of the embryos showed yolk sac defects. Reductions in somite number, crown-rump length and yolk sac diameter occurred at toxic levels of these drugs (> 35 µg/ml and ≥150 µg/ml, respectively).

In vitro, VPA has been shown to induce a dose-dependent inhibition of neuronal process formation in dorsal root ganglia of the chick; the concentration which produced 50% inhibition of control values was 38µg/ml. The effect was similar to that seen with PHT, while PB and CBZ had a much less toxic effect (Dow and Riopelle, 1988). Using embryonic chick brain, Sedowofia and Clayton (1985) noted that PHT, more than VPA or PB, caused a dissociation of neuronal clusters resulting in cell death, and that all three drugs caused an alteration in biochemical markers of neurons and glia, such as the accumulation of GABA and the activity of carbonic anhydrase.

In the CD-1 mouse, Eluma et al. (1984) reported that CBZ was associated with a slight decrease in fetal weight, while the effects of PHT and VPA were more marked.

9. *Summary*

In summary , in vivo and in vitro experimental studies indicate that AED induce growth retardation, although non-specific effects from maternal toxicity may also be a contributing factor in in vivo studies. Carbamazepine appears to be less growth-inhibiting than PHT, PB or VPA. The growth disturbance appears to affect weight, length and brain,

although some studies indicate that the brain is affected without a reduction in body weight.

P. Mental Development

1. *Studies in Epileptic Patients*

The literature concerning mental development in the offspring of epileptic women is controversial, with the majority of studies showing an increased risk of developmental delay (Fujioka et al., 1984 ; Hanson et al., 1976; Hättig et al., 1987; Hill et al., 1974,1982; Jäger-Roman et al. 1982; Majewski et al., 1981; Nelson and Ellenberg, 1982; Nomura et al., 1984; Shapiro et al., 1976; Speidel and Meadow, 1972; Vert et al., 1982), while other studies show negative findings (Andermann, 1982b; Andermann et al., 1985; Gaily et al., 1988b; Granström, 1982). Causes for the developmental retardation are difficult to determinc and may be due to a number of factors, such as socioeconomic status, genetic predisposition, type and severity of the epilepsy, type and number of AED, mother/child interactions, and perinatal complications. That some studies have shown developmental retardation and others have not may be due to differences in these factors. Most studies of mental development in offspring of epileptic women do not appear to have been carried out blind to the exposure status of the child. Several longitudinal studies have looked at the developmental sequelae of small head circumference noted at birth.

There have been three reports from the Collaborative Perinatal Project concerning mental development in the offspring of epileptic women. Shapiro et al., (1976) compared offspring exposed to AED in utero and control offspring of nonepileptic mothers with respect to

mean scores of mental and motor development at 8 months, and mean IQ scores at 4 years of age. The comparisons were standardized for socioeconomic class, ethnic group and hospital of birth. Overall , the scores were lower among children born to epileptic mothers, but not in children born of epileptic fathers. Within maternal epilepsy , there was no significant difference in scores according to the number or type(s) of AED treatment. An unexposed epileptic group was not defined. Antenatal exposure to PB in nonepileptic women had no effect on the offsprings' scores.

Nelson and Ellenberg (1982) reclassified women in the Collaborative Perinatal Project according to new criteria for the diagnosis of a seizure disorder (See section II.M2a, Birth weight). An increased frequency of mental retardation (IQ < 70) was found in the offspring of women with a seizure disorder, as compared with offspring of women who never had a seizure. This difference remained after taking into account the presence of mental retardation or toxemia in the mother. Although treated epileptic women with a seizure disorder were of lower socioeconomic class than seizure free women, this was not taken into account. No differences were noted among seizure free women who did or did not receive PB, confirming the results of Shapiro et al., 1976.

Hanson et al. (1976) identified 104 offspring exposed to hydantoins with or without barbiturates from the Collaborative Perinatal Project and 100 control children matched for socioeconomic status, maternal age, race and institution of birth. He found significantly lower WISC full-scale performance scores at 7 years of age in the epileptic group. The mean IQ of the epileptic children was within the normal range (91.7). Eleven children were identified with multiple features of the

hydantoin syndrome, including microcephaly, prenatal and /or postnatal growth deficiency. One-half of the children showed reduced intellectual ability (IQ < 85).

Majewski et al (1981) ascertained epileptic mothers from outpatient medical records of university hospitals in West Germany. The parents and children (n=194) were those who complied with a request to come for clinical and psychological examinations, and therefore may be a selected group. Seven children with features of the hydantoin-barbiturate syndrome were identified, of whom 4 were mentally retarded (IQ< 85). Neurological and/or developmental abnormalities including mental retardation, without dysmorphic features, were twice as common in children of treated epileptic women (13.8%) as in those of untreated women (6.5%), while the prevalence of these abnormalities among offspring of epileptic males (2.8%) was not different from the general population. Among women taking AED, the abnormalities were higher in children of mothers who had seizures during pregnancy (16.5%) as compared with those who did not (5.7%).

Hill et al. (1974, 1982) followed the mental development of 59 infants and 252 controls, all from a middle to high socioeconomic class, who were ascertained prior to delivery at the Texas Medical Centre. Parental age and education were similar between the groups, however, no matching was done with respect to other parameters (smoking, alcohol, etc.). Psychological testing was performed without knowledge of exposure. Fourty-two of the children of epileptic mothers were exposed to two or more AED in utero, while the rest were exposed to PHT or PB alone.

In the epileptic group, there were four children who were

proportionately small at birth (length and/or weight and head circumference less than the 10th percentile). Three of these had major malformations. In two children who could be followed, head circumference was still small at 7 to 9 years of age, and IQ was 60 in one child and 112 in the other. In the control group, two children had reduced head circumference at birth, one of whom also had reduced weight and length. Head circumference of the growth retarded child later normalized, and the child had an IQ of 101 and a learning disability. The second child showed normal growth and an IQ of 127 at a later age.

Children of epileptic mothers had lower developmental scores than controls in the first three years of life. Infants who were exposed to PB and PHT or other multiple AED regimens had lower scores than those exposed to these drugs alone. Psychometric testing at an older age showed that 19% of the children of epileptic mothers had IQ's <90, as compared to 4% of the children of nonepileptic parents. Twenty-two percent of the children of epileptic mothers showed a language learning disability as compared to 8% of controls, although global IQ was >90. There were several factors which appeared to be related to the risk of developmental delay. These were intrauterine growth retardation, failure to thrive in the first few months of age, a major malformation, nine or more minor anomalies, and exposure to multiple AED in utero. It should be noted that the epileptic group also had a higher prenatal Hobel score (prenatal risk factors found to be predictive of perinatal morbidity and mortality in the general population) (Hill and Tennyson, 1982a) than did controls, but this was not studied in relation to pregnancy outcome in the epileptic group.

In a prospective study of children born to women who were ascertained from obstetric clinics in West Berlin, Jäger-Roman et al (1982) evaluated 47 offspring of treated epileptic mothers, 11 offspring of untreated epileptic mothers and 28 controls, with the Denver Developmental test at ages 3 to 36 months. They found evidence of a higher frequency of slight psychomotor retardation in offspring of treated epileptic mothers as compared with controls, which appeared to normalize with advancing age. The authors suggested that poor mother child interaction due to the mother's sedation might be a contributing factor to the psychomotor retardation.

Deblay et al. (1982) ascertained 80 infants who had been exposed to AED in utero (mostly PB with or without other AED) from consecutive admissions to a neonatal ward, and followed them longitudinally to evaluate their development. The majority were followed for 3 years or less although some were followed for up to 6 years. Twenty-six percent had developmental scores between 80 and 90 and 7.5% had scores less than 80. Sixty percent of the children who had small head circumferences at birth (less than two standard deviations below the mean) had scores less than 90, as compared with 25% of those whose head circumferences were within two standard deviations of the mean. Seventy-six to 87% of children with poor head growth and/or weight growth postnatally (less than or equal to two standard deviations below the mean) had scores less than 90.

Steinhausen and colleagues (Huth et al., 1982; Steinhausen et al., 1982) compared 19 children of treated epileptic mothers with 19 control children matched for age (4-10 years), sex, and socioeconomic status, ascertained from records of the Department of Obstetrics at the

Free University of Berlin. The epileptic children were found to have normal psychological functioning , but hyperactivity was increased. In a later longitudinal study (Hättig et al., 1987), Bayley Mental Scales were administered at the age of 18 months. Children exposed to AED polytherapy had lower scores than those exposed to monotherapy. The scores were not associated with the occurrence of maternal seizures during pregnancy. In the monotherapy group, only children exposed to VPA monotherapy had significantly lower scores than controls, matched on a number of variables including socioeconomic status and maternal smoking.

Two Japanese studies have found evidence for developmental retardation in children exposed to AED in utero (Fujioka et al., 1984 ; Nomura et al.1984). Fujioka et al. (1984) investigated 45 infants exposed to AED in utero and unmatched controls. The children of epileptic women were exposed to polytherapy (2 to 5 drugs) in most cases. Psychomotor retardation was more evident in older children (greater than 24 months) than in younger children. Developmental quotients particularly for social and linguistic abilities were lower in children born to women with partial seizures, than in those with generalized seizures. Scores for speech and /or language understanding were lower in offspring of women who had partial seizures during pregnancy than in those who did not, and in those with high drug "scores" (based on the number and dosage of AED). A positive correlation was noted between speech development and head circumference at birth but not with birth weight. When developmental quotients were stratified by maternal education, differences between the epileptic and control groups were noted for children of women who went to junior high or

high school, but not in those who went to university or college. Many of the epileptic mothers were noted to be negligent in the care of their children. These results suggested complex interrelationships among a variety of factors which were associated with developmental outcome, but the independent association of the factors with outcome was not determined.

At the Hirosaki University Hospital, Nomura et al (1984) followed 21 infants exposed to AED in utero and unmatched controls, from 3 months to 3 years. Head circumferences in the offspring of epileptic women were smaller than in controls in the first 3 postnatal months. Length was reduced from 3 months to 18 months of age. There was a higher prevalence of fetal distress, neonatal asphyxia and neurological abnormalities, including hypotonia, in the neonatal period in the epileptic as compared to the control group. Developmental quotients for motor development; manipulation/exploration; socialization; feeding; toilet raining; daily routine; and understanding/language were lower in the epileptic group at 18 to 30 months. The scores had normalized by three years of age, except for motor development and feeding/toilet training/daily routine. The frequency of neurological abnormalites and the developmental quotients were positively correlated with the "drug scores". The authors found that children exposed to AED, who showed hypotonia or had decreased head circumference at birth or in the early neonatal period, had lower developmental quotients than did controls.

Beck-Mannagetta and Janz (1982) assessed developmental status by interviewing the parents and found an increased prevalence of psychomotor retardation (8.8%) and neurological abnormalities, such as cerebral palsy (1.8%), in children of epileptic mothers as compared to

children of epileptic fathers (3.8%, 0.5%). The increased prevalence of retardation in offspring of epileptic mothers was associated with the presence of partial seizures and perinatal complications. There was no indication that there were any differences in mental development at school age.

Andermann, Dansky and colleagues (Andermann, 1982b; Andermann et al., 1985) did not find evidence of developmental delay in 50 offspring of treated epileptic mothers, tested at ages 3 to 30 months with the Griffith Developmental Scale. The mothers of the offspring had been followed prospectively during pregnancy. There was no significant correlation of the scores with head circumference. Only in the older groups (3 to 5 years), was there a suggestion of impairment in visual-motor coordination and practical reasoning.

In the Finnish regional-based prospective study, Granström (1982) evaluated 66 children exposed to AED and 66 controls born in the same obstetric department with the Denver Developmental Screening Test at 18 months of age. The obstetric department provided services to the majority of women in the Uusimaa District (Hiilesmaa, 1982). The children of epileptics achieved lower scores on two sub-items only. In a follow-up of the study, Gaily et al. (1988b) assessed the intelligence of 121 children of epileptic women and 105 control children at 5.5 years. Seventy-five percent of the women with epilepsy in the Uusimaa District were included in the study. About 50% of the women were on monotherapy during pregnancy, 14% took no drugs, and the remainder were on drug combinations, usually involving 2 drugs. They found that the prevalence of mental deficiency and of borderline intelligence was not very different from the general population. The greatest differences

between epileptics and controls were in the lower social class, which also had the highest prevalence of women with mental deficiency. There was no difference in intelligence scores between those on polytherapy or monotherapy, those with relatively high versus low plasma AED levels, or between women with or without generalized seizures. There was no association between typical minor anomalies usually associated with AED exposure and intelligence quotients. The authors concluded that their population-based study may provide a better estimate of the overall risk of mental deficiency, than other more selected clinic-based studies.

2. ***Experimental Animal Studies***

Alterations in activity, neurological function and intelligence-related behavior have been described in mice and rats exposed prenatally to PHT (Elmazar and Sullivan, 1981; Mullenix, et al., 1983; Vorhees,1983, 1985,1986,1987a); PB (Fishman and Yanai, 1983; Renisch and Sanders, 1982; Vorhees,1983,1985); or trimethadione (Vorhees,1983, 1985). In the rat model of the fetal hydantoin syndrome, functional disturbances in the offspring were evident at maternal PHT concentrations in the human therapeutic range (Elmazar and Sullivan, 1981; Vorhees, 1986, 1987a). Although functional deficits were noted at doses which induced a reduction in body weight, brain weight or brain protein (Elmazar and Sullivan, 1981; Mullenix et al., 1983; Vorhees, 1983,1985), they also occurred without such effects on growth (Vorhees, 1986, 1987a). At maximum doses which did not produce gross structural defects, PHT appeared to have the greatest behavioral teratogenic effect, followed by trimethadione and then PB (Vorhees,

1985). The effects were stage and dose-dependent, with the greatest period of vulnerability during the period of organogenesis (Vorhees, 1986, 1987a).

Various behavioral studies of PHT and PB suggest that there may be differential effects on males and females (Fishman and Yanai, 1983; Mullenix et al., 1983; Renisch and Sanders,1982), and it has been postulated that such findings may be related to the effects of AED on sex steroid metabolism. It has also been suggested that drug-induced behavioral alterations may be mediated by changes in the development of neurotransmitter systems (Lauder and Krebs, 1984; Leonard, 1982; Seth et al., 1986; Yanai et al., 1982).

Q. Other Pregnancy Outcomes in Epileptic Women

1. *Spontaneous Abortion*

a. Human Studies

In general, it is difficult to estimate the number of spontaneous abortions because many early abortions are missed. Without biochemical tests, the only sign of an early pregnancy resulting in spontaneous abortion may be a late menstrual period. There are also difficulties in the way studies of pregnancy outcome in epileptic women have been carried out. Most of the studies citing the frequency of spontaneous abortions in epileptic women have been historical cohort studies, relying on patient recall in addition to medical records of the events. In prospective cohort studies, women ascertained during pregnancy may have entered the study later than the first trimester, when most spontaneous abortions occur. The vast majority of historical and prospective studies reporting the frequency of spontaneous abortions in epileptic and nonepileptic women have not taken into account the time of recognition of the pregnancy and of the occurrence of spontaneous abortion. This may result in potential bias, as epileptic women who are pregnant may be followed earlier in pregnancy than nonepileptic women.

Studies surveying spontaneous abortion in epileptic women (number of pregnancies ranging from 33 to 788) have reported frequencies of 4.2% to 22% (Akhtar and Millac, 1987; Andermann et al., 1982b; Annegers et al., 1989; Bossi et al., 1980; Dansky et al., 1980, 1982a; Knight and Rhind, 1975; Koch et al., 1982a, Lindhout et al., 1982; Martin,1978; Meyer,1973; Nakane et al., 1980; Nelson and Ellenberg, 1982; Pardi et al., 1982, Satish and Kalyanaraman, 1984; Speidel and

Meadow, 1972; Starreveld-Zimmerman,1974; Weber et al., 1977). The high frequencies of spontaneous aborton among epileptic women (19-22%) (Martin, 1978; Starreveld-Zimmerman et al., 1974) reported in some uncontrolled historical cohort studies may not be excessive in view of the fact that the upper estimate of recognized spontaneous abortion in the general population is about 20%. (Huisjes, 1984).

An historical cohort study by Dansky and colleagues (Andermann et al., 1982b; Dansky et al., 1980,1982a) did not find an increased frequency of spontaneous abortion among 182 pregnancies of epileptic women (12.6%) as compared with 114 pregnancies in spouses of epileptic men (14.2%). Annegers and colleagues (1989) reported the results of a detailed historical cohort study of spontaneous abortion rates in pregnancies of women with epilepsy and of spouses of epileptic males, using life-table methodology to compute gestational age-specific rates. They did not find an association between spontaneous abortion and maternal epilepsy or AED therapy. Other studies citing control or general population figures have also not found an elevated frequency of spontaneous abortion in epileptic women (Andermann et al., 1982b; Bossi et al., 1980; Dansky et al., 1980,1982a; Hiilesmaa et al.,1982a; Knight and Rhind,1975; Koch et al., 1982a; Nelson and Ellenberg, 1982; Speidel and Meadow, 1972; Teramo and Hiilesmaa, 1982; Weber et al., 1977).

Several historical and prospective cohort studies have not found an increased frequency of spontaneous abortions in epileptic women taking AED during pregnancy, as compared with untreated epileptic women, spouses of epileptic men or nonepileptic controls (Annegers et al., 1989; Andermann et al., 1982b; Dansky et al., 1982a; Koch et al., 1982a;

Martin,1978; Meyer,1973; Speidel and Meadow, 1972; Starreveld-Zimmerman et al., 1974). However, an historical cohort study by Dansky and colleagues (Andermann et al., 1982b; Dansky et al., 1982a) showed a trend towards a higher risk of spontaneous abortion among 114 pregnancies of treated epileptic women (22.8%), as compared with that among 50 pregnancies of untreated epileptic women (10.0%). Similar results were shown by Satish and Kalyanaraman (1984). As well, Ogawa et al. (1984) reported a higher proportion of epileptic women on AED with a history of previous spontaneous abortion as compared with nonepileptic women.

More detailed prospective studies of spontaneous abortion rates in epileptic women on AED could provide more information on the full spectrum of possible embryotoxic effects associated with maternal epilepsy and AED, that is of defects which are not compatible with life.

b. *Animal Studies*

Most AED tested have been shown to result in an increased frequency of embryolethality to varying extents, when given in sufficiently high doses. In rodents, the incidence of embryolethality has been shown to increase with increasing doses of PHT (Gibson and Becker, 1968a; El-Sayed et al., 1983; Fritz et al., 1976; Finnell, 1981; Harbison and Becker, 1969, 1972; McClain and Langhoff, 1980; Skalko et al., 1984; Tanaka et al., 1984). A number of these studies showed an increase in embryolethal effects only at doses which produced toxic effects in the mother (Gibson and Becker,1968a; El-Sayed et al., 1983; Fritz et al., 1976; McLain and Langhoff, 1980; Skalko et al., 1984). Other studies indicated that plasma levels were within the human therapeutic range

and/or that there were no toxic effects in the mother (Finnell, 1981; Tanaka et al., 1984). Gibson and Becker (1968a) demonstrated strain and time differences in the susceptibility to resorptions. Oral and subcutaneous administration resulted in lower resorption rates than did intraperitoneal injection (Harbison and Becker, 1969).

In mice, Olivecrona (1964) reported a high degree of embryolethal effects when PB was administered intraperitoneally at doses which induced maternal sedation. An increased incidence of resorptions was not noted in mice administered PB, at doses which did not induce maternal toxicity (Finnell et al., 1987b).

Primidone has generally not been shown to produce embryolethal effects in mice (Sullivan and McElhatton 1975,1977; McElhatton et al., 1977).

In several mouse studies, CBZ was not found to induce an increase in resorptions or fetal deaths (Fritz et al., 1976; Paulson et al., 1979; Sullivan and McElhatton, 1977; Wray et al., 1982), while one study (Finnell et al., 1986) reported a dose-dependent increase in resorptions, indicative of early embryonic loss. This study used pair-fed controls and showed that plasma CBZ levels were within the human therapeutic range.

The dose-dependent embryolethal effects of VPA have been well documented (Finnell et al., 1988; Hendrickx et al., 1988; Kola and Folb, 1986; Mast et al., 1986; Nau et al., 1981b; Ong et al., 1983; Paulson et al., 1985; Petrere et al.,1986; Vorhees, 1987). In some studies, the effects appeared to occur at doses which induced maternal sedation, weight loss or death (Finnell et al., 1988 ; Ong et al., 1983, Petrere et al., 1986, Vorhees, 1987). Nau et al. (1981b) showed that application of

VPA via implanted minipumps, yielded therapeutic steady state concentrations and an increase in embryolethality, but to a lower degree than with subcutaneous injection once daily, which yielded higher fluctuating plasma concentrations.

In the CD-1 mouse, Eluma et al. (1984) showed that an increase in fetal deaths was dependent on dose, time of administration and type of AED administered, whereas an increase in resorptions was only time dependent. Carbamazepine was less embryolethal than VPA or PHT. In vitro, Bruckner et al. (1983) demonstrated dose-dependent embryolethality for PHT and VPA. Phenytoin was embryolethal only at highly toxic doses, whereas VPA produced these effects at levels within the therapeutic range.

2. ***Perinatal Death***

Many authors have reported an increased risk of perinatal mortality (defined as stillbirths and neonatal deaths occurring within the first week of life) in the offspring of epileptic women. This has usually ranged from 2 to 3 times higher than in nonepileptic controls (Akhtar and Millac, 1987; Andermann et al.,1982b; Bjerkedal and Bahna,1973; Dansky et al., 1980, 1982a; Fedrick,1973; Gopfert-Geyer et al., 1982; Higgins and Comerford, 1974; Hillesmaa, 1982; Hillesmaa et al., 1982a, 1985; Hill and Tennyson, 1982a; Kallen, 1986; Knight and Rhind, 1975; Koch et al., 1983; Monson et al.,1973; Nelson and Ellenberg, 1982; Niswander and Gordon, 1972; Sabin and Oxorn, 1956; Satish and Kalyanaraman, 1984; Speidel and Meadow,1972; Starreveld-Zimmerman et al., 1974; Weber et al.,1977; Yerby et al., 1985), although a smaller relative risk of 1.2 was noted in a large historical cohort study of 3773

births to epileptic mothers in Norway (Bjerkedal,1982a). The excess of perinatal deaths associated with maternal epilepsy persisted when the influence of socioeconomic class was taken into account in some studies (Fedrick,1973; Gopfert-Geyer et al., 1982; Hiilesmaa, 1982; Hiilesmaa et al., 1982a, 1985; Koch et al., 1983).

Findings cited in association with 96 perinatal deaths reported in the literature have included fetal factors, such as congenital malformations (n=20) (most frequently congenital heart disease or central nervous system anomalies); prenatal and intrapartum obstetric complications (n=9) (most frequently infection of fetal membranes); spontaneous hemorrhage (n=8); prematurity (n=5); serious maternal illness (n=5); placentopathies (n=11) (most frequently abruptio placenta), maternal grand mal seizures (n=3); and other complications (n=2). Details were not given in 33 cases (Cvetko,1969; Fedrick, 1973; Göpfert-Geyer et al., 1982; Higgins and Comerford, 1974; Hiilesmaa et al., 1985; Hill and Tennyson, 1982 a,b; Knight and Rhind, 1975; Koch et al., 1983; Lindhout et al., 1982, 1985b; Loiseau et al.,1974; Maroni and Markoff, 1969; Millar and Nevin, 1973; Nelson and Ellenberg, 1982; Sabin and Oxorn, 1956; Speidel and Meadow, 1972; Starreveld-Zimmerman et al., 1974)

In three historical cohort studies of viable births to epileptic women receiving AED (number of births ranging from 141 to 550), the frequency of perinatal deaths (2.2 to 4.3%) was higher than that among a small number of births to untreated epileptic women (0-1.1% among 56 to 93 births) (Annegers et al., 1974; Kallen, 1986; Speidel and Meadow, 1972). Kallen (1986) found a higher prevalence of perinatal deaths in offspring of women who took polytherapy as compared with those who

took monotherapy, but mothers on monotherapy were also younger than those on polytherapy.

In a study based on the data of the U.S. Collaborative Perinatal Project, Nelson and Ellenberg (1982) reported a higher relative frequency of stillbirths among women with seizures during pregnancy (5.1%) than among women with a current seizure disorder, but with no seizures during pregnancy (2.4%); however, there was no clear relationship to AED treatment. In contrast, among women without a history of convulsive disorder, the frequency of stillbirths was significantly elevated among women who were taking PB for indications other than toxemia, as compared to untreated women.

In an historical cohort study by Dansky and colleagues, perinatal deaths were increased in 155 offspring of epileptic women (6.0%), as compared to 98 offspring of epileptic men (1.0%), and to the general population, but this was not correlated with maternal AED during pregnancy (Dansky et al., 1982a).

Hiilesmaa and colleagues (1985) did not find a relationship between the type or plasma levels of AED and the occurrence of perinatal deaths. Miyakoshi and Seino (1984) did not find an association between the occurrence of spontaneous abortions and stillbirths considered together, and the type of epilepsy, duration of AED therapy, number of AED or the total "drug score", based on the number and dosage of AED.

3. *Infant Mortality*

Annegers and associates (1974) found that postneonatal deaths (from one month to one year of age) occurred more frequently among treated epileptic women than among untreated epileptic women, or women

without a convulsive disorder. All of these were attributed to congenital malformations. Bjerkedal showed a 21% increase in infant deaths (from one week to one year of age), as well as an increase in deaths beyond one year of age, in epileptic women as compared with nonepileptic controls (matched for parity and number of multiple births, but not socioeconomic class) (Bjerkedal and Bahna, 1973; Bjerkedal, 1982a; Egenaes,1982). No explanation was given for this increase in mortality.

4. *Prematurity*

Most studies have not found an increase in premature labor (≤ 37 weeks of gestation) in offspring of epileptic women (Akhtar and Millac, 1987; Andermann et al., 1982b; Fedrick, 1973; Göpfert-Geyer et al., 1982; Hillesmaa et al., 1985; Janz and Beck-Mannagetta, 1982b; Kallen, 1986; Koch et al., 1983; Monson et al., 1973; Niswander and Gordon, 1972; Ogawa et al., 1982,1984; Robertson et al., 1986; Speidel and Meadow, 1972; Watson and Spellacy, 1971; Weber et al., 1977, Yerby et al., 1985). However, Bjerkedal (1982a) showed a small increased risk of prematurity (10.8%) in a large series of offspring of epileptic women as compared with matched controls (9.6%), ascertained from the Norwegian Birth Registry. Svigos (1984) found an increased risk of premature labor and rupture of the membranes in epileptic women (10.7%) as compared to controls (4%) of similar age, parity, and socioeconomic status.

5. *Other Findings in the Newborn*

An increased risk of spontaneous hemorrhage has been reported in neonates of epileptic women who took AED during pregnancy. This has been due to a decreased level of vitamin K-dependent clotting factors in the newborn induced by AED (Mountain et al., 1970; Vert et al., 1979). Montouris (1979) suggested administration of vitamin K to the newborn infant, followed by clotting studies.

The newborn infant may manifest a drug withdrawal syndrome postnatally. Symptoms include hyperirritability, tremor, jitteriness, vomiting, poor sucking, and sleep disturbances (Bossi, 1982; Bossi et al.,1980, 1982b; Kaneko et al., 1982b; Vert et al., 1979). These abnormalities may be due to the decrease in the concentration of AED postnatally, or to the direct effects of the drugs and/or seizures on the fetal brain. Koch et al. (1985) observed sedation and hyperexcitability with other symptoms of withdrawal more often in infants exposed to AED in utero than in controls, matched for socioeconomic class, parental age, parity, institution of birth and maternal smoking. The presence of the symptoms and the time of their appearance did not appear to depend on the type of AED, the concentration of AED in cord blood, nor on the rate of disappearance of the drug from the infant's plasma.

Although some studies have reported an increase in neonatal asphyxia in offspring of epileptic women (Bjerkedal and Bahna, 1973; Fedrick, 1973; Janz and Beck-Mannagetta, 1982b; Yerby et al., 1985), others have not found a difference in apgar scores between children of epileptic women and controls (Bossi et al., 1980; Göpfert-Geyer et al, 1982; Hillesmaa et al., 1985; Watson and Spellacy, 1971).

R. Putative Mechanisms For the Teratogenic Effects of Anticonvulsants

A number of hypotheses for the potential teratogenic effects of AED have been postulated, and there has been much testing of these various hypotheses, mostly in experimental models, in recent years. Some of the postulated mechanisms are listed in Table II.10. In general, the mechanisms involve direct effects of the drug and/or its metabolites on the embryo and fetus or indirect effects, such as drug-induced metabolic changes. These putative mechanisms will be discussed in more detail below.

1. *Toxic Metabolites of Anticonvulsants*

Much attention has been directed towards determining whether the ultimate teratogens of AED are the parent compounds or their metabolites, particularly reactive arene oxide metabolites. Most AED undergo oxidation by the hepatic cytochrome P-450 monooxygenase system (Eadie and Tyrer, 1980). Epoxides (or arene oxides which are epoxides formed from aromatic compounds) are presumptive intermediates in the oxidation of most aromatic rings to dihydrodiols. Evidence for the formation of arene oxide intermediates of AED is indirect due to the fact that the metabolites are short-lived, and includes the detection of dihydrodiol metabolites found in the urine of rodents, monkeys, humans and the human neonate. The unstable reactive intermediates are capable of covalently binding to DNA, proteins and other macromolecules and have been implicated in drug toxicity, mutagenesis, carcinogenesis, and teratogenesis. Major pathways for the

Table II.10

Putative Mechanisms For the Teratogenic Effects Associated with Anticonvulsant Therapy

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Metabolism of AED to reactive intermediates

Folate metabolism

Hormonal effects (T_3, T_4, TSH, thyrotropin; corticosteroids, insulin, sex steroids)

Heavy metals (e.g. Zinc)

Bone metabolism (vitamin D, vitamin K, calcium)

Placental changes

- Endocrine
- Vascular

Maternal hypoxia

Alteration of DNA, protein synthesis

Embryonic intracellular pH

detoxification of epoxides are conversion to dihydrodiol, catalyzed by the enzyme epoxide hydrolase, or conjugation with glutathione, catalyzed by glutathione-s-transferase. It is thought that the extent of covalent binding and resultant effects on fetal outcome depend on the relative extent of bioactivation of AED to reactive metabolites, catalyzed by the cytochrome P-450 - dependent monooxygenase system, and of detoxification by enzyme systems such as epoxide hydrolase (Jerina and Daly, 1974; Juchau, 1981; Spielberg,1982; Wells and Harbison, 1980).

It is postulated that the putative arene oxide metabolites implicated in AED teratogenesis are of maternal, placental and/or fetal origin (Martz et al., 1977; Wells and Harbison, 1980). The human embryo and fetus have been shown to be capable of drug metabolism, although, in general, enzyme activity is lower than in the adult (Juchau,1981). The early rat embryo has been shown to have cytochrome P-450 enzymes, implying that potentially toxic intermediates may be formed at critical periods of embryonic development (Brown et al, 1986a,b; Flint and Brown, 1987).

a. *Phenytoin and Phenobarbital*

A woman with epilepsy and a family history of twinning who took PHT during pregnancy gave birth to heteropaternal dizygotic twins (Phelan et al., 1982). One twin presented with the full spectrum of the "fetal hydantoin syndrome", while the other was only mildly affected. Studies of the activity of epoxide hydrolase were carried out in a double blind manner on fibroblasts derived from the mother and twins (Buehler,

1984). Epoxide hydrolase activity was lowest in the severely affected twin, intermediate in the mother, and highest in the mildly affected twin. The data suggested that three levels of epoxide hydrolase activity might be found in humans.

In later studies, Buehler (1986) found that epoxide hydrolase activity in fibroblasts and amniocytes correlated with the presence of features of the fetal hydantoin syndrome: individuals with the syndrome had low enzyme activity whereas those without clinical features had higher levels.

Strickler, Dansky and colleagues (Dansky et al., 1987b; Strickler et al., 1985) correlated the presence or absence of major and/or minor congenital anomalies and newborn growth parameters in 24 children, who had been exposed to PHT in utero, with the capacity to detoxify PHT metabolites. Lymphocytes drived from the children and their parents were exposed in vitro to PHT metabolites, generated by a murine microsomal enzyme system. This in vitro assay provided an indirect measure of epoxide hydrolase activity: lymphocytes with the lower epoxide hydrolase activity would be more susceptible to cell death (positive response) than lymphocytes with higher enzyme activity (no significant increase in cell death = negative response). Twelve of 14 children with positive assays had one or more major birth defects, while only 2 of 10 children with negative assays were affected. In particular, cardiac malformations and microcephaly occurred significantly more often among positive responders than among negative responders. In contrast, birth weight and length did not differ between the two groups. At least one of the parents of children with a positive

assay was also a positive responder, and there was no difference according to sex of the parent. This suggests that the metabolism of the affected offspring may have been more important than maternal metabolism of PHT. The extent of the detoxification impairment in malformed children and their parents was comparable to that seen in parents of patients with PHT hepatotoxicity (an intermediate range of toxicity). The findings suggested autosomal co-dominant inheritance of the detoxification defect. Minor anomalies such as craniofacial defects were not associated with detoxification defects, suggesting that the etiology of minor defects may be different from that for major defects. However, the lack of correlation with minor anomalies may have been due to the greater degree of subjectivity in the ascertainment of these defects than with major congenital malformations.

In the mouse (Finnell and Chernoff,1987; Finnell et al., 1987b), epoxide hydrolase levels in three inbred mouse strains were negatively correlated with the incidence of PHT-induced fetal abnormalities in each strain. However, the strain with the highest enzymatic activity still had a 14-fold increase in abnormal fetuses as compared with controls. In contrast to the findings for PHT, there was no relationship between epoxide hydrolase activity and strain sensitivity to the teratogenic effects of PB (Finnell et al., 1987b). Thus epoxide hydrolase activity may not have been the only factor influencing the response to in utero exposure to PHT and PB. The unchanged drugs may also have played a role.

A number of animal studies have attempted to determine whether PHT, the parent drug, or one of its metabolites, particularly those

involving an arene oxide intermediate, is responsible for PHT-induced abnormal growth and morphogenesis (Barcellona et al., 1987 ; Flint and Orton, 1984; Harbison and Becker, 1970, 1974; Lum and Wells, 1986 ; Martz et al., 1977; Wells et al., 1982; Wong and Wells, 1988). These studies have either administered metabolites directly to pregnant dams or to embryos in vitro, or have utilized inhibitors or inducers of PHT metabolism, in order to alter the relative concentrations of the parent compound and its metabolites.

Harbison and Becker (1974) showed that PHT, the parent compound, but not some of its metabolites, diphenylhydantoic acid; 5-(*p*-hydroxyphenyl)-5-phenylhydantoin; α-aminodiphenylacetic acid, significantly increased the incidence of orofacial anomalies and affected fetal growth. The metabolites were less embryolethal than PHT. These results were taken as evidence for the parent compound as the ultimate teratogen. However, the parent compound is metabolized through an arene oxide intermediate, while none of the metabolites tested are further metabolized to the arene oxide stage (Hartsfield, 1986).

The results of studies employing inducers or inhibitors of PHT metabolism have provided conflicting evidence for the role of either the parent drug or its arene oxide metabolite. These findings may reflect the fact that when inducers or inhibitors of metabolism are administered they affect both rates of formation of reactive intermediates and rates of inactivation of these intermediates; thus the net effect on reactive intermediates may be difficult to predict (Juchau, 1981). For example, initial studies showed that pretreatment with PB

which enhanced PHT metabolism antagonized PHT-induced teratogenicity, whereas pretreatment with SKF 525A, an inhibitor of PHT metabolism, potentiated the teratogenic effects (Harbison and Becker, 1970). These results were interpreted as evidence for the teratogenic role of the parent compound as opposed to metabolites. However, PB not only induces the cytochrome P-450 monooxygenase, but also the liver microsomal epoxide hydrolase (Oesch, 1976). It is therefore difficult to correlate specific metabolic changes with observed effects in the offspring. Alternatively, evidence for both the parent compound and its metabolite may indicate that the etiology of PHT-induced abnormalities is multifactorial.

In an in vitro sytem utilizing rat embryo midbrain and limb bud cells, Flint and Orton (1984) found that PHT caused a dose-dependent inhibition of differentiation, with minimum concentrations at the upper limit of the human therapeutic range. Addition of metabolizing enzymes did not increase toxicity, thus suggesting that the formation of toxic metabolites was not necessary for the induction of adverse effects. However, biotransformation to toxic metabolites by the cells themselves could not be ruled out, as cytochrome P-450 metabolizing enzymes were detected in rat embryos (Brown et al., 1986a,b). In vitro, the hydroxy metabolites of PHT (*p*-hydroxyphenytoin and *m*-hydoxyphenytoin) were found to be of similar toxicity to PHT (Brown et al., 1986b). Co-incubation with various inhibitors of cytochrome P-450 activity enhanced the toxicity observed with PHT alone, while co-incubation with TCPO (Trichloropropene oxide, an epoxide hydrolase inhibitor) did not alter PHT-induced toxicity (Brown et al., 1986a,b).

These results suggested that PHT is the more potent teratogen in vitro, but that the hydroxy metabolites also pose a risk.

Some of these studies suggest that there may be a dissociation between resorptions, structural malformations and fetal growth retardation with respect to the ultimate teratogen responsible for their occurrence. For example, in one study (Lum and Wells, 1986), pretreatment wih acetaminophen was used as a means to inhibit detoxification of the PHT arene oxide. Acetaminophen depletes glutathione within cells, which is postulated to conjugate with the arene oxide metabolite. Combined treatment of acetaminophen and PHT increased the incidence of fetal resorptions, cleft palate and postpartum mortality as compared to treatment with PHT alone. In contrast, there was no significant change in fetal weight in the acetaminophen + PHT group as compared to the PHT only group.

Martz and co-workers (1977) noted a significant potentiation of PHT-induced cleft lip and palate following injection of TCPO into random bred Swiss mice, treated with a single subcutaneous injection of PHT on day 11. This was associated with increased covalent binding of labeled PHT to fetal tissue which was interpreted to be binding of arene oxide metabolites. In an inbred mouse strain (C57BL/6J), Hartsfield (1986) found that chronic oral treatment with PHT prior to and during gestation, yielding therapeutic plasma levels, resulted in decreased fetal weight and length, cranial measurements and tibial length, but did not result in major malformations. Treatment with TCPO + PHT did not potentiate the reduction in growth measurements. The author concluded that the effect on growth and development from in

utero exposure to PHT did not appear to be related to the activity of epoxide hydrolase, suggesting that there may be different mechanisms for the different expressions of PHT teratogenicity. The results of the study by Martz et al. (1977) indicated that major malformations appear to be related to the arene oxide metabolite, whereas impaired growth and development, as found in the Hartsfield study (1986), might be related to levels of the unchanged drug or some other mechanism.

In studies by Hansen and Hodes (1983a, 1983b), A/J mice (susceptible strain) exhibited an increased incidence of resorptions, cleft lip with or without cleft palate, and decreased fetal weight and length following exposure to PHT in utero (intraperitoneal injection on days 10,11,12). C57BL/6J mice, on the other hand, were resistant to the effects of PHT. In reciprocal crosses between these two strains, an increased incidence of orofacial clefts in the offspring was observed when the mother was from the susceptible strain, but not when the mother was from the resistant strain. On the other hand, fetal measurements were not decreased in any of the crosses. The maternal effect observed was not found to be attributable to differences in the maternal hepatic production of metabolites involving intermediary arene oxides (e.g dihydrodiol). In contrast to these findings, Brown et al. (1985) did not find evidence for a maternal effect in reciprocal crosses between two other susceptible (CL/Fr) and resistant (Star) mouse strains.

b. *Carbamazepine*

Epoxide intermediates are formed during the biotransformation of

CBZ. One of these is a stable epoxide metabolite (10, 11- epoxide), but reactive epoxide metabolites may also be formed. Lindhout and colleagues (1984,1987) observed a high risk of congenital malformations in children of mothers who took a combination of CBZ + VPA + PB ± PHT (58%). This combination was associated with a high CBZ-10, 11-epoxide/ CBZ serum concentration ratio (which appeared to be due to metabolic interaction), and hyponatremia in the patients. In order to elucidate possible mechanisms for the high risk of congenital abnormalities in children of mothers taking these AED, whole rat embryos were exposed to sera from patients and from nonepileptic controls. Abnormal neural tube closure was found to correlate with hyponatremia and with an increase in the CBZ-10, 11-epoxide/ CBZ serum concentration ratio but not with CBZ or CBZ-10,11-epoxide levels. The authors hypothesized that the correlation with the ratio and not with the individual drugs might indicate that the ratio is a proxy for some other teratogenic agent, such as other short-lived CBZ epoxides, which also might be increased due to metabolic interaction. Kerr and associates (1989) showed that VPA at therapeutic concentrations inhibited human liver microsomal epoxide hydrolase and the conversion of CBZ-10,11-epoxide to the dihydrodiol. Induction of the metabolism of VPA to reactive metabolites [e.g., 2-propyl-4-pentanoic acid (4-en-VPA)] by other AED such as PB is also another possible mechanism (Kaneko et al., 1988).

In a murine model, Finnell et al. (1986) found that there was no significant relationship between the levels of CBZ or its epoxide metabolite and the incidence of fetal abnormality, in agreement with

the findings of Lindhout and colleagues (1987). There was also no strain difference in the incidence of abnormality, despite variation in the levels of epoxide hydrolase in different strains.

c. Valproic Acid

There is evidence that both VPA and some of its metabolites, particularly 4-en-VPA, may account for the teratogenic effects induced by this drug (Di Carlo et al., 1986; Klug et al., 1985; Nau and Löscher, 1986; Rettie et al., 1986). The teratogenicity of these compounds is associated with particular structural features (Nau and Loscher, 1986). The active metabolite 4-en-VPA can be biotransformed into reactive intermediates capable of binding to cellular constituents, and has been strongly implicated in hepatotoxicity associated with VPA. Whether or not the mechanism for liver toxicity and teratogenesis are similar is not known (Di Carlo et al., 1986).

2. *Folate Metabolism*

As the active derivatives of folic acid are intimately involved in the synthesis of nucleic acid and proteins, folic acid deficiency in pregnancy may especially affect rapidly growing trophoblastic and embryonal tissues. Meadow (1968), in England in the late 1960's, first speculated that AED might cause congenital malformations by inducing folate deficiency. This hypothesis has been suggested by studies showing a reduction in serum and red cell folate levels in patients on long term AED treatment (Baylis et al., 1971; Dellaportas et al., 1982; del Ser et al., 1983; Houben et al., 1971; Krause et al., 1982;

Muñoz-Garcia et al., 1983; Reizenstein and Lund, 1973; Reynolds et al., 1972), and by studies implicating folate deficiency as a risk factor for congenital defects in the nonepileptic population (Hibbard, 1975; Meadow, 1979; Smithells et al., 1976) In addition, experimental studies have shown that folate deficiency produces adverse pregnancy outcome (Jordan et al., 1977; Kinney and Morse, 1964; Nelson et al., 1955,1956; Skalko and Gold, 1974).

a. *Folate in Relation to Anticonvulsants*

The frequency of subnormal serum folate levels in outpatient epileptics receiving AED has varied from 8-76%, presumably due to the definition of deficiency, duration and type of AED regimen, and dietary background (Dellaportas et al., 1982; Klipstein, 1964; Krause et al., 1982; Muñoz-Garcia et al., 1983). Occasionally, chronic treatment with AED can result in megaloblastic anemia. Berg et al. (1988) showed an early decrease of serum folates in healthy male volunteers administered PHT. PHT, PB, PRM, VPA, and CBZ have all been shown to result in folate depletion in animals (Carl et al. , 1987; Carl and Smith,1983, 1984; Smith and Carl , 1982). In man , the antifolate effects of PHT, PB, and PRM are well established, while there is less data concerning CBZ and VPA. Hendel and colleagues (1984) found evidence that VPA and CBZ interfere with the intestinal absorption of folate , but others have found equivocal results with respect to the association of CBZ therapy with decreased serum and red cell folate levels (Dellaportas et al., 1982; Hiilesmaa, 1982; Hilesmaa et al., 1983; Reizenstein and Lund, 1973).

Various mechanisms have been proposed to explain the antifolate effects of AED apart from interference with the intestinal absorption of folate, and these include increased urinary loss of folate , and enzyme induction of metabolic pathways involved in the utilization or catabolism of folate (Chanarin, 1979), including use of folate as a co-factor in AED metabolism (Berg et al., 1988). Recent evidence suggests that PHT decreases the activity of the enzyme 5,10-methylenetetrahydrofolate reductase involved in liver folate metabolism (Billings and Hansen,1984; Hansen and Billings,1985), and this effect is postulated to account for the biochemical changes observed with PHT treatment, such as alteration in the distribution of particular hepatic folate forms and the decrease in plasma folate levels.

b. *Folate Levels During Pregnancy*

In normal pregnancy, without folate supplementation, serum folate concentrations frequently decline to lowest levels near term and/or the puerperium. Red cell folate levels also fall, although the decrease may not be as great as that in serum, and in some women there may be no change at all (Ball and Giles, 1964; Chanarin et al., 1968, Chanarin, 1979; Ek and Magnus, 1981; Fleming et al., 1974b; Molina et al., 1974; Willoughby and Jewell, 1968) The decrease in blood folate levels in normal pregnancy is thought to be due to the expansion of plasma volume and of red cell mass; greater utilization of folate due to increased metabolism; increased urinary excretion of folate; and high fetal demand, particularly in the last few weeks of pregnancy Subsequently

additional folate is lost in lactation (Chanarin, 1979).

Pregnant women taking AED may be especially at risk of developing folate deficiency. Two small preliminary Japanese studies of epileptic women showed a decline in serum folate levels during pregnancy and/or in the puerperium (Ueda et al., 1984; Yamamoto et al., 1984). Serum levels in pregnant epileptic women were significantly lower than in pregnant controls (Ogawa et al.1985). In one study, 9 of 10 pregnant epileptic women monitored had at least one subnormal serum folate level during pregnancy or the puerperium (Ueda et al., 1984).

c. *Folate and Pregnancy Outcome in the General Population*

In humans, the use of folate antimetabolites as abortifacients has resulted in a high frequency of embryonic deaths, or delivery of offspring with multiple congenital anomalies and intrauterine growth retardation, as in experimental studies (Warkany, 1978).

The role of spontaneously occurring maternal folate deficiency in the development of adverse pregnancy outcome is controversial. Several historical and prospective cohort studies did not find that spontaneous abortions and congenital malformations were correlated with maternal folate status, as assessed by serum folate, red cell folate, and or megaloblastic bone marrow changes (Avery and Ledger, 1970; Chanarin et al., 1968; Daniel et al., 1971; Fleming et al., 1975; Fletcher et al., 1971; Hall, 1972; Loewenstein, et al., 1966; Pritchard et al., 1970; Scott et al., 1970). In contrast, a prospective inquiry in England showed significantly lower erythrocyte folate levels in early pregnancy in women who subsequently had spontaneous abortions or children with

neural tube defects (Hibbard, 1975; Smithells et al., 1976). These conflicting results may be due, in part, to variable methods and times of determining maternal folate status. Recent studies have shown that the offspring of women who take folic acid-containing multivitamins before conception and/or early in pregnancy have a lower risk of neural tube defects than do children of unsupplemented women (Milunsky et al., 1989; Smithells et al., 1989)

A decrease in maternal blood folate levels at term (Ek, 1982; Whiteside et al., 1968) or cord levels (Baker et al , 1977; Ek, 1982) has been associated with small birth weight and / or length for gestational age. Mothers who receive folate supplements during pregnancy have been shown to give birth to heavier babies than women who do not take these supplements (Rolshcau et al., 1979; IYengar, 1971; IYengar and Rajalakshmi, 1975), although other studies have not found evidence of such a relationship (Blot et al., 1981; Chanarin et al., 1968, Fleming et al., 1974a; Giles et al., 1971). One study did not find a difference in the head circumference of babies born to folate supplemented as compared with unsupplemented women (Blot et al., 1981).

d. *Folate and Pregnancy Outcome in Epileptic Women*

Adverse outcome of pregnancy in epileptic women, such as spontaneous abortion, congenital malformation , or growth retardation might occur if folate levels were inadequate at critical stages during pregnancy.

In a preliminary Japanese study (Ueda et al., 1984) of 10 pregnant

epileptic women receiving AED, no association was found between maternal serum folate levels during pregnancy and newborn growth parameters -length, weight, and head circumference. In a later report by the same group of 47 epileptic women treated with AED, no relationship was found between the type of AED or the "drug score" (based on the number and dosage of all AED taken) and folate levels, or between folate levels in the first trimester and the occurrence of congenital malformations (Ogawa et al., 1985). The number of children with congenital malformations, however, was small and included 2 major and 4 minor malformations.

Hiilesmaaa and co-workers (1983) in Finland, also did not find an association between low serum folate levels in 133 pregnancies of 125 epileptic women taking AED , and the occurrence of structural birth defects, the fetal hydantoin syndrome or perinatal deaths in the offspring. However, the Finnish study excluded pregnancies ending before 24 weeks. Folate levels were not measured before 8 weeks of gestation, the major period of organogenesis, while only approximately one quarter of the patients were monitored by 12 weeks of gestation. Furthermore 91% of the women took folate supplements, beginning from the 6th to 16th week of pregnancy, and red cell folate levels were studied only if serum folate levels or haematological indices were abnormal. Serum folate levels rise relatively quickly in response to vitamin therapy, while there is a lag in red cell response. All of these factors, may have masked any pre-existing deficiency early in pregnancy.

Biale and associates (Biale and Lewenthal, 1984; Biale et al., 1975)

compared the condition of 33 children born to 22 epileptic women followed prospectively during pregnancy who were taking AED and folate supplements, and 66 children of 24 unsupplemented treated epileptic women ascertained retrospectively. In the retrospective group, blood folic acid measurements had been carried out in two women in the 7th month of pregnancy who delivered two malformed infants, one with anencephaly and cleft lip and palate and the other with hypospadias and dysmorphic features, and were found to be low. Fifteen percent of the children in the retrospective group had major congenital malformations and several were of low birth weight, while none of the children in the prospective group were malformed. No difference in mean birth weight was found between the two groups. In this study, differences in the ascertainment of the two patient groups may have introduced unknown bias in the findings.

As the early embryo may be particularly sensitive to nutrient deficiencies before the development of a mature placenta (see section H.3b, Etiology of Abnormal Intrauterine Growth in the General Population), further prospective studies of epileptic women are needed, in order to elucidate the contribution of low folate levels around the time of conception or early in gestation to the occurrence of spontaneous abortions and congenital malformations. In addition, little is known about the possible relationship between maternal folate deficiency during pregnancy and prenatal growth retardation in offspring exposed to AED in utero.

e. *Folate, Anticonvulsants, and Pregnancy Outcome in Experimental Animal Studies*

Folate deficiency induced experimentally in animals by folate antimetabolites and/or a folate deficient diet produces intrauterine death, growth retardation and congenital malformations (Johnson, 1963; Jordan et al., 1977 ; Kinney and Morse, 1964; Nelson et al., 1955, 1956; Skalko and Gold, 1974). Any organ system may be affected, but the type and incidence of anomalies depend on the severity, duration and timing of the deficiency, and the species studied (Jordan et al., 1977; Nelson et al., 1955). In the rat, folate deficiency for a period of two days early in gestation can produce such effects with little or no overt signs of deficiency in the mother (Nelson et al., 1955)

In rodents, PHT and VPA have been shown to produce dose-related antifolate and teratogenic effects (Devore and Woodbury, 1977; Hansen and Billings, 1985 ; Marden et al., 1981; Netzloff et al.,1979; Trotz et al., 1987a,b). In mouse embryos exposed to PHT in utero, Netzloff and co-workers (1979) showed a dose-related depression in serum folate levels and oxygen consumption, with a corresponding increase in congenital malformations. Similar effects were obtained with the use of 9-methylpteroylglutamic acid, a folic acid antagonist, suggesting that PHT teratogenicity may be mediated by folate deficiency.

Devore and Woodbury (1977) found that subcutaneous administration of PHT (15/mg/kg daily) to rats throughout pregnancy resulted in increased maternal serum AED concentrations throughout pregnancy as compared to nonpregnant animals, and prevention of the normal rise in

maternal serum folate concentrations usually observed on day 14, a critical time of embryogenesis.

Marden and co-workers (1981), in a rat experimental model, showed that VPA at its anticonvulsant ED50 value (656 mg/kg) produced resorptions, malformations, a reduction in fetal brain and body weights, and a decrease in fetal liver and placental folate. In this model, PHT, PB and CBZ had no such effects.

Valproic acid induces exencephaly in the mouse embryo. Trotz et al. (1987a,b) studied the possible protective effects of folinic acid (metabolically active tetrahydrofolic acid derivative), administered either by multiple injections or by constant rate application via osmotic minipumps at the time of neural tube closure. Both methods, but particularly constant rate infusion, were found effective in reducing the incidence of exencephaly at all doses employed (ranging from 300-600 mg/kg). Folinic acid treatment was accompanied by an increase in plasma levels of folinic acid, but no decrease in plasma levels of VPA. In another experiment, teratogenic doses of VPA were found to decrease several folate metabolites in the embryo during organogenesis.

Hansen and Billings (1985) determined the effect of PHT on folate metabolism in pregnant mice and in the embryos, using chronic dosing of PHT in the diet before and throughout pregnancy. This resulted in murine therapeutic plasma AED levels, and the production of teratogenic effects in the embryo without embryotoxicity. As in nonpregnant animals (Billings and Hansen, 1984), maternal levels of the enzyme 5,10- methylenetetrahydrofolate were found to be decreased. There was also an alteration in the distribution of folate forms in the liver

(decreased concentration of 5-methyltetrahydrofolate and increased concentration of tetrahydrofolate) and a reduction in maternal plasma folate levels , both of which could be attributed to the decreased enzyme levels. The decrease in plasma folate levels could be a direct result of decreased hepatic concentration of 5-methyltetrahydrofolate, the folate form which circulates in the plasma. Although these changes were not seen in total embryos, embryonic tissues were not analyzed in order to see whether folate and enzyme levels were reduced in specific tissues (Hansen and Billings, 1985).

Efforts to reduce the teratogenic effects of various AED experimentally, using folic acid or folinic acid (active folate derivative), have shown both positive (Marsh and Fraser, 1973, Sullivan and McElhatton,1975; Trotz et al., 1987a,b) and negative results (Kernis et al., 1973; Mercier-Parot and Tuchmann-Duplessis, 1974; Schardein et al., 1973; Sullivan and McElhatton 1975). As noted previously, AED such as PHT and VPA may have important effects on the metabolism of folate. Studies of folate supplementation, however, have generally been done without examining possible specific effects of different AED on folate metabolism, and therefore the supplements used may not correct the biochemical alteration responsible for the folate deficiency (Hansen and Billings, 1985). Other possible drug-induced deficiency states, which may not have been corrected by the addition of folinic acid alone, may also explain these negative findings.

In an experimental study in the rat (Zhu and Zhou, 1989), intraperitoneal injection of 75 mg/kg of PHT between 9 to 11 days of gestation resulted in decreased weight and length of the fetuses; an

increased rate of subcutaneous bleeding; a retardation of skeletal ossification (in the sternebrae, caudal, metacarpus, and distal phalanx bones); and an increased number of internal malformations such as hydrocephalus and cardiac septum defects. Addition of folic acid alone, or folic acid with vitamins and amino acids, resulted in a significant reduction in these abnormalities. Folic acid with vitamins and amino acids was more effective than folic acid alone in reducing the incidence of subcutaneous bleeding. Only the former supplementation decreased the incidence of internal malformations and ossification abnormalities of the distal phalanx bones. The authors concluded that nutritional deficiency appears to be an important factor in PHT-induced teratogenicity. The authors mentioned that the appetites of rats fed the supplemented diets were normal, but no mention was made of the appetite of rats given PHT alone. In this study, rats given PHT alone gained less weight during pregnancy than other experimental groups, although no significant differences were noted among the various experimental groups.

Chatot et al. (1984) cultured rat embryos on serum samples from epileptic patients receiving different monotherapies (n=120) or no drugs (n=8), and found that the proportion of embryos with abnormalities of growth and morphogenesis was similar for PHT (72%), CBZ (78%) and VPA (75%), and that these were higher than the corresponding proportions for PB (60%) and no drug (58%). The latter relative frequencies were three times those observed for embryos cultured in sera from nonepileptic controls. No dose response relationship could be found between serum AED levels, ranging from

below to within the human therapeutic range, and abnormalities in the embryo. Addition of vitamins, including folic acid, and/or amino acids to the serum corrected the abnormalities in 60% of 53 samples tested, including samples of untreated epileptics (but not in the case of embryos exposed to VPA). The profile of supplementation required to correct the abnormalities differed for individual serum samples, thus suggesting that individual deficits in nutrition may be a factor in teratogenicity. The relative importance of drug-induced nutritional changes versus diet-related deficits was not answered by this study.

3. *Hormonal Effects*

a. *Thyroid Function*

Various studies have shown that AED alter thyroid function tests, although the patients paradoxically appear clinically euthyroid. Mild hypothyroid states, however, cannot be ruled out, as the symptoms of mild hypothyroidism may not be easily distinguishable from the variation seen in the euthyroid state. For example, the patient treated with PHT has generally been shown to have decreased total and free T_4 and in some studies decreased total and free T_3 as well, without a compensatory rise in TSH levels. It has been suggested that the induction of the metabolism of thyroid hormone by PHT appears to be the principle mechanism for the decrease in serum thyroid levels (Smith and Surks, 1984; Surks et al.1983). The lack of increase of TSH in response to lower serum thyroid hormone levels has been attributed to a drug-induced inhibition of TSH-regulation (Surks et al.1983). There

is also evidence indicating that PHT produces a dose-dependent competitive inhibition of specific T_3 nuclear binding and attenuates certain cellular responses to T_3; for example, growth rate of a rat pituitary cell line responsive to T_3 was slowed by the addition of PHT. It has been postulated that PHT may exert such effects by acting as a weak thyroid agonist (Smith and Surks, 1984).

Three studies have investigated indices of thyroid function in pregnant epileptic women taking AED and in cord blood, as compared with those in nonepileptic pregnant women and their infants. Strauss and associates (1979) found that epileptic women showed significantly lower levels of total and free T_4 in serum in the third trimester. Carriero and colleagues (1985) also found a trend towards lower total and free T_4 (calculated as free thyroxine index-FTI) and total T_3 in maternal serum in the third trimester, as well as significantly reduced total T_4 and FTI in cord blood. Kaneko and co-workers (1982a, 1983a) found lower levels of total T_4 and TSH in maternal serum in the first, second and third trimesters, and in cord blood . The decreased levels were correlated with a reduction in the head circumference of the offspring. However, in these studies, the tests of thyroid function did not show hypothyroid indices. It is unknown whether levels of thyroid hormone required to maintain normal fetal growth may be lower than that needed to prevent clinical evidence of hypothyroidism.

b. *Glucocorticoids*

It has been suggested that PHT-induced cleft palate has a common genetic and biochemical pathway with that produced by glucocorticoids. Susceptibility to glucocorticoid or PHT-induced cleft palate is influenced by the same genes within the H-2 and H-3 histocompatibility loci (Goldman, 1984; Goldman et al.,1983). Genes which control the levels of glucocorticoid receptors map within the H-2 region. The teratogenic effects of glucocorticoids are thought to be mediated via glucocorticoid receptors, and there is evidence that increased susceptibility to glucocorticoid or PHT-induced cleft palate in mouse strains is related to elevated levels of glucocorticoid receptors (Goldman, 1984; Katsumata et al., 1982). Katsumata and colleagues (1982) showed that PHT and glucocorticoids bind to a common glucocorticoid receptor which mediates the inhibition of prostaglandin synthesis. The latter is postulated to participate in normal palatal development (Goldman, 1984).

In man, Goldman et al. (1987) found an elevated level of glucocorticoid receptors in lymphocytes of children with the fetal hydantoin syndrome as compared with unaffected children exposed to PHT in utero. They suggested that the elevated level of receptors may be a marker for susceptibility to the fetal hydantoin syndrome.

c. Other Effects

Because of their induction of hepatic microsomal enzymes, AED increase the metabolism of corticosteroids and sex hormones. In addition, PHT inhibits the secretion of insulin in response to glucose

(Reynolds, 1975). It is not known what these effects may have on growth and development in offspring of epileptic women.

4. *Zinc Deficiency*

Zinc is an essential membrane ion, and like folate, it is necessary for many enzymatic reactions including nucleic acid and protein synthesis. In humans an inverse association has been reported between plasma, muscle or leukocyte zinc levels during gestation and the birth of malformed , low-birth-weight or small-for-gestational age babies (Jameson 1976, Meadows et al. 1981). Phenytoin and VPA may be effective chelators of zinc, producing hypozincemia and hyperzincuria (Hurd et al., 1983, Palm et al., 1986). In animal studies, both zinc deficiency and VPA intake during pregnancy produce, among other anomalies, neural tube defects in the offspring (Hurd et al., 1983). These defects have been associated with maternal use of VPA in human pregnancies (Lindhout and Schmidt, 1986). However, zinc supplementation did not prevent the induction of neural tube defects by VPA in rat embryo cultures (Coakley and Brown, 1986)

5. *Bone Metabolism*

In epileptic patients, antiepileptic drugs, particularly PHT or PB, have been shown to impair bone and mineral metabolism, leading in some cases to clinical evidence of bone disease. Common biochemical abnormalities include hypocalcemia and altered serum levels of active vitamin D metabolites, as well as changes in vitamin K metabolism in some patients. Similar biochemical changes have been noted in

maternal blood during pregnancy and in cord blood (Markestaad et al., 1984). Effects of AED on bone formation may lead to prenatal and postnatal growth retardation, as observed in experimental animals and in humans (Keith, et al., 1982).

6. ***Placental Changes***

Effects on placental structure and function must also be considered as a possible mechanism for abnormal growth and dysmorhogenesis associated with AED. No significant differences in placental weight, serum placental lactogen, or serum estriol and urinary estriol excretion were found between women with epilepsy taking AED and control women (Battino et al., 1982b; Göpfert-Geyer et al., 1982; Hiilesmaa, 1983). In a small study of 13 placentas of treated women with epilepsy (Eeg-Olofsson et al., submitted for publication), no specific morphological changes or alterations in insulin and insulin-like growth factor (IGF-I and IGF-II) receptor activity were found. In these studies (Eeg-Olofsson et al., submitted for publication; Hiilesmaa, 1983), plasma AED levels ranged from below to within the therapeutic range. It is not known whether higher levels might affect some of these biochemical parameters.

In vitro studies have shown that physiological concentrations of VPA and clonazepam markedly inhibit the placental inactivation of cortisol, while PHT does not have this effect, even at extremely toxic concentrations. Other AED tested at very toxic levels showed slight (PB, PRM, ethosuximide) or moderate (CBZ) inhibition of cortisol metabolism in the placenta (Carriero, et al., 1983).

Landon et al. (1987) showed that PHT even at high concentrations did not depress protein synthesis by the human trophoblast, and this may have been due to the fact that PHT showed negligable uptake by these cells.

In sheep (Ayromlooi, et al., 1981), intravenous administration of a clinical dose of PHT to pregnant ewes in the third trimester, resulted in transient fetal hypoxia without any change in uterine blood flow.

In A/J mice genetically susceptible to orofacial anomalies, the administration of PHT results in a depression of maternal cardiac function (potentially leading to uterine ischemia), while in resistant mice (C57BL/6J), cardiac function is less affected (Watkinson and Millicovsky, 1983). Furthermore, hyperoxia reduces the incidence of cleft palate in A/J mice (Millicovsky and Johnston, 1981).

7. *DNA, Protein Synthesis*

Anticonvulsants such as PHT may affect DNA and protein synthesis (MacKinney et al., 1988). PHT has been shown to alter DNA and protein synthesis in mouse embryonic palates, and this has been correlated with the differential susceptibility of various mouse strains to PHT induction of cleft palate (Salomon and Pratt, 1979; Sonawane and Goldman, 1981). Decreased cell number has been correlated with PHT-induced growth retardation in rats exposed to PHT in utero (Tassinari et al., 1981).

8. *Embryonic Intracellular pH*

Acids such as VPA accumulate in the embryo because of the

relatively basic milieu of the embryo, and may alter embryonic intracellular pH, which is a critical determinant of cell proliferation and differentiation (Nau and Hendrickx, 1987).

III. OUTLINE AND OBJECTIVES OF THE PRESENT INVESTIGATION

The studies reported in this thesis were designed to assess the prevalence of abnormal pregnancy outcomes in epileptic women, and the genetic and environmental factors responsible for adverse outcomes, with particular emphasis on the effects of maternal anticonvulsant therapy during pregnancy. The specific objectives of these studies are :

1. To evaluate the frequency of spontaneous abortions, perinatal deaths, and major congenital anomalies (malformations, deformations).

2. To determine the distribution of newborn growth parameters (weight, length and head circumference).

3. To explore the association of a number of potential risk factors, including type, dosage and plasma levels of different anticonvulsant drugs, with the outcomes 1 and 2 above.

4. To explore the interrelationships among folate , anticonvulsants and pregnancy in epileptic women , and to evaluate whether folate deficiency adversely affects the outcome of pregnancy (1 and 2 above).

5. To evaluate the contribution of genetic differences in the capacity to detoxify arene oxide metabolites of phenytoin to the distribution of newborn growth parameters and the occurrence of congenital anomalies.

6. To determine which risk factors remain independently associated with each adverse pregnancy outcome - spontaneous abortions, major

congenital anomalies, and both these outcomes combined - after controlling for the potential confounding effects of other risk factors by stepwise multiple logistic regression analysis. The relative risks of adverse pregnancy outcomes for risk factors which remain independently associated with the outcomes following multivariable analysis will then be determined.

7. To determine which risk factors are important in explaining the variation of each newborn growth parameter (weight, length and head circumference), and to evaluate which risk factors remain independently correlated with these parameters, once the possible confounding effects of other factors are taken into account by stepwise multiple regression analysis.

Part 1 of the thesis deals with the frequency of spontaneous abortions and major congenital anomalies and the results of the univariate and multivariable analyses of potential etiological factors for these adverse outcomes. Part 2 of the thesis is concerned with growth measurements of newborns of epileptic women, and the results of univariate and multivariable analyses of genetic and environmental risk factors for the variation in growth parameters.

IV. PATIENT MATERIAL AND METHODS

A. Number of Patients and Period of Study

An ongoing prospective study of the outcome of pregnancy in epileptic women began in the Neurogenetics Unit of the Montreal Neurological Hospital in 1972. This thesis comprises a consecutive series of 119 pregnancies in 94 women followed prospectively during the period from January 1972 to January 1984. There were 74 mothers with one pregnancy, 15 mothers with two pregnancies, and five mothers with three pregnancies during this period.

B. Ascertainment

Table IV.1 shows the source of ascertainment of the epileptic women. Fifty-one women with 65 pregnancies were referred from private neurological and other medical practices, and 21 women with 29 pregnancies were referred from the Seizure Clinics of the Montreal Neurological and Montreal Children's Hospitals. Three other out-patients, each with one pregnancy, were referred from other departments of the Montreal Neurological Hospital (e.g. Neuropsychology) and Royal Victoria Hospital (e.g. Obstetrics Clinic), while 3 women, each with one pregnancy, were inpatients on the neurological and obstetrics wards of these hospitals. Eleven women with 13 pregnancies were ascertained from a prospective study on the pharmacology of antiepileptic drugs (AED) during pregnancy, while it was being conducted by Dr. Allan Sherwin of the Neuropharmacology Unit at the Montreal Neurological Hospital, and 6 women, each with one pregnancy, were ascertained from a pilot prospective study begun by Dr. Robert Kinch at the Montreal General Hospital on the course and

Table IV.1

Ascertainment of Women

	Number of Women	Number of Pregnancies
A. Outpatients from private neurological practices	48	62
B. Outpatients from other private medical practices	3	3
C. Outpatients from the Seizure Clinics of the Montreal Neurological and Montreal Children's Hospitals	21	29
D. Other outpatients	3	3
E. Inpatients on neurological and obstetrical wards (Montreal Neurological and Royal Victoria Hospitals)	3	3
F. Women in study of effect of pregnancy on plasma AED levels- Neuropharmacology Unit, Montreal Neurological Hospital	11	13
G. Women in study of epilepsy and pregnancy, Dept. of Obstetrics, Montreal General Hospital	6	6
TOTAL	94[a]	119

[a] Number of women not additive as one woman with 2 pregnancies falls into 2 groups

outcome of pregnancy in epileptic women. None of the women were selected for any particular factors in their medical, reproductive, or family history.

The women were ascertained either prior to or as early as possible during pregnancy. Thirty women were first seen prior to pregnancy (Figure IV.1). Figure IV.1 also shows the cumulative number of patients who entered the study at various stages of gestation. By the end of 12 weeks of gestation, 58 of 119 pregnancies (48.7%) had entered the study.

C. General Protocol

In one or more interviews, detailed social, medical, family and reproductive histories were obtained by the author from the women and their husbands, if available, according to a standardized protocol (see Appendix A). Detailed pedigrees of both the proband's and spouse's families were obtained, with special emphasis on a history of major congenital anomalies and of one or more seizures. Pregnant women were then followed at monthly intervals during gestation, and in the postpartum period, whenever possible. Any major or minor seizures were noted by the woman on a seizure calender. At each visit, medication intake, seizure history and antenatal progress were reviewed, and plasma AED levels and blood folate levels were monitored. The patients were followed during pregnancy by the author and Dr. Eva Andermann. The medical, neurological and antenatal histories of the woman were verified by the author by writing to attending physicians and hospitals, and by reviewing the Seizure Clinic chart and antenatal records.

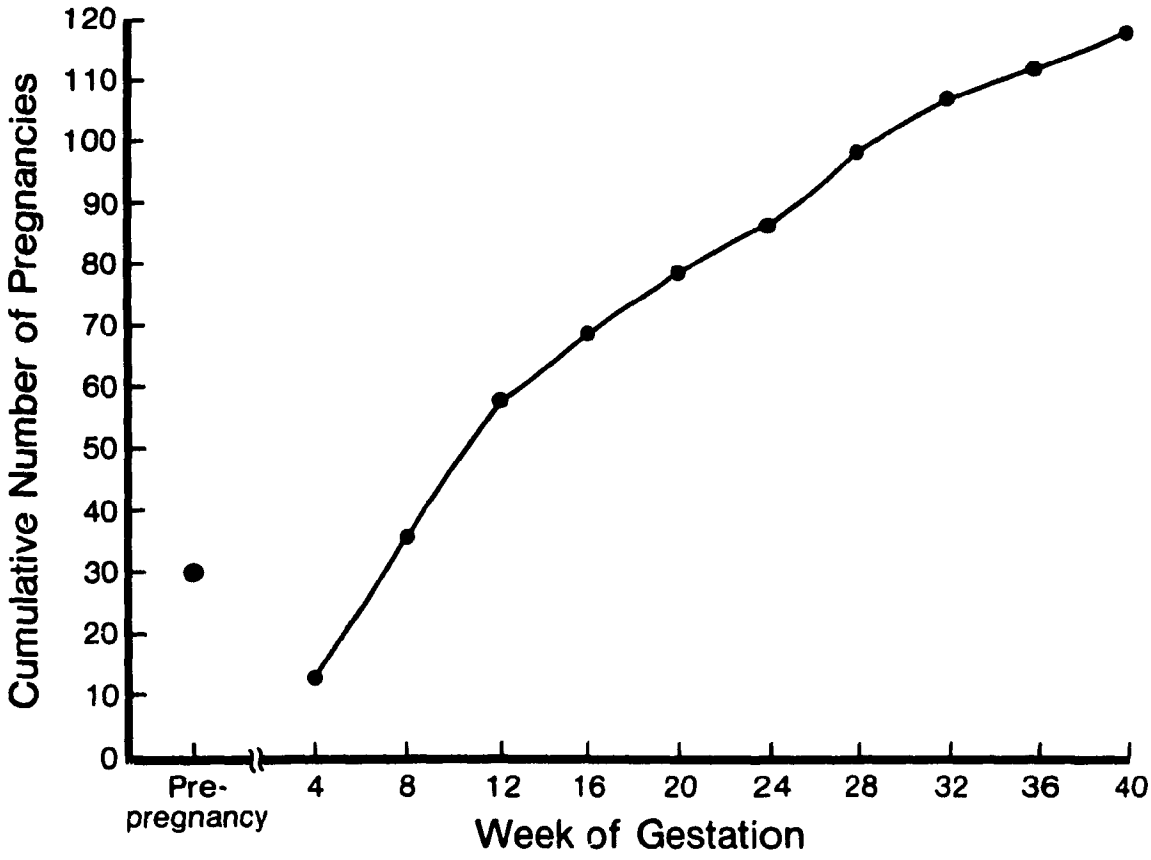


Figure IV.1

Cumulative number of patients who entered the prospective study at various stages of gestation. Thirty patients were initially seen prepregnancy.

The diagnoses of epilepsy and its different types were made or confirmed by a neurologist in all cases. Epilepsy was defined as two or more seizures not provoked by an acute cause, such as drug withdrawal or febrile convulsions. The type of epilepsy and frequency of major and minor seizures was first determined by Dr. Guy Rémillard, neurologist at the Seizure Clinic of the Royal Victoria Hospital, and this was later updated and reviewed by the author with Dr. Michelle Sammaritano, Fellow in the Dept of Neurology and Neurosurgery at the Montreal Neurological Hospital. Major seizures were defined as those with loss of postural tone and falling, because of their potential harmful effect on the mother and fetus (e.g. grand mal attacks). Minor seizures were usually absence attacks, partial complex seizures, or simple partial seizures.

Attempts were also made to confirm the medical histories of the husband and pertinent family members with the medical records. As well, all cases of cardiac defects in the families of epileptic women and their spouses were reviewed by the author with Dr. James Gibbons, cardiologist at the Montreal Children's Hospital, to confirm the diagnosis of congenital heart disease.

The outcome of pregnancy (spontaneous or therapeutic abortion, perinatal death, infant death, major congenital anomaly, newborn growth parameters) was determined by the author in interview with the parent (s) as well as by review of the newborn charts, hospital records, and reports of private pediatricians. Eighty-four percent of the children were examined at various intervals by Dr. Eva Andermann with the author in order to confirm the reports of major congenital anomalies and to detect any unreported major congenital defects and minor

stigmata. Specialized investigations and appropriate consultations were carried out whenever indicated. Follow-up of the children ranged from the neonatal period to 12 years of age, with a median age of 1.2 years.

A diagnosis of congenital heart disease in the children was made on the basis of consultation reports; X-ray, electrocardiogram, echocardiogram and/or catheterization investigations; and autopsy in one child who died in the neonatal period. All findings were reviewed by the author with a paediatric cardiologist, Dr. James Gibbons, of the Montreal Children's Hospital.

D. Type and Duration of Maternal Epilepsy

Table IV.2 shows the distribution of the epileptic women and their pregnancies according to the type of epilepsy. The most common type was primary generalized epilepsy. Three patients , each with one pregnancy, showed evidence of focal epilepsy, but the focus was undetermined. Seven patients with 7 pregnancies could not be classified as to the type of epilepsy, because of inconclusive clinical and electrographic findings. In the course of the study, a diagnosis of epilepsy was found to be unlikely in one woman who was prescribed anticonvulsant medication.

The age of the women at the last menstrual period (LMP) ranged from 17 to 41 years, with a mean of 26 ± 5 years. The duration of epilepsy from the first seizure to the LMP ranged from 1.4 months to 36 years with a mean of 13.7 ± 6.8 years.

Table IV.2

Type of Epilepsy

	Number of Women	Number of Pregnancies
Primary Generalized	35	48
Secondary Generalized with or without Focalization	9	14
Partial Complex	25	27
Partial Simple	14	19
Partial (Focus Undetermined)	3	3
Epilepsy Unclassified	7	7
Epilepsy Unlikely	1	1
Total	94	119

E. Type and Duration of Maternal AED Therapy

Anticonvulsant medication had been prescribed to the patients by their attending physicians, usually a neurologist. At the LMP, the patients had been treated with AED for 2.6 months to 32 years, with a median duration of 9.5 years. All but 3 of the patients were taking AED during the first trimester of pregnancy. Tables IV.3 and IV.4 show the type of AED(s) taken by the women in monotherapy or in polytherapy, in the first trimester and anytime during pregnancy. The AED regimen was not modified during pregnancy unless clinically indicated.

For specific AEDs, the daily dose at the LMP (mg/kg), the daily dose (mg/kg) at the time of monitoring plasma AED levels and the daily dose (mg), averaged for each trimester and the entire pregnancy, are shown in Appendix B.

F. Monitoring of Maternal Blood AED and Folate Levels

Plasma AED levels and blood folate levels (both serum [SF] and red blood cell [RCF]) were monitored concurrently prior to pregnancy, at monthly intervals during pregnancy, at term and postpartum.

Plasma AED levels were determined either by gas-liquid chromatography or enzyme-mediated immunoassay (Emit^{R}) in duplicate in the Neuropharmacology Unit of the Montreal Neurological Hospital, under the direction of Dr. Allan Sherwin. Plasma AED levels were determined in all 119 pregnancies: 48% percent of the pregnancies were monitored in the first trimester; 64% in the second trimester, and 75% in the third trimester. Blood measurements were performed from 1 to 10 times during pregnancy (median 3 determinations). Phenytoin was monitored in 88 pregnancies; phenobarbital in 56; carbamazepine in 24 ;

Table IV.3

Type of AED Taken During Pregnancy

	Trimester I[a]			Anytime[a]		
	Monotherapy	Polytherapy	Total	Monotherapy	Polytherapy	Total
Phenytoin	24[b]	63	87	23	66	89
Phenobarbital	7	44	51	6	49	55
Primidone	1	19	20	1	22	23
Carbamazepine	10	14	24	10	15	25
Valproic Acid	3	6	9	2	6	8
Other	1[c]	1[d]	2	3[e]	1[d]	4
TOTAL	47	69	116	46	72	118

[a] Three women were not taking AEDs in the first trimester, and one woman was not taking AEDs at anytime during pregnancy

[b] Number of pregnancies

[c] Phenobarbital alternating with primidone in monotherapy

[d] Phenylethyl- N- methylbarbituric acid taken in combination therapy

[e] More than one drug taken consecutively in monotherapy: Valproic acid and carbamazepine; phenytoin and carbamazepine; phenobarbital and primidone

Table IV.4

Combinations of AEDs Used in Polytherapy

		Trimester I		Anytime	
		No. of Pregn.	% of Pregn.	No. of Pregn.	% of Pregn.
Two Drugs	PHT + PB	28	23.5	27	22.7
	PHT + PRM	5		3	
	PHT + VPA	4		4	
	PHT + ETH	4		4	
	PB + PRM	2		2	
	PRM + CBZ	1		1	
	CBZ + VPA	1		1	
	CBZ + ETH	1		1	
	> 1 combination of 2 drugs	-		2	
	TOTAL	53	44.5	52	43.7
Three Drugs	PHT + PB + PRM	9	7.6	13	10.9
	PHT + PB + CBZ	2		2	
	PHT + PB + ETH	1		1	
	PHT + PB + PEMB	1		1	
	PHT + CBZ + VPA	1		1	
	PHT + PRM + ETH	1		1	
	PB + PRM + CBZ	1		1	
	TOTAL	16	13.4	20	16.8

PHT = phenytoin, PB = phenobarbital, PEMB = phenylethyl-N-methylbarbituric acid, PRM = primidone, CBZ = carbamazepine; ETH = ethosuximide; VPA = valproic acid

primidone in 18, valproic acid in 8, and ethosuximide in 7 Mean or median plasma levels of the individual drugs for each trimester and the entire pregnancy are given in Appendix B On the whole, the blood levels tended to be low, ranging from below to within the therapeutic range. Only one woman showed a toxic level of phenytoin.

Blood folate determinations were carried out in collaboration with Dr David Rosenblatt of the Medical Research Council Genetics Group, Montreal Children's Hospital. SF and RCF levels were determined by a microbiological assay with Lactobacillus casei Normal values were defined to be 4 to 15 ng/ml for SF and greater than 175 ng/ml for RCF

Fifty nonpregnant epileptic women had plasma AED and blood folate levels measured concurrently. The nonpregnant women were not lactating, nor were they taking folate supplements or oral contraceptives Twenty-six women were taking AED in monotherapy (PHT, 10, CBZ, 7, VPA, 6, PB, 3) Twenty-two women were taking AED in combinations consisting of PHT (17), PB (11), PRM (5), CBZ (7), VPA (6), and ETH (2) Twenty-one of the 50 women were also monitored during pregnancy

Blood folate and AED levels were monitored in a total of 49 pregnancies in 46 epileptic women Initial levels were measured at 1 to 37 weeks after the LMP, at a median gestational age of 12 weeks. Blood measurements were carried out from one to nine times during pregnancy (median, 3.6 determinations) Eleven (22 4%) pregnancies were being monitored by 8 weeks of gestation, the major period of organogenesis, and 29 (59 2%) pregnancies were being monitored by the end of the first trimester In the first trimester, 15 women were receiving AED in monotherapy (PHT,7; CBZ, 4, VPA, 2; PB, 2), and 13 women were on

polytherapy with PHT (10), PB (8), CBZ (4), VPA (3), PRM (2), and ETH (1). Vitamin use was carefully recorded for each patient. Combined vitamin tablets containing 100 to 5000 µg of folic acid (median daily intake, 300 µg) were taken in 31 of 49 pregnancies, beginning at 6 to 30 weeks of gestation until delivery or after term.

Median SF and RCF levels, by each trimester and all trimesters combined, for all pregnant women are given in Appendix B.

G. Determination of Detoxification Defects

In vitro studies of the contribution of pharmacogenetic differences in the detoxification of arene oxide metabolites of phenytoin to the occurrence of congenital anomalies were carried out in collaboration with Ms. Susan Strickler, M. Sc. and Dr. Stephen P. Spielberg, pediatric pharmacologist at the Toronto Hospital for Sick Children. Subjects in this study were derived from the present prospective study. In this study, the present author was responsible for providing the subjects, and for obtaining and classifying data on AED exposures and pregnancy outcome.

The capacity to detoxify arene oxide metabolites was measured by exposing lymphocytes derived from children with or without malformations and their parents to challenge with PHT metabolites, generated by a a microsomal enzyme system. A positive assay response was defined as a statistically significant increase in cell death in the presence of PHT and the microsomal system, as compared with that for the microsomal system alone. The parent compound was not found to be toxic in the assay. Details of the assay methods are given elsewhere (Strickler et al., 1985; Dansky et al., 1987).

In a previous report (Strickler et al., 1985; Dansky et al., 1987), a total of 24 children exposed to phenytoin in utero from 16 families were studied with their parents. These were children derived from pregnancies followed prospectively and their sibs. The present study reanalyzed the data concerning the in vitro response to phenytoin metabolites, using the criteria for congenital anomalies in this study (developmental anomalies, deformation or hernia). Only those children who were ascertained prospectively were studied, irrespective of the type of AED to which they they had been exposed in utero. The 20 children were from 16 families. Seventeen of the children had been exposed to phenytoin with or without other anticonvulsants throughout pregnancy, and three had been exposed to primidone and/or phenobarbital.

H. <u>Classification of Pregnancy Outcomes</u>

For the purposes of this investigation, abortions are defined to include spontaneous or induced termination of pregnancy before 24 weeks from the LMP. Viable births are defined as those at 24 weeks or more of gestation, and perinatal deaths as those occurring from age 24 weeks of gestation to one week of postnatal age.

Major congenital anomalies are defined as disfiguring or potentially life-threatening structural abnormalities present or presumed to be present at birth, although they may be detected later than the neonatal period. Minor congenital anomalies or variations such as craniofacial dysmorphic features and digital hypoplasia, as well as disorders of function , such as mental retardation, were excluded. Limb defects, e.g. talipes equinovarus, were included as major congenital anomalies when

they required correction (casts, splints or corrective shoes), while minor limb deformities not requiring such correction were excluded, because they could most likely be explained by malposition of the limb in utero. All inguinal and umbilical hernias were included, irrespective of whether or not they were surgically treated.

Newborn growth measurements (weight, length, and head circumference) in the children of epileptic mothers were assessed using the normal standards of intrauterine growth of Usher and McLean (1969). These standards are based on a sample of the neonatal population of the Royal Victoria Hospital, where many of the epileptic patients in this study delivered. For each child, standard scores for weight, length, and head circumference adjusted for gestational age were calculated employing the mean and standard deviation at each gestational age of the normal group. This permitted a comparison of growth parameters in newborns of epileptic women with the newborn measurements of Usher and McLean. If the distributions were similar, then one would expect a normal distribution with a peak around 0. If the distributions were dissimilar, one would expect a skewing of values below or above the mean value of 0.

Overall, eight outcomes of pregnancy (see Table IV.5) were evaluated in relation to a number of potential risk factors. The first five outcomes (A, B, C, D, E) pertained to major congenital anomalies and spontaneous abortions and the last three outcomes (F, G, H) were the newborn somatic parameters of head circumference, weight, and length in standard score form. In A, all major congenital anomalies were considered together with spontaneous abortions (Total abnormal outcomes). Spontaneous abortions were studied separately in B. All

Table IV.5

Pregnancy Outcomes Studied (A-H)

A. Total abnormal outcomes including spontaneous abortion (outcome B) and total major congenital anomalies (outcome C)

B. Spontaneous abortions

C. Total major congenital anomalies (outcomes D and E).

D. Developmental defects

E. Congenital postural deformities, inguinal and umbilical hernias only

F. Number of standard deviation units (standard score)[a] of head circumference (HC) from the mean

G. Number of standard deviation units (standard score)[a] of birth weight (WT) from the mean

H. Number of standard deviation units (standard score)[a] of birth length (LT) from the mean

[a]Adjusted for gestational age

major congenital anomalies without spontaneous abortions were also studied (C). In D, specific major congenital malformations were examined, excluding individuals in which the only major anomalies were congenital postural deformities (congenital limb deformation, congenital dislocation of the hip), inguinal hernia, and/or umbilical hernia . In this way attempts were made to study more closely a group of developmental abnormalities ("Developmental" group), excluding those in which mechanical or stress factors may play a role. Inguinal and umbilical hernias, and congenital postural deformities of the limb and hip, were evaluated separately in E ("Congenital Postural Deformity, Hernia" group).

I. Risk Factors Studied

A number of potential risk factors were selected by the author for evaluation as they may relate to the outcome of pregnancy (see Table IV.6 and Appendix C). These included the main variables of interest: type of epilepsy; duration of epilepsy and of AED treatment; frequency of major and minor seizures during pregnancy; type, number, dosage and plasma levels of specific AEDs during pregnancy; detoxification defects of arene oxide metabolites of phenytoin; and blood folate levels monitored during pregnancy. Other factors (referred to as " background factors") pertained to social data of the parents; personal characteristics of the parents such as age and head circumference; reproductive history of the parents; genetic factors such as family history of major congenital malformations or seizures, in the families of both the epileptic woman and her husband; characteristics and complications of the pregnancy; and environmental factors such as

Table IV.6

Risk Variables Studied

Social Data

- -father's occupation
- -parental education

Personal Characteristics

- -sex of child
- -length of follow-up of child
- -parental age
- -parental growth measurements

Maternal and Paternal Family History (in 1st, 2nd, and/or 3rd degree relatives of child)

- -one or more seizures
- -major congenital anomalies

Reproductive History

- -pregnancy order
- -birth order
- -previous fetal loss
- -previous therapeutic abortion

Pregnancy Characteristics

- -trimester of 1st obstetric visit
- -number of obstetric visits
- -duration of gestation
- -maternal weight gain

Maternal Illnesses and Pregnancy Complications

- -endocrine disorder
- -infection, treated
- -fever
- -anemia
- -bleeding
- -toxemia
- -nausea +/-vomiting, treated
- -premature contractions, treated
- -oligohydramnios, polyhydramnios
- -blood group sensitization

Physical Agents

- -x-ray exposure

Drugs Other Than AED

- -sex hormones
- -other hormones
- -alcohol
- - cigarette smoking

Table IV.6

Risk Variables Studied

Maternal Epilepsy

- -type of epilepsy
- -duration of epilepsy
- -frequency of major seizures
- -frequency of minor seizures

Folate

- -folate supplements
- -serum and red cell folate levels

AED Therapy

- -duration of therapy
- -number of AED
- -type(s) of AED
- -dose (mg/kg , total daily mg)
- -plasma levels
- -detoxification defects

cigarette smoking, and chronic maternal diseases other than epilepsy. These variables were chosen on the basis of previous evidence or suspicion of an adverse effect on morphogenesis and growth.

All of the data, with the exception of type and severity of maternal epilepsy were classified and coded by the author. If the information about a particular risk factor was insufficient to classify the individual on that factor, then the variable was coded as missing for that particular individual.

Adverse pregnancy outcomes were studied in relation to the most frequent types of maternal AED monotherapies and/or polytherapies. The specific comparisons analysed are shown in Appendix C, Variable Nos. 85-102).

When appropriate, particular risk factors (e.g. frequency of seizures) were evaluated for both the first trimester and the entire pregnancy. Dose and plasma levels of AED and blood folate levels were evaluated for each trimester of pregnancy and over all trimesters combined.

For each pregnancy, mean dose and plasma levels of AED, and mean blood folate levels were calculated for each trimester and for the entire pregnancy. The mean value for the entire pregnancy was calculated from the mean values for the three trimesters of pregnancy.

The dose of each AED was coded in two ways, for each pregnancy. In the first, an average was calculated of the daily mg for each trimester and over all trimesters combined. The second method calculated the dose in mg/kg at the LMP and the mean mg/kg for each trimester and over all trimesters combined. In the second method, the patient's weight and AED dose were recorded at the time the patient's plasma AED levels were monitored. The two different methods of measuring

dose were employed because it was not known a priori which might be better correlated with adverse pregnancy outcome. Also, more data were available for the dose in mg than in mg/kg.

Because of the problem of missing data in some individuals, variables such as frequency of seizures and family history were coded as both dichotomous variables (presence or absence of the factor), as well as the actual number of seizures or of affected relatives. For example, in some individuals, it was not possible to accurately assess the frequency of minor attacks, and where the information was adequate, minor attacks were coded as a yes/no variable. Similarly, in some cases, it was known that for example, an aunt had epilepsy (coded therefore as having a positive family history of epilepsy) but the total number of second degree relatives was not known.

In order to evaluate the proportion of relatives of the offspring of epileptic women with a positive history of major congenital anomalies or seizures (Appendix C, Variable Nos. 16-42), an individual with an uncertain diagnosis was omitted from the analysis. As the main aim with respect to family history of major congenital anomalies was the evaluation of the contribution of multifactorial inheritance of single major defects to the occurrence of major congenital anomalies in the offspring of epileptic women, individuals with syndromes (eg. Down's), possible single gene disorders (eg., congenital glaucoma affecting a mother and two daughters), or defects possibly attributable to drugs reported to have been taken during pregnancy (eg. cleft lip and other anomalies in child exposed to cortisone), were omitted from the analysis. Thus sibs of the offspring were not considered as the majority of them were exposed to AED(s) in utero. Similarly,

individuals with only seizures in which there is little or no genetic component (neonatal seizures, alcohol withdrawal seizures, and seizures associated with CNS infection) were not counted as having a positive history of seizures.

Family history of major congenital anomalies and of seizures were analyzed in relation to adverse pregnancy outcomes by two methods. In the first, a crude analysis was carried out in which the question was asked of whether adverse pregnancy outcomes were associated with a family history of major malformations or of seizures (yes/no variable) This analysis was performed for first degree relatives of the offspring (i.e., epileptic mother and her mate), first and second degree relatives considered together, and first, second and third degree relatives combined. As the contribution of family history varies according to the number and degree of affected relatives (See Section II.G4, Literature Review), the second method analyzed the proportion of relatives affected for each offspring according to the degree of relationship to the offspring. This was done for first, second and third degree relatives separately, and for different degrees of relatives combined , as in the first method.

The two methods of analyzing family history of seizures and of congenital anomalies were carried out for maternal and paternal relatives of the offspring separately and for both of these combined.

For the purpose of describing the head circumferences of the parents, head circumference measurements were converted to standard scores using the normal measurements for males and females at 18 years of age , calculated by Nellhaus (1968), as the comparison groups. As pointed out by Nellhaus (1968), less than 2% of total head circumference

growth occurs after 18 years of age, and this is accounted for largely by increase in skull and scalp thickness. Using these comparison groups may have therefore resulted in some under - or over - estimation of the proportion of mothers and fathers with particular standard scores; however, the overall distributions of the head circumferences were not affected.

Heights of the parents were converted to percentile ranks, using the normal data of the National Center for Health Statistics Growth Charts, USA (1976).

Mean or median values of continuous risk factors for all pregnancies in the present study are given in Appendices B and D. The proportion of all pregnancies having specific unordered categorical and ordinal risk factors are given in Appendix E.

J. Statistical Analyses

Univariate and multivariate analyses were performed using SAS statistical package on the McGill University computer. The statistical analyses were carried out in collaboration with Dr. Christina Wolfson, Assistant Professor, Depts. of Epidemiology and Biostatistics, and of Neurology and Neurosurgery, McGill University.

Tests of normality were performed, where appropriate, on both the pregnancy outcome (dependent) and risk (independent) variables. Variables found to have distributions not significantly different from a normal distribution are summarized using the mean and standard deviation of values, while those found not to be normally distributed are described by the median and range of values.

1. ***Risk Factors in Relation to Growth Outcomes***

All potential risk factors, described in detail in Appendix C , were first examined univariately in relation to each of the newborn growth parameters (Figure IV.2; Table IV.5 Outcomes F-H) This involved both calculation of correlation coefficients (Pearson's or Spearman's) for continous variables such as mother's head circumference or ordinal variables such as the frequency of maternal seizures, as well as analysis of categorical variables (t-test, analysis of variance, Wilcoxon rank sum test) such as the presence or absence of major seizures or a family history of seizures.

In section VIB concerning the results of risk factors significantly correlated with newborn growth measurements, a positive correlation indicates an increase in the growth parameter with an increase in the risk factor, while a negative correlation refers to a decrease in the parameter with an increase in the risk factor.

All factors which were found to be statistically significant at less than or equal to the 0.05 level by univariate analysis were then entered into stepwise multiple regression analyses, in order to assess the independent effect of the factor after controlling for the intercorrelation of this factor with other factors, that were also significantly associated with the outcome. If no correlation remains between , for instance, factor A and the outcome after controlling for the effect of factor B, then all of the association between factor A and the outcome can be explained by its correlation with factor B. If the correlation of factor A with the outcome remains after removing the

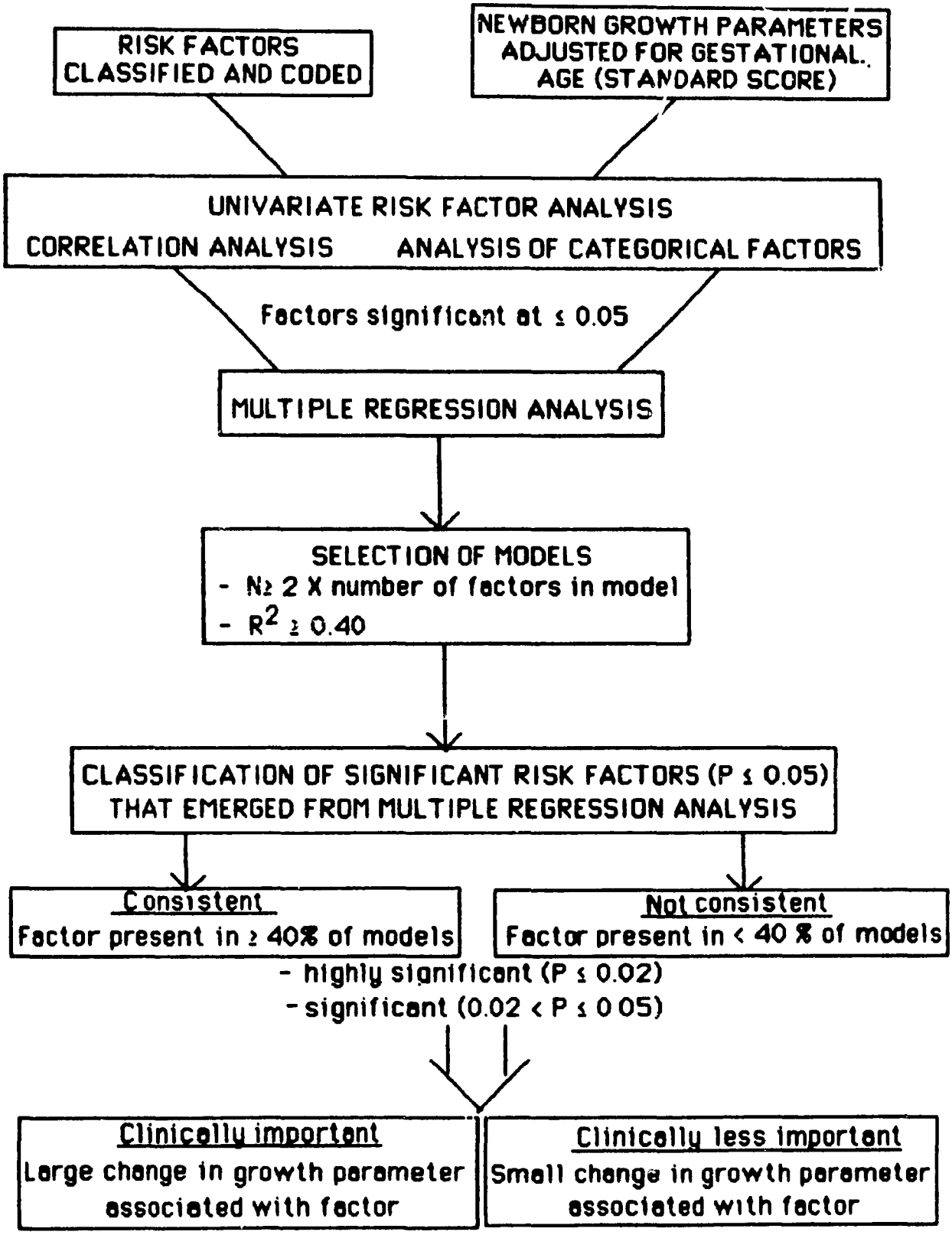


Figure IV.2

Univariate and multivariable statistical analysis of newborn growth parameters - head circumference, weight, and length - in relation to genetic and environmental risk factors.

effects of factor B, then factor A can be said to have an association with the outcome that is over and above the association of factor B with the outcome.

A limitation in this analysis, was the fact that not all women were taking the same AED, and not all women had complete data for all risk factors. Therefore, not all significant risk factors could be evaluated at once in relation to each of the growth outcomes in the same sample of women. Different models having different combinations of factors and involving different subsets of women had to be proposed for the evaluation of the independent association of risk factors with the outcomes by multiple regression analysis. An example of this would be a model with factors involving personal characteristics; family history; pregnancy complications; and type of maternal epilepsy, all of which may be referred to as background factors, and such factors as frequency of major or minor seizures at anytime in pregnancy, and the number of AED at anytime. A different model might be the background factors and such factors as frequency of major or minor seizures anytime; number of AED anytime; mean dose of PHT over all trimesters combined, and mean plasma levels of PHT over all trimesters combined. It would be expected that the sample size of women contributing to these models would be different.

Each different model was analyzed with various combinations of family history variables either as dichotomous variable(s) or continuous variable(s) involving proportions of affected relatives, and with major and/or minor seizures as a continuous (frequency of seizures) or dichotomous variable (presence or absence of at least one seizure). In this way sets of models were generated.

Following multiple regression analysis, models were selected for evaluation of which risk factors remained significantly associated with the outcome variable. The models selected were those which had a sample size that was greater than or equal to two times the number of factors in the model and which had an R^2 of ≥ 0.40, that is the linear combination of factors in the model had to explain at least 40% of the variation in the growth outcome.

In each set of models, these criteria were applied. Finally, one or two models were chosen out of each model set which reflected the consistency of results in the model set, and which had the highest R^2.

Because the risk factors were evaluated in different models, involving different subsets of patients, the effect of one factor in relation to others may not have remained the same from model to model. In other words, the factor may have remained significantly associated with the growth outcome in one model after multiple regression analysis involving a particular subset of patients, but in another model involving a different subgroup of patients, it may not have been maintained in the model. Therefore, in order to make an overall assessment of the factors which remained correlated with the outcome following regression analysis, a procedure was adopted which evaluated the consistency of results over the models.

Risk factors were considered to be consistently associated with the outcome if they were present in 40% or more of the models, and inconsistently associated with the outcome if they were present in fewer than 40% of the models. Inconsistent factors could have been related to the outcome only in the particular subset of patients

analyzed.

Both consistent and inconsistent factors were either statistically significant with a p-value between 0.02 and 0.05 or were highly significant at $p \leq 0.02$. Because of the large number of statistical analyses performed, a certain number of statistically significant asscIations would be expected to occur by chance, and this is more likely at the significance level 0.05 then at a lower level. Thus, a risk factor which is both consistent and highly significant suggests that this factor is less likely to be a spurious finding. In contrast, a risk factor which is inconsistent and significantly associated with the growth outcome at the 0.05 level suggests that the factor could have been significant by chance in the subset of patients analyzed.

Finally, the actual clinical importance of the factor has to be distinguished from the consistency or statistical significance of the variable. A clinically important factor is one associated with a large change in the growth parameter, while a clinically less important factor is one associated with a small change in the growth parameter.

These points are best illustrated by looking at an example of simple linear regression (Figure IV.3). Simple linear regression is a special case of multiple regression, in which only one independent variable is evaluated. In line 2, there is a very small rate of increase in the y variable as the x variable increases, but the slope is still statistically different from 0. Because there is very little variation in the data determining the line, the slope is highly statistically different than 0. This would be an example of a less important clinical factor that is highly statistically significant. On the other hand, line 1 represents a more important clinical variable which is less statistically significant.

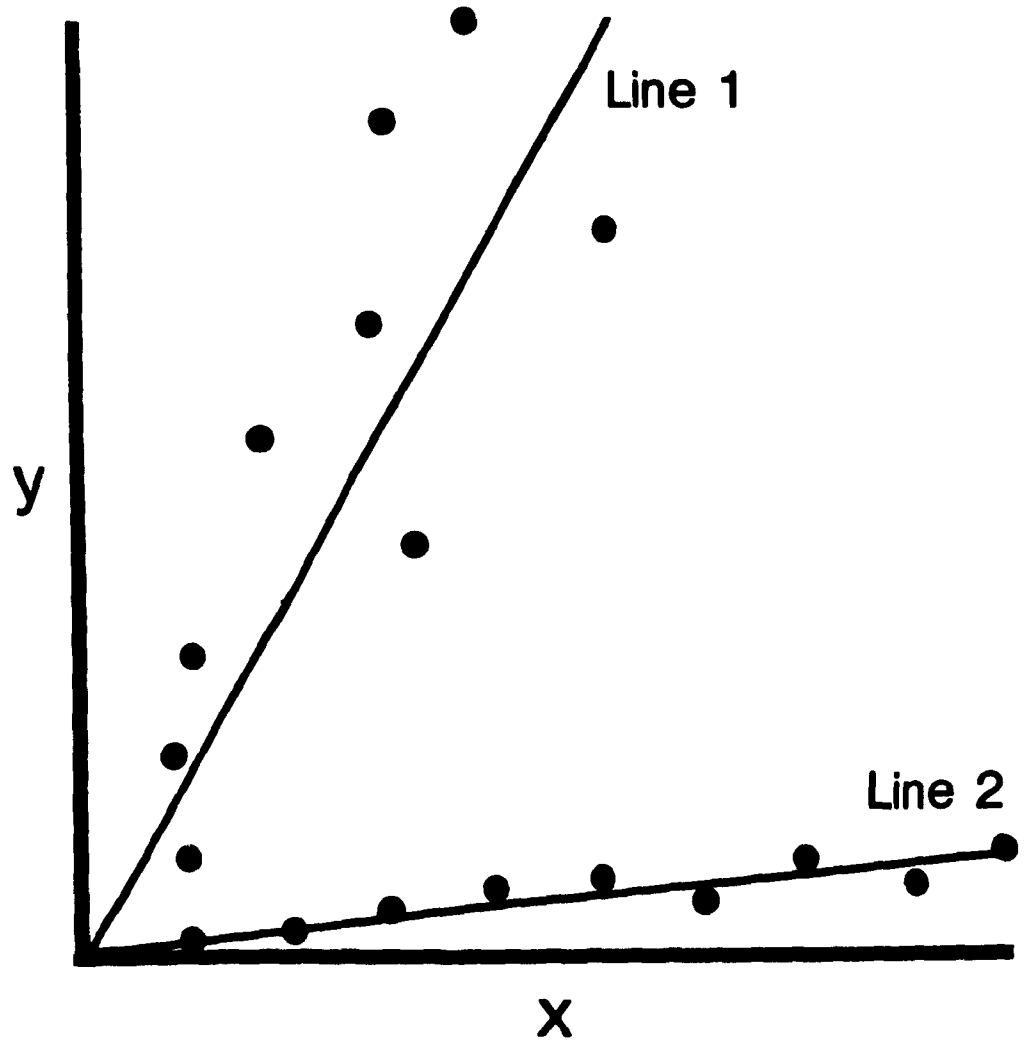


Figure IV.3

Two illustrations of simple linear regression. See text for explanation.

Here there is a greater rate of change in y with change in x but, because of the greater variability in the data, the location of the line and the estimate of its slope is less reliable and the relationship between y and x is less statistically significant.

2. ***Risk Factors in Relation to Major Congenital Anomalies and Spontaneous Abortions***

Univariate analyses were first carried out in order to detect whether there was any significant association between any particular risk factor outlined in Table IV.6 and Appendix C and each of the outcomes pertaining to major congenital anomalies and/or spontaneous abortions (Figure IV.4; Table IV.5, outcomes A-E). Both analyses of unordered categorical (e.g. type of AED, sex of child); ordinal factors (e.g. father's occupation, birth order); and of continuous factors (e.g. dose of AED, parental age) were done using the appropriate parametric (t-test) or nonparametric statistics (Spearman's correlation coefficient; Wilcoxon rank sum test; chi-square test; Fisher's exact probability test).

In the reporting of the univariate results, the proportion of pregnancies resulting in adverse pregnancy outcome in relation to a particular risk variable is referred to as the *risk* of abnormal pregnancy outcome.

Then to assess the separate effect of each factor on pregnancy outcome, while controlling for potential confounding factors, risk factors showing a significant association with pregnancy outcomes A-E ($p \leq 0.10$) by univariate analysis, were evaluated in relation to each other and the outcome of interest by stepwise multiple logistic regression analysis. The 0.10 significance level was chosen in this case

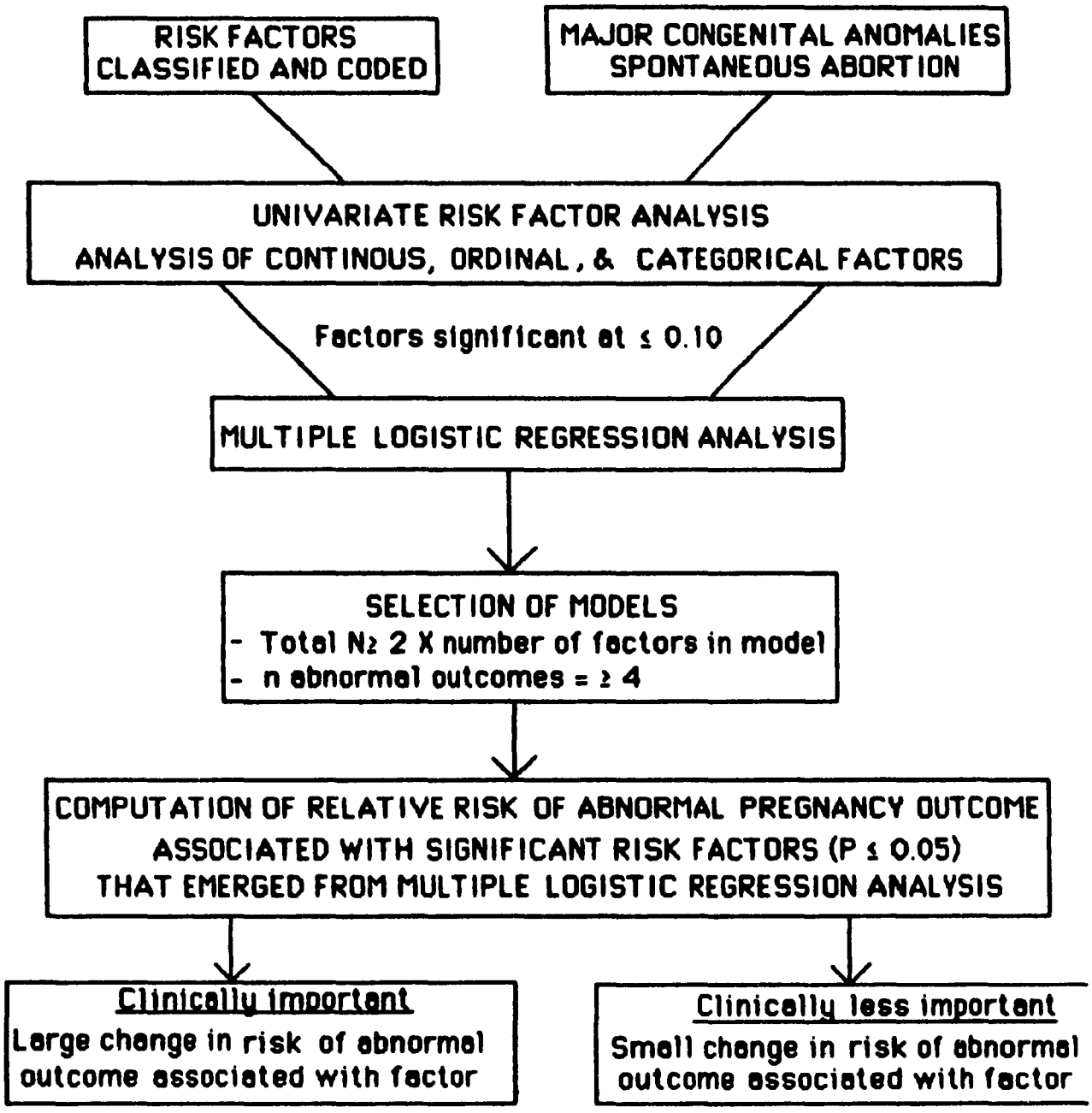


Figure IV.4

Univariate and multivariable statistical analysis of total abnormal pregnancy outcomes, spontaneous abortion, total major congenital anomalies, developmental anomalies, and congenital postural deformities or hernias in relation to genetic and environmental risk factors.

in order to more completely evaluate trends in certain risk factors such as dose of AED, which was found to be significantly associated with adverse pregnancy outcome at significance levels ≤ 0.10 in different trimesters of pregnancy. Multiple logistic regression analysis, rather than multiple regression analysis, was employed because of the dichotomous nature of the outcome variables.

Following stepwise multiple logistic regression analysis, models of risk factors which remained significantly associated with the outcome of interest were selected according to certain criteria (Figure IV.4): the total number of pregnancies comprising the model had to be at least twice the number of independent variables in the model, and there had to be at least four pregnancies resulting in abnormal outcomes contributing to the model.

Once these criteria were met, models were chosen to illustrate the consistency of results and important interrelationships among the independent and dependent variables.

Multiple logistic regression analysis permits the computation of the ratio of the odds of adverse pregnancy outcome* associated with a particular exposure variable or level of exposure, relative to that associated with a nonexposed or low exposure group. The odds ratio is an estimate of the relative risk of adverse pregnancy outcome. Examples of the latter would be the risk of adverse pregnancy outcome in women who have major seizures during pregnancy (exposed group) versus that of women who do not have major seizures (nonexposed

* odds = $\frac{\text{Probability of adverse pregnancy outcome}}{\text{Probability of normal pregnancy outcome}}$

group), or the risk associated with in utero exposure to high doses of AED (high exposure group) relative to that associated with in utero exposure to low doses (low exposure group). The relative risk is adjusted for the potential confounding influence of other exposure variables or risk factors which may also be significantly associated with the outcome of interest, and therefore gives a measure of the independent association of a particular risk factor with the pregnancy outcome. The relative risk also provides a measure of the increased risk associated with the risk factor. A relative risk of 1 indicates no increased risk, while a relative risk significantly greater than 1 indicates the magnitude of the increased risk.

Although the relative risk gives a measure of the increased risk, the actual clinical importance of the increased risk will depend not only on the relative risk, but also on the baseline risk in a nonexposed or low exposure group and the frequency of exposure. For example, suppose the relative risk of birth defects in infants of epileptic mothers who took 300 or 400 mg of PHT was 2 and 4 respectively, as compared with infants of epileptic mothers who did not take AED (reference group). If the risk of birth defects in the reference group is the same as that in the general population (4%), then the absolute risks of birth defects in infants of mothers who took 300 or 400 mg of PHT would be 8 and 16%, respectively. As PHT is a common drug taken by epileptic women, exposure to PHT in utero, particularly at high doses, would have an important impact on the frequency of birth defects among offspring of epileptic women.

V. PART I OF THE PRESENT INVESTIGATION: SPONTANEOUS ABORTION, PERINATAL MORTALITY, AND MAJOR CONGENITAL ANOMALIES

A. Introduction

Although the association of maternal epilepsy with major congenital malformations and minor anomalies has been recognized for two decades, the etiology of abnormal pregnancy outcome in epileptic women is controversial. These abnormalities may be due to the teratogenic effects of anticonvulsant medication or other factors related to maternal epilepsy. If anticonvulsant drugs are teratogenic , one would suspect this effect to be determined in part by the amount of drug(s) taken by the epileptic woman during pregnancy. Few studies have investigated a dose-response relationship, and the vast majority of these studies have not included monitoring of maternal plasma AED levels. Furthermore, only some studies have assessed the role of putative risk factors, other than the effects of anticonvulsant therapy. In order to evaluate the teratogenic potential of anticonvulsant medication more precisely, a prospective study was carried out to assess the outcome of pregnancy (major congenital malformations, spontaneous abortion) in relation to both the dosage and the plasma levels of anticonvulsant drugs monitored during pregnancy, after taking into account the potential confounding effects of other variables, such as maternal seizures and family history of major congenital malformations and seizures. The present study also explored putative mechanisms (blood folate levels, detoxification defects of phenytoin metabolites) for the potential teratogenic effects of anticonvulsants.

B. Results

1. Outcome of Pregnancy

a. *All Outcomes*

As outlined in section IV. A, 119 pregnancies were followed prospectively in 94 women with epilepsy. Six of the 119 pregnancies ended in spontaneous abortions, all occurring by fourteen weeks of gestation (Table V.1). The relative frequency of this outcome among pregnancies ascertained by the fourteenth week (n= 62) was 9.7%, which is at the lower limit of the range of 10-20% reported for the general population.

There was 1 ectopic pregnancy and 3 therapeutic abortions. In one of the latter cases, this was carried out after ultrasound revealed an 18 week old fetus with hydrocephalus.

There were 110 offspring, including the malformed aborted fetus (57 males, 53 females). Twenty-eight of these had major congenital anomalies (25.5%), as compared with the general population rate of 2-4% ($p < 0.001$). Thirteen children (11.8%) had major congenital malformations which were included in the "Developmental" anomaly group (DA). Fifteen children (13.6%) had isolated congenital postural deformities (limb deformities, congenital dislocation of the hip), inguinal hernia and/or umbilical hernias ("Congenital postural deformity, hernia" group [PDH]). Both sexes were equally affected by each type of defect (Tables V.1 and V.2).

There were 4 women who each had more than abnormal pregnancy outcome (Table V.2). One woman had 2 spontaneous abortions. Two other mothers each had 1 spontaneous abortion and 2 children with major congenital anomalies: 1 woman had 2 children with clubfoot, and

Table V.1

Pregnancy Outcome

No. Women	94	
Total No. Pregnancies	119	
Outcomes	**No.**	**%**
Spontaneous abortion	6[a]	5.0 (9.7[b])
Therapeutic abortion	3	2.5
Ectopic pregnancy	1	0.8
Live birth	109	91.6
Total children with major congenital anomalies	28	25.5[c]
- Developmental anomaly	13	11.8
-Congenital postural deformity, hernia	15	13.6
Perinatal death	1	0.9[d]

[a] One women had two spontaneous abortions

[b] Out of 62 pregnancies monitored by 14 wks of gestation

[c] Out of 109 live births plus one therapeutic abortion; two mothers each had two children with major congenital anomalies and one spontaneous abortion; another mother had two children with major congenital anomalies

[d] Out of 109 live births

Table V.2: Specific Characteristics of Pregnancies Resulting in Abnormal Outcomes

Case No. (Sex of child)	Pregnancy Outcome (DA/PDH)[e]	Type Maternal Epilepsy	No. Maternal Seizures		Maternal AED[a]		Family History[d]	
			Major	Minor	Dose (mg/day)	Mean[b,c] Plasma Level (µg/ml)	Major Congen. Anomaly	Seizures
1. (M)	VSD Hypospadias Umbilical hernia (DA)	Primary Generalized	?	?	PHT 300 PB - PRM 500 ETH 1000	5.5[c] 6 1[c] 4 7[c] 45 2[c]	Brother- inguinal hernia	Father, paternal aunt
2. (M)	VSD (DA)	Partial Complex	0	0	PHT 300 PB 120	7.7 7 3	–	-
3 (M)	VSD (DA)	Secondary Generalized with or without Focalization	0	?	PHT 300 PB 90 PRM 625-750	10 3 49.2 6 4	–	–
4. (F)	Aortic stenosis Endocardial fibroelastosis Patent ductus arteriosus (DA)	Secondary Generalized with or without Focalization	2	1/day	PHT 350	13 3[b]	-	Maternal aunt, paternal uncle
5 (M)	Coarctation of aorta (DA)	Unclassified	0	?	PHT 300 PB - PRM 750	6 4[c] 6 0[c] -	Maternal uncle- polydactyly	Maternal great aunt

Table V.2: Specific Characteristics of Pregnancies Resulting in Abnormal Outcomes

Case No. (Sex of child)	Pregnancy Outcome (DA/PDH)[e]	Type Maternal Epilepsy	No. Maternal Seizures		Maternal AED[a]		Family History[d]	
			Major	Minor	Dose (mg/day)	Mean[b,c] Plasma Level (µg/ml)	Major Congen. Anomaly	Seizures
6 (F)	Hypertrophic cardiomyopathy with endocardial fibroelastosis and conduction defect (DA)	Partial Simple	0	occasional	PHT 200 PB 15	5 5 4.3	Mother- moderate scoliosis	Maternal aunt + three 1st degree cousins
7. (F)	Metatarsus adductus (PDH)	Partial Simple	0	0	PB 60	7.0	-	Maternal uncle
8. (F)	Metatarsus adductus (PDH)	Primary Generalized	0	0	PHT 100	2.3	Maternal aunt- horeshoe kidney	-
9 (F)	Bilateral metatarsus varus (PDH)	Primary Generalized	0	0	PHT 300	13.9[b]	Sib (case 10) + maternal 1st degree cousin- congenital limb deformities	Maternal aunt + grandmother
10. (M)	Bilateral talipes calcaneovalgus (PDH)	Primary Generalized	0	0	PHT 200	1 3[c]	See Case No. 9	

Table V.2: Specific Characteristics of Pregnancies Resulting in Abnormal Outcomes

Case No. (Sex of child)	Pregnancy Outcome (DA/PDH)[e]	Type Maternal Epilepsy	No. Maternal Seizures		Maternal AED[a]		Family History[d]	
			Major	Minor	Dose (mg/day)	Mean[b,c] Plasma Level (µg/ml)	Major Congen. Anomaly	Seizures
11. (F)	Talipes calcaneovalgus Scaphocephaly with cranial lacunae and small posterior fossa (DA)	Primary Generalized	0	0	PHT 300 PB 30 PRM 500	4 2[b] 26 9[b] 3.6[b]	Sib (Case 25) Hypospadias, polydactyly, syndactyly	Paternal 1st degree cousin
12 (F)	Bilateral talipes equinovarus Inguinal hernia Umbilical hernia (PDH)	Partial Simple	0	2-3/mo	PHT 300 PB 120 PRM 1000	5 9[b] 29 7[b] 6.0[b]	Sister- omphalocoele, Sister-inguinal hernia	Maternal uncle + grandmother
13 (F)	Bilateral internal tibial torsion (PDH)	Partial Complex	0	0	PHT 300 PB - PRM 125 - 250	4 4 5 0 2 6	Father and paternal great uncle congenital limb deformities	Father
14 (M)	Inguinal hernia Umbilical hernia (PDH)	Primary Generalized	0	3	PB - PRM 250-500	5 6[b] 5 9	Sib (case 15)- inguinal hernia Maternal aunt- branchial cyst, Paternal great grandfather- CHD	-
15 (M)	Inguinal hernia (PDH)	Primary Generalized	1	3/wk	PB[f] 30-60 PRM[f] 125	5 9 -	See Case No 14	

Table V.2: Specific Characteristics of Pregnancies Resulting in Abnormal Outcomes

Case No. (Sex of child)	Pregnancy Outcome (DA/PDH)[e]	Type Maternal Epilepsy	No. Maternal Seizures		Maternal AED[a]		Family History[d]	
			Major	Minor	Dose (mg/day)	Mean[b,c] Plasma Level (µg/ml)	Major Congen. Anomaly	Seizures
16 (M)	Inguinal hernia (PDH)	Partial Complex	0	7	PHT 300 PB 45-90	1 8 7.8	Maternal aunt-umbilical hernia	-
17 (M)	Inguinal hernia (PDH)	Primary Generalized	1	0	PHT 400 PB 100	8 0[b] -	-	-
18 (M)	Inguinal hernia (PDH)	Primary Generalized	6	?	PHT 300 VPA 750	6 6 -	Maternal grandmother-Sprengel's deformity maternal first degree cousin (Case 24)-congenital subluxation of hip	Maternal grandfather + aunt
19 (M)	Umbilical hernia (PDH)	Partial Complex	0	0	PHT 100 PB 100 PEMB 40	2 3 15 0 -	-	Paternal 1st degree cousin
20 (F)	Umbilical hernia (PDH)	Primary Generalized	2	daily	PHT 400 ETH 1250	2 8 37 0	-	-
21 (M)	Cleft lip (DA)	Partial Complex	1	2/wk	PHT 300 PB 90-120 PRM 625	3.4[b] 42 1[c] 8.6[c]	Maternal 1st degree cousin-CHD	-

Table V.2: Specific Characteristics of Pregnancies Resulting in Abnormal Outcomes

Case No. (Sex of child)	Pregnancy Outcome (DA/PDH)[e]	Type Maternal Epilepsy	No. Maternal Seizures		Maternal AED[a]		Family History[d]	
			Major	Minor	Dose (mg/day)	Mean[b,c] Plasma Level (µg/ml)	Major Congen. Anomaly	Seizures
22 (M)	CLeft lip + cleft palate (DA)	Unclassified	0	10	PHT 300-400 PB 50-200 PRM 250-375	13 3 37 6 6 2	Maternal uncle-pyloric stenosis,	Both maternal grandparents and great-grand father
23 (M)	Congenital subluxation of hip (PDH)	Partial Simple	1	1	PB 100	10 6[b]	Maternal uncle-CHD; maternal great-uncle-agenesis of rectum	-
24 (F)	Congenital subluxation of hip (PDH)	Primary Generalized	0	1	CBZ 800	7 9	See Case No 18	
25 (M)	Hypospadias Polydactyly Syndactyly (DA)	Primary Generalized	0	1	VPA 1000	40 0[b]	See Case No 11	
26 (M)	Hypospadias (DA)	Partial Complex	0	0	CBZ 600	6 6[b]	Maternal uncle-cryptorchism, maternal aunt-club-foot	-

Table V.2: Specific Characteristics of Pregnancies Resulting in Abnormal Outcomes

Case No. (Sex of child)	Pregnancy Outcome (DA/PDH)[e]	Type Maternal Epilepsy	No. Maternal Seizures		Maternal AED[a]		Family History[d]	
			Major	Minor	Dose (mg/day)	Mean[b,c] Plasma Level (µg/ml)	Major Congen. Anomaly	Seizures
27 (M)	Hydrocephalus (Dandy-Walker cyst) (DA)	Partial Complex	0	0	PHT 300	11 5	-	-
28 (F)	Polycystic kidney Ureteral atresia (DA)	Partial Complex	1	0	PHT 400	11 0[b]	-	-
29	Spontaneous Abortion	Partial Complex	0	2/wk	PB 30-90 PRM 750 CBZ 600	22.0 12.2 4.9	-	-
30	Spontaneous Abortion	Partial Complex	3	2	PB - PRM 250-750 CBZ 200-600	11.2 5.8 7.0	-	-
31	Spontaneous Abortion	Primary Generalized	0	0	PHT 200	4.6	Father-inguinal hernia	-

Table V.2: Specific Characteristics of Pregnancies Resulting in Abnormal Outcomes

Case No. (Sex of child)	Pregnancy Outcome (DA/PDH)[e]	Type Maternal Epilepsy	No. Maternal Seizures		Maternal AED[a]		Family History[d]	
			Major	Minor	Dose (mg/day)	Mean[b,c] Plasma Level (µg/ml)	Major Malformation	Seizures
32	Spontaneous Abortion	Primary Generalized	0	0	PHT 300 PB 30 PRM 500	9 9 27 5 8 4	See No. 11	
33	Spontaneous Abortion	Primary Generalized	0	0	PHT 300 PB ?	15.0 29.4	See No 9	
34	Spontaneous Abortion	Partial Simple	0	daily	PHT 200-300 PB 60-120	29 0 37 4	Mother- inguinal hernia, Maternal uncle- club-foot, Maternal uncle- congenital bladder diverticulum, Maternal great-grandfather- lung agenesis: Paternal great-grandmother- CHD	-

Multiple pregnancies in four women Case Nos 9,10, 33 , Case Nos 11, 25, 32, Case Nos 29, 30, Case Nos 14, 15

Case Nos 16 and 24 are maternal first degree cousins

[a] In the first trimester, except for mean plasma levels not available for the first trimester, in which case levels are given for the second [b] or third trimester[c]

[d] Among first to third degree relatives of child or abortus

[e] Included in the "Developmental" anomaly (DA) or " Congenital postural deformity, hernia" (PDH) groups.

[f] Case No 15 took PB and PRM alternately in monotherapy

PHT = phenytoin, PB = phenobarbital PRM = primidone, CBZ = carbamazepine, VPA = valproic acid, PEMB = phenylethyl methylbarbituric acid

the other had 1 child with clubfoot, scaphocephaly, and cranial lacunae, and a second child with polydactyly, syndactyly, and hypospadias. The fourth woman had two children with hernias, one with inguinal and umbilical hernias, and the other with inguinal hernia.

There were 4 deaths among offspring of epileptic women, 3 among those with major congenital anomalies. One child with cyanotic congenital heart disease died of cardiac failure on the first day of life. A second child with cardiomyopathy died as a consequence of viral pneumonia at age 18 months. A third child with congenital subluxation of the hip was diagnosed at one year of age to have acute promyelocyte leukemia. She died at age 2 years. There was also one case of sudden death in an infant at age 2 1/2 months.

The remainder of this chapter, concerning the relationship between genetic and environmental factors and pregnancy outcome, excludes the ectopic pregnancy and the 2 therapeutic abortions with unknown embryopathology.

b. *Specific Major Congenital Anomalies*

The specific types of congenital anomalies are shown in Tables V. 2 and V.3. The numbers are not additive as 7 children had multiple major defects involving the same or different organ systems. Sixteen infants with major congenital anomalies (12 DA; 4 PDH) first presented with some abnormality in the neonatal period, and 12 infants (1DA; 11 PDH) showed evidence of abnormalities after this time.

Congenital heart disease, clubfoot, and inguinal hernia were the most common major congenital anomalies, each occurring in 6 offspring (5.5 %) (Table V. 3).

Table V.3

Specific Major Congenital Anomalies in 110 Offspring of Epileptic Women

	No.[a]	%
Congenital heart disease	6	5.5
-Ventricular septal defect	(3)	(2.7)
-Aortic stenosis endocardial fibroelastosis patent ductus arteriosus	(1)	(0.9)
-Coarctation of the aorta	(1)	(0.9)
-Hypertrophic cardiomyopathy with endocardial fibroelastosis and conduction defect	(1)	(0.9)
Clubfoot	6[b]	5.5
Inguinal hernia	6[c]	5.5
Umbilical hernia	5[d]	4.5
Hypospadias	3	2.7
Cleft lip ± cleft palate	2	1.8
Congenital subluxation of hip	2	1.8
Polycystic kidney with ureteral atresia	1	0.9
Hydrocephalus (Dandy-Walker cyst)	1	0.9
Scaphocephaly with cranial lacunae and small posterior fossa	1	0.9
Polydactyly with syndactyly	1	0.9
Bilateral internal tibial torsion	1	0.9

[a] Numbers are not additive. Total number of individuals with major congenital anomalies is 28.

[b] Talipes equinovarus (1), talipes calcaneovalgus (2), metatarsus adductus (2), metatarsus varus (1). All cases of clubfoot required correction.

[c] Four required surgical treatment

[d] One required surgical treatment

The risks for each specific type of defect (congenital heart disease, clubfoot, inguinal hernia, umbilical hernia, hypospadias, cleft lip ± cleft palate, congenital subluxation of the hip, were significantly elevated relative to those of the general population (Table V. 4).

The most common type of heart abnormality found was ventricular septal defect (3 cases), as in the general population (Table V.3). One of the children with this abnormality, also had hypospadias and umbilical hernia. A diagnosis of aortic valve stenosis, ductus arteriosus persistens, and severe endocardial fibroelastosis was established at autopsy in one child who died of cardiac failure on the first day of life. Other cardiac diagnoses were made on the basis of clinical, radiological and electrocardiographic investigations with follow-up over a period of at least one year. The child with hypertrophic cardiomyopathy died at age 18 months of cardiac arrest, precipitated by an episode of viral pneumonia.

All 6 children with clubfoot required some form of correction. Two of these children had additional major anomalies as well as neurological abnormalities. One had umbilical and inguinal hernias, spina bifida occulta at C1, and hypotonia. The second child at age two months showed evidence of a complex congenital anomaly involving dolichocephaly, partial fusion of the sagittal suture, small posterior fossa, and cranial lacunae. She also had pilonidal sinus.

Inguinal and/or umbilical hernias generally occurred as isolated anomalies, except in 2 children, in whom the associated anomalies were ventricular septal defect and hypospadias in 1 child, and clubfoot in the other . Four of the 6 cases of inguinal hernia and 1 of the 5 cases of umbilical hernia required surgical correction.

Table V.4

Prevalence of Specific Major Congenital Anomalies in Offspring of Epileptic Women as Compared with Studies of the General Population

	Prevalence (per 1000)		
	Present Study[a]	Heinonen et al.(1977)[b]	Leck et al.(1968)[b]
Congenital heart disease	54.5	8.0	4.2
Clubfoot	54.5	3.8	5.7
Inguinal hernia	54.5	13.6	-
Hypospadias[c]	52.6	7.4	1.8
Umbilical hernia	45.5	1.0[d]	-
Cleft lip ± cleft palate	18.2	1.0	1.3
Congenital subluxation of hip	18.2	1.8	0.9

[a] All prevalences are significantly higher than those reported in studies of the general population (Heinonen et al., 1977; Leck et al., 1968), at the 0.01 significance level.

[b] These populations were chosen as comparison groups as prevalences of specific malformations are based on large numbers of children with follow-up from birth to 6 or 7 years of age.

[c] Prevalence per 1000 males

d The prevalence of umbilical hernia as reported by Heinonen et al. may be low. Holmes (1975) reported a higher prevalence of 9 per 1000 newborns.

Table V.2 shows some of the individual characteristics of the 28 mothers who had 34 abnormal pregnancy outcomes (major congenital anomalies and/or spontaneous abortions), and of the 28 children with major anomalies. In 24 of 33 pregnancies, in which the seizure history was adequate, the mother had no major seizures during the first trimester of pregnancy. Overall, the mothers were taking higher than average amounts of AED during the first trimester. In 14 of 34 pregnancies, the women were on monotherapy (phenytoin 7, phenobarbital 2, carbamazepine 2, primidone 1, valproic acid 1, and phenobarbital and primidone taken alternately in monotherapy 1); in 11 pregnancies, the women were on 2 drugs; and in 9 pregnancies on 3 drugs. Plasma levels were within or below the therapeutic range. Only one woman, who had a spontaneous abortion, had a toxic plasma phenytoin level (case no. 34).

Two of the 6 children with clubfoot were siblings (case nos. 9 and 10). There was also a positive family history of congenital limb deformity in a first degree cousin of these siblings. A positive history of congenital limb deformity was also obtained for the father and paternal great uncle of a child with bilateral internal tibial torsion (case no. 13).

One child with both umbilical and inguinal hernias (case no. 14) and another (case no. 15) were siblings. In three other cases (case nos. 1, 12, and 16), there was a positive family history of inguinal or umbilical hernias in a previous sib(s), or an aunt.

Taken together, 7 of 15 children with PDH had a positive history of concordant anomalies among first-second-or third-degree relatives (case nos. 9, 10, 12, 13, 14 ,15 ,16; in 5 cases this involved a sibling of

the affected child), while 6 children with PDH had a history of discordant anomalies among their relatives (case nos. 8, 14, 15, 18, 23, 24).

There were no concordant anomalies among first to third degree relatives of children affected with DA. On the other hand, a positive family history of discordant anomalies was found in 8 of 13 children who had DA (case nos. 1, 5, 6, 11, 21, 22, 25, 26; in 3 cases this involved a sibling of the affected child).

Overall, 18 of the 28 families represented by 34 malformed children or abortuses had a first- second- or third-degree relative (sib, maternal or paternal relative) of the child with a major congenital anomaly. There was also a positive family history of seizures on the maternal or paternal side in 13 of the families.

It should be noted that, as discussed in Methods, siblings of the children in this study were not included in the statistical analyses of the relationship between family history of congenital anomalies and of seizures, and pregnancy outcome in the epileptic woman.

2. *Analysis of Abnormal Pregnancy Outcome in Relation to Risk Factors*

a. Univariate Risk Factor Analysis

(1). Personal characteristics

Spouses of women who miscarried were older than spouses of women who had viable pregnancies (n=6; median=30.0; range, 27.5-38.9 (yrs) versus n=80; median=27.3; range, 19.4-50.9 (yrs) , respectively) ($p<0.10$).

(2). Family history of major congenital anomalies and of seizures

The proportions of different degrees of relatives affected with seizures or any type of major congenital anomaly were compared between malformed children or abortuses and normal children (Tables V.5 and V.6). For example, the first row in Table V.5 shows a significantly increased proportion of second degree maternal relatives with a major congenital anomaly among children with PDH as compared to children without major anomalies. An increased proportion of relatives with major congenital anomalies was also noted in families where the index case was a spontaneous abortion (Table V.5).

All other statistically significant results were contrary to expectation, as these showed a decrease in the proportion of relatives affected with major congenital anomalies in families with abnormal pregnancy outcomes, as compared to normal outcomes (Table V.5).

Table V.6 shows that each type of abnormal pregnancy outcome, except spontaneous abortion, was associated with a significantly increased proportion of paternal and/or maternal relatives with a history of one or more seizures, although in some instances a significant decrease in affected relatives was also observed.

When the risk of abnormal pregnancy outcome was studied in relation to the presence or absence of a positive family history of seizures or major congenital anomalies, a higher risk of DA was associated with the presence of a positive family history of seizures among paternal first to third degree relatives, than with its absence (Table V.7).

Table V.5: Pregnancy Outcome in Relation to Family History of Major Congenital Anomalies.

Direction of arrows indicates a statistically significant increase or decrease in the proportion of relatives affected with any type of major congenital anomaly in families with abnormal pregnancy outcome (spontaneous abortion; total major congenital anomalies; developmental anomaly; congenital postural deformity, hernia), as compared with the proportion of affected relatives in families with normal outcomes. M = maternal, P = paternal , 123DEG = 1st, 2nd and 3rd degree relatives.* $0.05 < p < 0.10$; **$0.025 < p < 0.05$.

	PREGNANCY OUTCOME				
RELATIVES[a] AFFECTED	Total Abnormal	Spontaneous Abortion	Total Major Cong. Anom.	Developmental Anomaly	Cong. Postural Deform., Hernia
2DEGM	–	–	–	–	↑**
3DEGM	–	↑*	–	–	–
23DEGM	–	–	–	–	–
2DEGP	↓*	–	↓*	–	–
3DEGP	–	–	–	–	–
12DEGP	–	–	↓*	–	–
123DEGP	–	–	–	↓*	–
2DEGMP	–	–	–	–	–
3DEGMP	–	–	–	–	–
12DEGMP	–	–	–	–	–
123DEGMP	–	↑*	–	–	–

[a] Relationship to offspring of epileptic woman and her mate

Table V.6 : Pregnancy Outcome in Relation to Family History of Seizures.

Direction of arrows indicates a statistically significant increase or decrease in the proportion of relatives with seizures in families with abnormal pregnancy outcome (spontaneous abortion; total major congenital anomalies; developmental anomaly; congenital postural deformity, hernia), as compared with the proportion of affected relatives in families with normal outcomes. M = maternal, P = paternal, 123DEG = 1st, 2nd and 3rd degree relatives. * $0.05 < p < 0.10$; ** $0.025 < p < 0.05$.

	PREGNANCY OUTCOME				
RELATIVES[a] AFFECTED	Total Abnormal	Spontaneous Abortion	Total Major Cong. Anom.	Developmental Anomaly	Cong. Postural Deform., Hernia
2DEGM	–	–	–	–	↑*
3DEGM	↓*	–	–	–	↓*
23DEGM	–	–	–	–	–
2DEGP	–	–	–	–	–
3DEGP	↑*	–	↑*	↑**	–
12DEGP	–	–	–	–	–
123DEGP	–	–	–	↑**	–
2DEGMP	–	–	–	–	↑*
3DEGMP	–	–	–	–	–
12DEGMP	–	–	–	–	–
123DEGMP	–	–	–	–	–

[a] Relationship to offspring of epileptic woman and her mate

Table V.7

Pregnancy Outcome in Relation to Categorical Risk Factors

Risk Factor Abbreviation	Risk Factor	Abnormal Pregnancy Outcome	% Abnormal Pregnancy Outcome Risk Factor Absent(0)	Risk Factor Present(1)	N_0/N_1	P
	Family History					
SZ123DEGP	Seizures in paternal 1st, 2nd,3rd degree relatives	Developmental Anomaly	8.1	33.3	86/12	0.05
	Pregnancy Characteristics and Complications					
BLEEDI	Bleeding Trim I	Cong. Postural Deformity,Hernia	10.8	29.4	93/17	0.10
	Drugs Other Than AED					
ALC	Alcohol	Developmental Anomaly	14.9	3.0	74/33	0.10
	Maternal Seizures					
MAJSZA	Presence of major seizures Anytime	Total Major Cong. Anomalies	18.8	34.1	64/44	0.10
MINSZA	Presence of minor seizures Anytime	Developmental Anomaly	6.1	19.2	49/52	0.05

(3) Consanguinity

Cousin marriages between the epileptic woman and her mate were reported in 2 instances. In one case, the parents were third degree cousins and the child was normal. In the second case, the parents were second degree cousins and the child was found to have cardiomyopathy in the neonatal period (Table V.2, case no. 6). It should be noted that consanguinity was not analyzed along with other risk factors in relation to adverse pregnancy outcome in a multiple logistic regression model, as it was felt that the small number of cases would not have substantially influenced the findings concerning the association between adverse pregnancy outcome and other possible risk factors, such as AED.

(4). Reproductive history

There was a statistically significant but low positive correlation between the occurrence of abnormal pregnancy outcome and pregnancy order: the proportion of pregnancies resulting in abnormal outcomes for pregnancy order (1) = 12/55, 21.8%; (2) = 9/27, 33.3%; (3) = 9/24, 37.5%; (4) = 3/7, 42.9%; (5) = 1/2, 50.0%; (6) = 0/1 (Spearman rho = 0.159, $p<0.10$).

(5). Pregnancy complications

An increased frequency of children with PDH was found among women who reported spotting in the first trimester, as compared to mothers who did not bleed (Table V.7).

(6). Drugs other than anticonvulsants

Women who reported alcohol intake during pregnancy were all occasional to moderate drinkers, and there were no women who were chronic users. Contrary to expectation, a significantly higher frequency of children with DA was found among women who reported no alcohol intake, as compared to women who reported alcohol intake (Table V.7).

(7). Maternal seizures

Among 64 children of women who had no major seizures at anytime during pregnancy, there were 12 with major congenital anomalies (18.8%): 6 DA and 6 PDH. There were 15 children with major congenital anomalies (6 DA, 9 PDH) among 44 children of women who experienced major seizures at anytime during pregnancy, giving a relative frequency of 34.1%, almost twice that of women who had no seizures during pregnancy ($p < 0.10$) (Table V.7). Only 3 of 49 children whose mothers had no minor seizures during pregnancy had developmental anomalies (6.1%), while there were 10 children with such anomalies among 52 children of mothers who had at least one minor attack (19.2%)($p < 0.05$) (Table V.7).

(8). Type of anticonvulsant therapy

The outcome of pregnancy did not differ significantly among women who took different AED monotherapies in the first trimester (Table V.8), or at anytime during pregnancy. There were no DA among 7 children exposed to PB alone in the first trimester, although 2 of these children showed congenital subluxation of the hip or clubfoot. Another child whose mother took PRM and PB alternately in monotherapy in the first

Table V.8: Pregnancy Outcome in Relation to Type of Monotherapy in the First Trimester

Type of AED		Total Abnormal		Spontaneous Abortion		Total Major Cong. Anomalies		Developmental Anomaly		Congenital Postural Deformity, Hernia	
Monotherapy	N	No.	%[a]	No.	%[a]	No.	%[b]	No.	%[b]	No.	%[b]
PHT	24	7	29.2	1	4.2	6	26.1	3	13.0	3	13.0
PB	7	2	28.6	0	0.0	2	28.6	0	0.0	2	28.6
CBZ	8	2	25.0	0	0.0	2	25.0	1	12.5	1	12.5
VPA	3	1	33.3	0	0.0	1	33.3	1	33.3	0	0.0
PRM	1	1	–	0	–	1	–	0	–	1	–
PRM,PB[c]	1	1	–	0	–	1	–	0	–	1	–
ETH	1	0	–	0	–	0	–	0	–	0	–
Total	45	14	31.1	1	2.2	13	29.5	5	11.4	8	18.2

[a] Percent of all pregnancies; [b] Percent of viable pregnancies

c One child exposed to PRM and PB taken alternately in monotherapy

trimester had an inguinal hernia. Among 23 children exposed to PHT alone in the first trimester, 3 had DA (hydrocephalus; polycystic kidney; aortic stenosis), and 3 had clubfoot (Table V.8).

The occurrence of abnormal outcomes differed significantly among pregnancies of women who took particular combinations of AED during the first trimester (Table V.9). Those who took PRM in combination with other AED in the first trimester showed the highest risk of abnormality, and the differences were highly significant for total abnormal outcomes, total major congenital anomalies and DA. This trend was not observed among pregnancies resulting in children with PDH.

Similar findings were noted with respect to the relationship between the type of polytherapy taken at anytime during pregnancy and subsequent outcome of pregnancy, although differences in the occurrence of abnormal outcomes among different polytherapies tended to be not as marked as those observed for the first trimester.

Women who took PRM in combination with other AED most commonly were prescribed the specific drug combination PHT, PB and PRM, followed by the drug combination PHT and PRM (Table IV.4, Methods). Table V.10 shows that those who were taking PHT , PB and PRM in the first trimester were most likely to show abnormal pregnancy outcome, followed by those on PHT and PRM, as compared to women taking PHT and PB , PHT alone, or PB alone.

There were no significant differences in pregnancy outcome for other specific monotherapy versus polytherapy comparisons (e.g, PHT, CBZ vs PHT alone vs CBZ alone; see Appendix C, nos. 91-102 for specific comparisons analysed). Overall, pregnancy outcome did not differ significantly between women who took any type of monotherapy and

Table V.9: Pregnancy Outcome in Relation to Type of Polytherapy in the First Trimester

Type of AED Polytherapy	N	Total Abnormal No.	% [a] [††]	Spontaneous Abortion No.	% [a]	Total Major Cong. Anomalies No.	% [b] [***]	Developmental Anomaly No.	% [b] [***]	Congenital Postural Deformity, Hernia No.	% [b]
PHT + PB	27	6	22.2	2	7.4	4	16 0	2	8.0	2	8.0
PRM + other AED	19	11	57.9	3	15.8	8	50.0	6	37.5	2	12.5
CBZ + other AED[c]	10	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
VPA + other AED	6	1	16.7	0	0.0	1	16.7	0	0.0	1	16.7
Other combi-nations	6	2	33.3	0	0.0	2	33.3	0	0.0	2	33.3
Total	68	20	29.4	5	7.4	15	23.8	8	12.7	7	11.1

[a] Percent of all pregnancies ; [b] Percent of viable pregnancies; [c] Excluding PRM or VPA

*** $0.01 < P \leq 0.02$; †† $0.001 < p \leq 0.005$

Table V.10

Pregnancy Outcome in Relation to PHT, PB, PRM Taken Alone or in Combination in the First Trimester

Type of AED	N	Total Abnormal No.	Total Abnormal %[a]	Spontaneous Abortion No.	Spontaneous Abortion %[a]	Total Major Cong. Anomalies No.	Total Major Cong. Anomalies %[b]	Developmental Anomaly No.	Developmental Anomaly %[b]*	Congenital Postural Deformity, Hernia No.	Congenital Postural Deformity, Hernia %[b]
PHT,PB,PRM	9	6	66.7	1	11.1	5	62.5	4	50.0	1	12.5
PHT,PRM	5	2	40.0	0	0.0	2	40.0	1	20.0	1	20.0
PHT,PB	27	6	22.2	2	7.4	4	16.0	2	8.0	2	8.0
PHT	24	7	29.2	1	4.2	6	26.1	3	13.0	3	13.0
PB	7	2	28.6	0	0.0	2	28.6	0	0.0	2	28.6
Total	72	23	31.9	4	5.6	19	27.9	10	14.7	9	13.2

[a]Percent of all pregnancies; [b] Percent of viable pregnancies; * $0.05 < p \leq 0.10$

women on any combination of AED.

(9). Dose and plasma levels of anticonvulsants

(a) Phenytoin

The average daily maternal dose (mg) of PHT for each trimester of pregnancy and all trimesters combined are compared between pregnancies resulting in abnormal and normal outcomes in Table V.11. In each trimester and all trimesters combined, the dose (mg) was significantly higher for total abnormal outcomes, total major congenital anomalies, and DA.

Over all trimesters combined, the average dose (mg/kg) of PHT (not shown in Table V.11) taken by mothers who had any type of abnormal pregnancy outcome (median 5.2, range 1.7-6.0) or children with DA (median: 5.2, range 3.9-6.0) was significantly higher than that of mothers with normal pregnancy outcomes (median: 4.3, range 1.6-8.0) ($p<0.10$). No significant differences in PHT dose (mg or mg/kg) were noted for spontaneous abortion or PDH.

When the the outcome of pregnancy was studied in relation to increasing mean daily dose (mg) of PHT in the first trimester, the risk of total abnormal outcomes, total major congenital anomalies and developmental anomalies increased with increasing doses of PHT in the first trimester (Table V.12A). Similar results were observed for the relationship between pregnancy outcome and mean daily dose (mg) of PHT over all trimesters combined, except in the latter case, the risk of abnormality tended to plateau at 300- 349.9 mg per day (Table V.12B).

The average plasma level (µg/ml) of PHT for each trimester of pregnancy and all trimesters combined, are compared between

Table V.11: Pregnancy Outcome and Average Dose (mg/day) of Phenytoin in Each Trimester

Pregnancy Outcome	Trimester I N	Trimester I Median[a] (Range)	Trimester II N	Trimester II Median (Range)	Trimester III N	Trimester III Median (Range)	All Trimesters N	All Trimesters Median (Range)
Total Abnormal	25	300.0* (69.1-400)	20	300.0** (100.0-400.0)	20	300.0* (100.0-400.0)	26	300.0** (23.4-400.0)
Spontaneous Abortion	4	298.9 (200.0-300.0)	-	-	-	-	-	-
Total Major Anomalies	21	300.0** (69.1-400.0)	20	300.0** (100.0-400.0)	20	300.0* (100.0-400.0)	22	300.0** (23.4-400.0)
Developmental Anomaly	11	300.0** (200.0-400.0)	11	300.0* (200.0-400.0)	10	328.3** (200.0-400.0)	11	302.4† (200.0-400.0)
Cong. Postural Deformity, Hernia	10	300.0 (69.1-400.0)	9	300.0 (100.0-400.0)	10	300.0 (100.0-400.0)	11	300.0 (23.4-400.0)
Normal Outcome	60	300.0 (9.6-500.0)	57	300.0 (25.8-500.0)	57	300.0 (46.2-500.0)	62	297.5 (3.1-500.0)

[a] Median of average doses in each trimester and over all trimesters combined

* $0.05 < p \leq 0.10$; ** $0.02 < p \leq 0.05$; † $0.001 < P \leq 0.01$

Table V.12A

The Occurrence of Abnormal Pregnancy Outcome in Relation to Mean Dose Phenytoin (mg/day) in the First Trimester

Dose	N	Total Abnormal No	Total Abnormal % [a]	Spontaneous Abortion No	Spontaneous Abortion % [a]	Total Major Cong. Anomalies No.	Total Major Cong. Anomalies % [b]	Developmental Anomaly No.	Developmental Anomaly % [b]	Congenital Postural Deformity, Hernia No.	Congenital Postural Deformity, Hernia % [b]
<200.0	14	2	14.3	0	0.0	2	14.3	0	0.0	2	14.3
200.0-299.9	21	4	19.0	2	9.5	2	10.5	1	5.3	1	5.3
300.0-349.9	40	14	35.0	2	5.0	12	31.6	7	18.4	5	13.2
≥ 350	10	5	50.0	0	0 0	5	50.0	3	30.0	2	20.0
Total	85	25	29.4	4	4.7	21	25.9	11	13.6	10	12.3

[a] Percent of all pregnancies ; [b] Percent of viable pregnancies

Table V.12B

The Occurrence of Abnormal Pregnancy Outcome in Relation to Mean Dose Phenytoin (mg/day) Over All Trimesters Combined

Dose	N	Total Abnormal No.	Total Abnormal % [a]	Spontaneous Abortion No.	Spontaneous Abortion % [a]	Total Major Cong. Anomalies No.	Total Major Cong. Anomalies % [b]	Developmental Anomaly No.	Developmental Anomaly % [b]	Congenital Postural Deformity, Hernia No.	Congenital Postural Deformity, Hernia % [b]
<200.0	20	3	15.0	0	0.0	3	15.0	0	0.0	3	15.0
200.0-299.9	19	4	21.1	2	10.5	2	11.8	1	5.9	1	5.9
300.0-349.0	38	15	39.5	2	5.3	13	36.1	8	22.2	5	13.9
≥ 350.0	11	4	36.4	0	0.0	4	36.4	2	18.2	2	18.2
Total	88	26	29.5	4	4.5	22	26.2	11	13.1	11	13.1

[a] Percent of all pregnancies ; [b] Percent of viable pregnancies

pregnancies resulting in abnormal and normal outcomes in Figure V.1. Plasma PHT levels in the first trimester associated with spontaneous abortion tended to be higher than that for normal outcomes, but the difference was not significant. In the first and second trimesters, and over all trimesters combined, plasma PHT levels were significantly higher in pregnancies resulting in offspring with DA than in those with normal outcomes. In contrast, plasma levels of PHT in the first trimester tended to be lower in pregnancies resulting in infants with PDH than in pregnancies with normal outcomes, while little difference was noted later in pregnancy. Plasma PHT levels over all trimesters were significantly higher for total abnormal outcomes than for normal outcomes.

The risk of total abnormal outcomes, spontaneous abortion, total major congenital anomalies and DA increased with increasing mean plasma PHT levels in the first trimester and/or over all trimesters combined. This was not found for the risk of PDH (Table V.13A,B). In the first trimester, there was some suggestion of a threshold effect, in that the risk of abnormalities increased markedly when plasma levels entered the therapeutic range of ≥ 10 µg/ml (Table V.13A).

Women who had mean plasma PHT levels (µg/ml) ≥ 10 µg/ml over all trimesters combined and whose pregnancies resulted in abnormal outcomes were on monotherapy (n=4) or polytherapy, specifically PHT + PB (n=2) or PHT, PB and PRM (n=1).

(b) Phenobarbital

Women who had children with major congenital anomalies tended to take higher average daily doses (mg) of PB in each trimester of

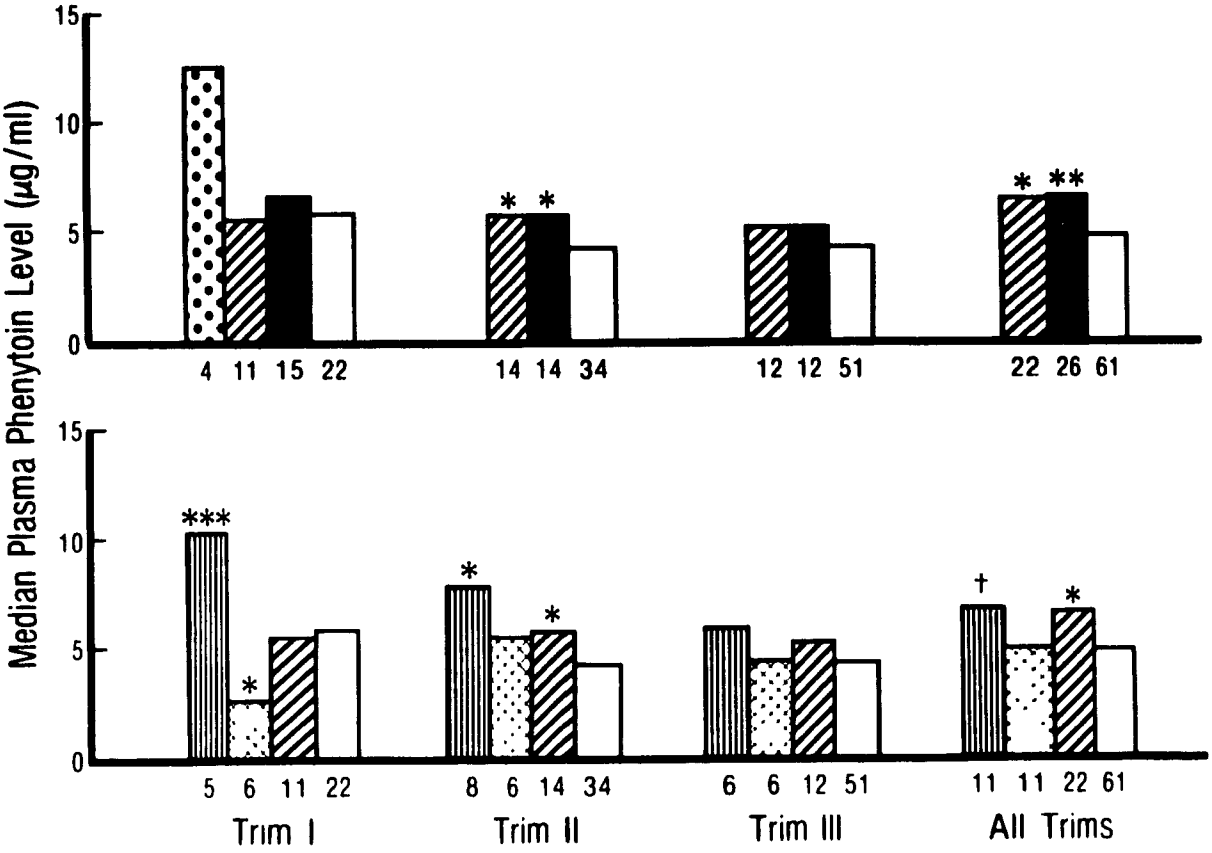


Figure V.1

Median of average values of plasma phenytoin levels in each trimester and over all trimesters combined for pregnancies resulting in total abnormal outcomes ■, spontaneous abortions ⁘, total major congenital anomalies ▨, developmental anomalies ▥, isolated congenital postural deformities or hernias ▨, as compared to normal outcomes □. Numbers below bars are number of pregnancies monitored. * p≤ 0.10; ** p ≤ 0.05; *** p≤ 0.02; † p ≤ 0.01.

Table V.13A

The Occurrence of Abnormal Pregnancy Outcome in Relation to Mean Plasma Phenytoin Levels (μg/ml) in the First Trimester

Plasma Level	N	Total Abnormal		Spontaneous Abortion		Total Major Cong. Anomalies		Developmental Anomaly		Congenital Postural Deformity, Hernia	
		No.	%[a]	No.	%[a]	No.	%[b]	No.	%[b]	No.	%[b]
< 5.0	17	6	35.3	1	5.9	5	31.3	0	0.0	5	31.3
5.0-9.9	13	4	30.8	1	7.7	3	25.0	2	16.7	1	8.3
≥ 10.0	7	5	71.4	2	28.6	3	60.0	3	60.0	0	0.0
Total	37	15	40.5	4	10.8	11	33.3	5	15.2	6	18.2

[a] Percent of all pregnancies; [b] Percent of viable pregnancies

Table V.13B

The Occurrence of Abnormal Pregnancy Outcome in Relation to Mean Plasma Phenytoin Levels (µg/ml) Over All Trimesters Combined

Plasma Level	N	Total Abnormal No.	Total Abnormal % [a]	Spontaneous Abortion No.	Spontaneous Abortion % [a]	Total Major Cong. Anomalies No.	Total Major Cong. Anomalies % [b]	Developmental Anomaly No.	Developmental Anomaly % [b]	Congenital Postural Deformity, Hernia No.	Congenital Postural Deformity, Hernia % [b]
<5.0	41	9	22.0	1	2.4	8	20.0	2	5.0	6	15.0
5.0-9.9	30	10	33.3	1	3.3	9	31.0	5	17.2	4	13.8
≥10.0	16	7	43.8	2	12.5	5	35.7	4	28.6	1	7.1
Total	87	26	29.9	4	4.6	22	26.5	11	13.3	11	13.3

[a] Percent of all pregnancies; [b] Percent of viable pregnancies

pregnancy and over all trimesters combined than did women with normal children (Figure V. 2). Doses were higher among mothers who gave birth to children with DA as well as those who had children with PDH (Figure V.2).

There was some suggestion that a mean daily dose of PB of > 90 mg in the first trimester was associated with a higher risk of total major congenital anomalies or total abnormal outcomes than were lower doses, but no clear trend was observed for more specific outcomes (Table V.14A). The frequency of total abnormal outcomes, total major congenital anomalies, and specifically PDH tended to increase with increasing mean daily doses (mg/kg) of PB over all trimesters combined (Table V.14B). Mothers who had children with the latter defect took significantly higher average doses (mg/kg) of PB over all trimesters (median 1.7, range 0.8-2.3), than did mothers of normal children (median 1.0, range 0.4-4.2) ($p<0.10$).

In the first trimester, plasma PB levels (µg/ml) were significantly higher in pregnancies resulting in spontaneous abortion than in pregnancies with normal outcomes (Figure V.3). Plasma PB levels in pregnancies resulting in children with DA were also higher than in those with normal outcomes, and the differences were statistically significant in the second trimester, and over all trimesters combined (Figure V 3). Significant differences were also found in the second trimester for total major congenital anomalies, and over all trimesters combined for total abnormal outcomes. This was due to the higher plasma PB levels associated with DA and spontaneous abortion.

The risk of spontaneous abortion increased with increasing mean plasma PB levels (µg/ml) in the first trimester, but not for other

Figure V.2

Median of average values of daily dose of phenobarbital in each trimester and over all trimesters combined for pregnancies resulting in total abnormal outcomes , **spontaneous abortion** , **total major congenital anomalies** , **developmental anomalies** , **isolated congenital postural deformities or hernias** , **as compared to normal outcomes** . **Numbers below bars are number of pregnancies monitored. * ps 0.10.**

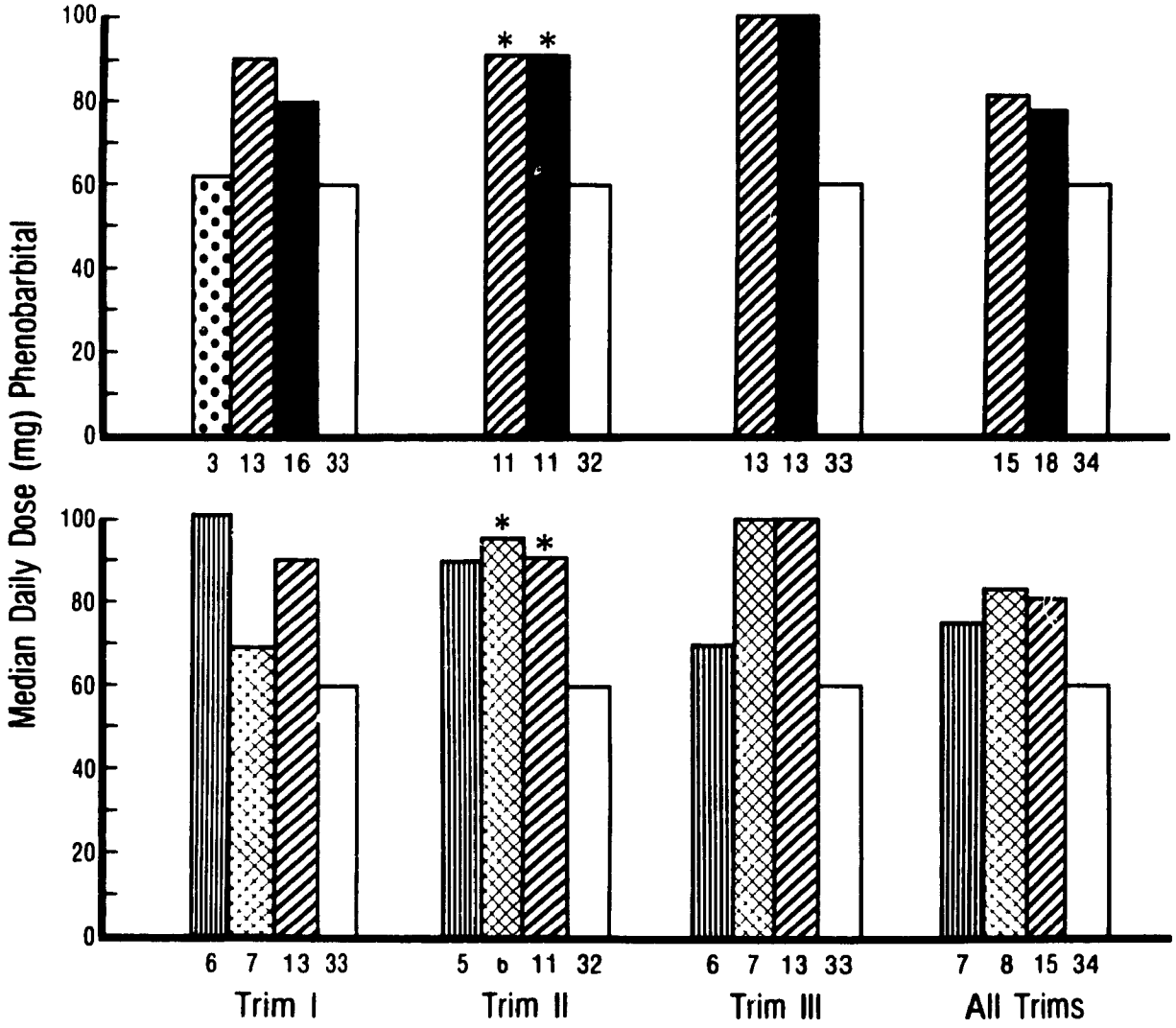

Median Daily Dose (mg) Phenobarbital
100
80
60
40
20
0
* *
3 13 16 33
11 11 32
13 13 33
15 18 34
*
*
6 7 13 33
5 6 11 32
6 7 13 33
7 8 15 34
Trim I
Trim II
Trim III
All Trims

Table V.14 A

The Occurrence of Abnormal Pregnancy Outcome in Relation to Mean Dose Phenobarbital (mg/day) in the First Trimester

Dose	N	Total Abnormal No.	Total Abnormal % [a]	Spontaneous Abortion No.	Spontaneous Abortion % [a]	Total Major Cong. Anomalies No.	Total Major Cong. Anomalies % [b]	Developmental Anomaly No.	Developmental Anomaly % [b]	Congenital Postural Deformity, Hernia No.	Congenital Postural Deformity, Hernia % [b]
≤ 30.0	11	3	27.3	1	9.1	2	20.0	2	20.0	0	0.0
30.1-60.0	15	3	20.0	0	0.0	3	20.0	0	0.0	3	20.0
60.1-90.0	10	3	30.0	1	10.0	2	22.2	1	11.1	1	11.1
>90.0	13	7	53.8	1	7.7	6	50.0	3	25.0	3	25.0
Total	49	16	32.7	3	6.1	13	28 3	6	13.0	7	15.2

[a] Percent of all pregnancies ; [b] Percent of viable pregnancies

Table V.14B

The Occurrence of Abnormal Pregnancy Outcome in Relation to Mean Dose Phenobarbital (mg/kg/day) Over All Trimesters Combined

Dose	N	Total Abnormal		Spontaneous Abortion		Total Major Cong. Anomalies		Developmental Anomaly		Congenital Postural Deformity, Hernia	
		No.	%[a]	No.	%[a]	No.	%[b]	No.	%[b]	No.	%[b]
< 0.50	7	1	14.3	0	0.0	1	14.3	1	14.3	0	0.0
0.50-0.99	14	3	21.4	1	7.1	2	15.4	1	7.7	1	7.7
1.00-1.49	16	6	37.5	1	6.3	5	33.3	3	20.0	2	13.3
≥ 1.50	14	6	42.9	1	7.1	5	38.5	1	7.7	4	30.8
Total	51	16	31.4	3	5.9	13	27.1	6	12.5	7	14.6

[a] Percent of all pregnancies; [b] Percent of viable pregnancies

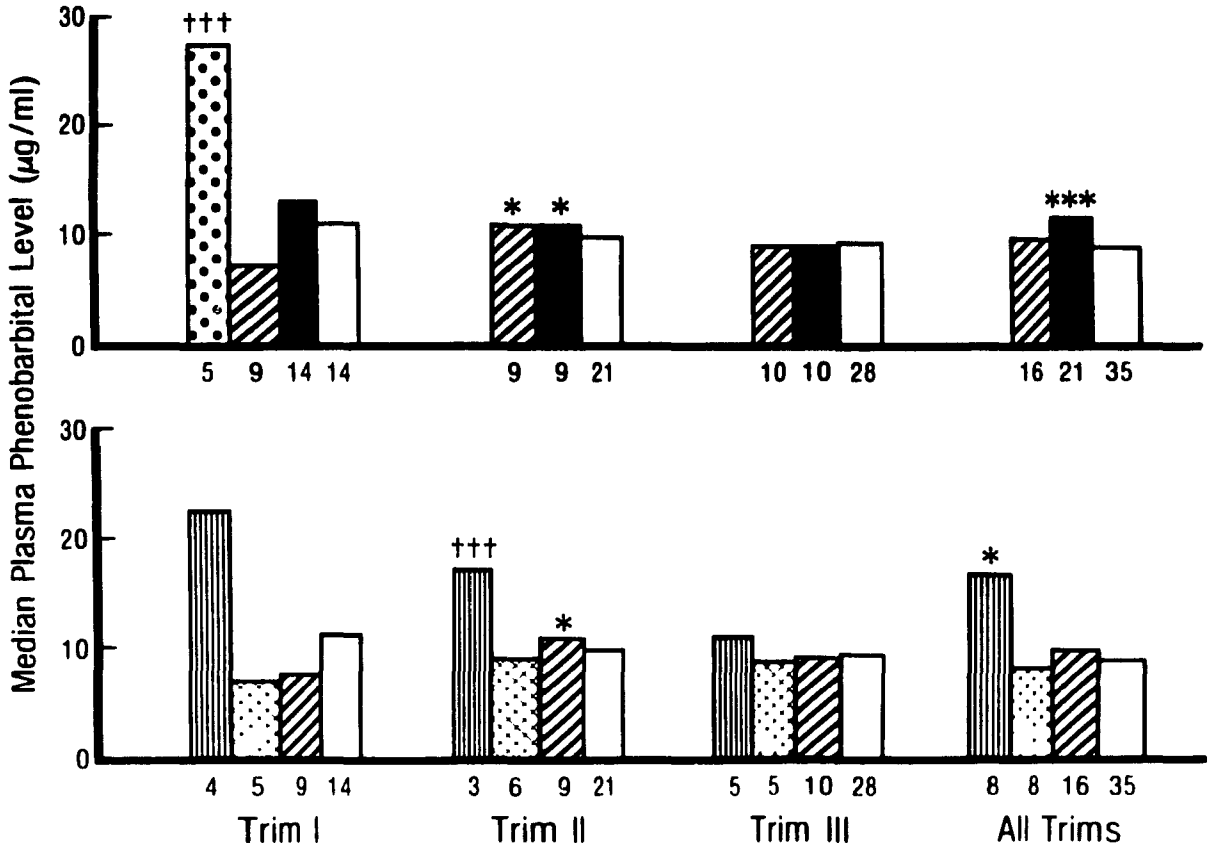


Figure V.3

Median of average values of plasma phenobarbital levels in each trimester and over all trimesters combined for pregnancies resulting in total abnormal outcomes ■, spontaneous abortions ⁘, total major congenital anomalies ▨, developmental anomalies ▥, isolated congenital postural deformities or hernias ▩, as compared to normal outcomes □. Numbers below bars are number of pregnancies monitored. * p≤ 0.10; *** p ≤ 0.02; ††† p ≤ 0.001.

abnormal outcomes (Table V.15A.). Over all trimesters combined, the data suggested a threshold effect with a high frequency of abnormal pregnancy outcomes noted in women with mean plasma PB levels greater than or equal to 20 µg/ml (Table V.15B). Women who had mean plasma PB levels ≥ 20 µg/ml over all trimesters combined were all on polytherapy, specifically PHT, PB and PRM (n=6); PHT and PB (n=3) and PB, PRM and CBZ (n=1).

(c) Primidone

Mean daily doses (mg) of PRM in the second and third trimesters and over all trimesters combined were significantly higher in women who had infants with DA than in women with normal infants (Figure V.4).

The risk of DA tended to increase with an increase in the mean daily dose (mg) of PRM in the first trimester or over all trimesters combined: < 250 (n=9), 0.0%; 250-500 (n=8), 42.9%; > 500 (n=6), 60.0%. This was not observed for PDH.

In the first trimester, PRM doses (mg/kg) for all women ranged from 3.0 to 14.3 (median: 9.3), and plasma PRM levels (µg/ml) ranged from 2.6 to 12.2 (median: 6.2). Two of three pregnancies resulting in spontaneous abortion showed the highest mean dose (mg/kg) and plasma levels (µg/ml) of PRM in the first trimester (9.3, 13.5, 14.3 mg/kg; 5.8, 8.4, 12.2 µg/ml), but the data were too few to make statistical comparisons with normal pregnancy outcomes (n=1, 4.6 mg/kg, 3.9 µg/ml).

The median average daily dose (mg/kg) of PRM over all trimesters combined was 9.7 (range 3.0-16.6) for total abnormal outcomes (n = 12) and 9.3 (range 5.7-12.5) for DA (n=6), as compared with 8.0 (range 2.0-9.5) for normal outcomes (n=9) ($p<0.10$).

Table V.15A

The Occurrence of Abnormal Pregnancy Outcome in Relation to Mean Plasma Phenobarbital Levels (µg/ml) in the First Trimester

Plasma Level	N	Total Abnormal No.	Total Abnormal %[a]	Spontaneous Abortion No.	Spontaneous Abortion %[a]	Total Major Cong. Anomalies No.	Total Major Cong. Anomalies %[b]	Developmental Anomaly No.	Developmental Anomaly %[b]	Congenital Postural Deformity, Hernia No.	Congenital Postural Deformity, Hernia %[b]
<10.0	13	6	46.2	0	0.0	6	46.2	2	15.4	4	30.8
10.0-19.9	9	2	22.2	1	11.1	1	12.5	0	0.0	1	12.5
≥20.0	6	6	100.0	4	66.7	2	100.0	2	100.0	0	0.0
Total	28	14	50.0	5	17.9	9	39.1	4	17.4	5	21.7

[a] Percent of all pregnancies; [b] Percent of viable pregnancies

Table V.15B

The Occurrence of Abnormal Pregnancy Outcome in Relation to Mean Plasma Phenobarbital Levels (µg/ml) Over All Trimesters Combined

Plasma Level	N	Total Abnormal		Spontaneous Abortion		Total Major Cong. Anomalies		Developmental Anomaly		Congenital Postural Deformity, Hernia	
		No.	%[a]	No.	%[a]	No.	%[b]	No.	%[b]	No.	%[b]
< 10.0	29	8	27.6	0	0.0	8	27.6	3	10.3	5	17.2
10.0-19.9	17	4	23.5	1	5.9	3	18.8	1	6.3	2	12.5
≥ 20.0	10	9	90.0	4	40.0	5	83 3	4	66.7	1	16.7
Total	56	21	37.5	5	8.9	16	31.4	8	15.7	8	15.7

[a] Percent of all pregnancies; [b] Percent of viable pregnancies

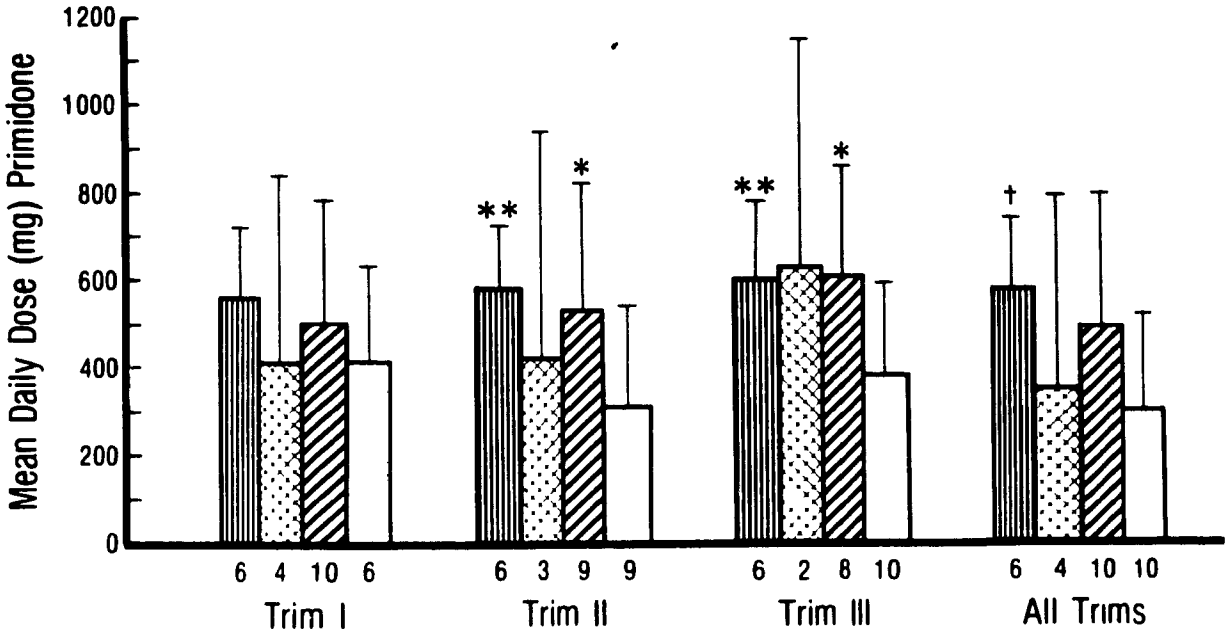


Figure V.4

Mean of average values of daily dose ± SD of primidone in each trimester and over all trimesters combined for pregnancies resulting in total major congenital anomalies ▨, developmental anomalies ▥, isolated congenital postural deformities or hernias ▩, as compared to normal outcomes |_. Numbers below bars are number of pregnancies monitored. * p≤ 0.10; ** p ≤ 0.05; † p ≤ 0.01.

The only significant differences in mean PRM plasma levels (µg/ml) were found in the second trimester for total major congenital anomalies (n=4, 5 ± 1.4 µg/ml) and PDH (n=2, 6.0 ± 0.1 µg/ml), as compared with normal outcomes (n=4, 2.7 ± 1.3 µg/ml) ($p<0.05$). The risk of DA tended to increase with mean plasma PRM levels for the entire pregnancy: 1 of 6 pregnancies (16.7%) with plasma levels below the therapeutic range (< 4 µg/ml) resulted in offspring with DA, as compared with 4 of 10 pregnancies (40.0%) with plasma levels in the therapeutic range.

(d) Other anticonvulsants

No significant correlations were noted between total abnormal pregnancy outcomes, spontaneous abortion, or total major congenital anomalies and mean maternal dose (mg or mg/kg) or plasma levels of CBZ, VPA, or ETH, among a small number of women who took these drugs during pregnancy. Numbers were too small to evaluate the association between these drugs and the specific outcomes DA or PDH.

(10) Folate studies

Blood folate levels [serum folate (SF) and red cell folate (RCF)] were studied in both pregnant (n=49) and nonpregnant (n=50) epileptic women in relation to AED, pregnancy, and pregnancy outcome, as outlined in Section F of Methods.

(a) Blood folate versus anticonvulsant therapy

Blood folate levels, outside of and during pregnancy, showed significant negative correlations with plasma phenytoin and phenobarbital levels among women who were taking one or more AED

(Table V.16). These trends were not observed in a small number of nonpregnant and pregnant women who were taking carbamazepine or valproic acid.

Nonpregnant patients on polytherapy had significantly lower SF ($p=0.05$) and RCF levels (ng/ml) ($p=0.003$) than did those on monotherapy (Table V.17). In women on polytherapy, the median RCF was 276 as compared with 602 for women on monotherapy. The median SF associated with polytherapy was 3.8, compared with 4.5 for monotherapy. Deficient SF (<4 ng/ml) and RCF (<175 ng/ml) levels also occurred more frequently in nonpregnant women on polytherapy (Table V.18).

There were no significant differences in blood folate concentrations among nonpregnant women taking specific AED monotherapies. Women taking PHT together with PB or PRM, with or without other AED, tended to show the lowest folate levels (Table V.17).

As folate intake differed in pregnant women receiving AED in monotherapy or polytherapy, the relationship between blood folate and the number of AED during pregnancy was analyzed according to folate use. In the group without folate supplementation, women on polytherapy more frequently exhibited deficient SF levels than did those on monotherapy (see Table V.18). There was also a trend towards lower folate levels (in the normal range) in women taking folate supplements and more than one AED.

(b) Blood folate during pregnancy

In women taking folate supplements during pregnancy (Figure V. 5), blood folate levels rose progressively to levels that were markedly

Table V.16: Serum (SF) and Red Cell Folate (RCF) Levels (ng/ml) in Relation to Plasma AED Levels (µg/ml) in Nonpregnant and Pregnant Epileptic Women (Spearman Rank Correlation Coefficient).

	Plasma Level[a]	
	Phenytoin	Phenobarbital
Nonpregnant		
N	27	17
SF	-0.59[g]	-0.13
RCF	-0.46[e]	-0.52[d]
Trimester I		
N	17	11
SF	-0.31	-0.21
RCF	+0.10	-0.06
Trimester II		
N	18	10
SF	-0.61[f]	-0.66[d]
RCF	-0.28	-0.68[d]
Trimester III		
N	18	11
SF	-0.24	0.0
RCF	-0.45[c]	-0.13
Puerperium		
N	11	4
SF	-0.51[b]	+0.32
RCF	-0.48[b]	0.0

[a] Percent plasma levels in therapeutic range: phenytoin 41%; phenobarbital 82%. Includes both monotherapy and polytherapy.

[b]$p<0.10$; [c]$p<0.05$; [d]$p<0.025$; [e]$p<0.01$; [f]$p<0.005$; [g]$p<0.001$.

Table V. 17: Serum and Red cell Folate Levels (ng/ml) in Nonpregnant Women According to AED Monotherapy and Polytherapy

		Serum Folate			Red Cell Folate		
	N	Median	Range	%<4	Median	Range	%<175
PHT Alone	10	4.5	1.8 - 8.9	40.0	611	<175 ->1000	10.0
PB Alone	3	5.4	4.6 - 8.4	0.0	685	207 - 962	0.0
CBZ Alone	7	4.2	2.3 - 5.5	42.9	567	315 - 878	0.0
VPA Alone	6	5.1	1.1 ->15.0	50.0	574	215 - 803	0.0
Total Monotherapy[a]	26	4.5	1.1->15.0	38.5	602	<175 ->1000	3.8
PHT + PB or PRM ± other AED	9	2.6	<1.0 - 8.6	77.8	258	<175 - 647	22.2
PHT + other AED (excluding PB, PRM)	8	4.1	1.0 - 5.8	50.0	288	175 ->1000	12.5
PB +/or PRM ± other AED (excluding PHT)	5	4.8	1.1 - 6.8	40.0	281	244 ->1000	0.0
Total Polytherapy	22	3.8	<1.0 - 8.6	59.1	276	<175 ->1000	13.6

[a]Percent plasma levels in optimal range: PHT 30%; PB 100%; CBZ 86%; VPA 83%.

Wilcoxon Rank Sum Test:	Serum Folate	Red Cell Folate
Total Polytherapy (n=22) vs. Monotherapy (n=26):	p=0.05	p=0.003
Total PHT combinations (n=17) vs. PHT alone (n=10):	p=0.06	p=0.04
Total PB combinations (n=14) vs. PB alone (n=3):	p=0.10	p>0.10

PHT=phenytoin; PB=phenobarbital; PRM=primidone; CBZ=carbamazepine; VPA= valproic acid

Table V.18

Percent Subnormal Serum (SF) and Red Cell Folate (RCF) Levels (ng/ml) in Nonpregnant and Pregnant Epileptic Women not Taking Folate Supplements.

	Monotherapy			Polytherapy			Total[a]		
		SF	RCF		SF	RCF		SF	RCF
	N	%<4	%<175	N	%<4	%<175	N	%<4	%<175
Nonpregnant	26	38.5	3.8	22	59.1	13.6	50	46.0	8.0
Trimester I	11	27.3	0.0	9	66.7[b]	0.0	21	52.4	4.8
Trimester II	5	40.0	20.0	6	66.7	16.7	11	54.5	18.2
Trimester III	4	75.0	25.0	7	85.7	28.6	11	81.8[d]	27.3[c]
Puerperium	3	66.7	33.0	3	66.7	0.0	6	66.7	16.7

[a] Includes 2 nonpregnant women and 1 pregnant woman not taking anticonvulsants.

[b] Fisher exact test: monotherapy vs. polytherapy, $p=0.05$.

[c,d] χ^2 corrected for continuity: trimester III vs. nonpregnant women, [c] $p<0.10$; [d] $p<0.05$.

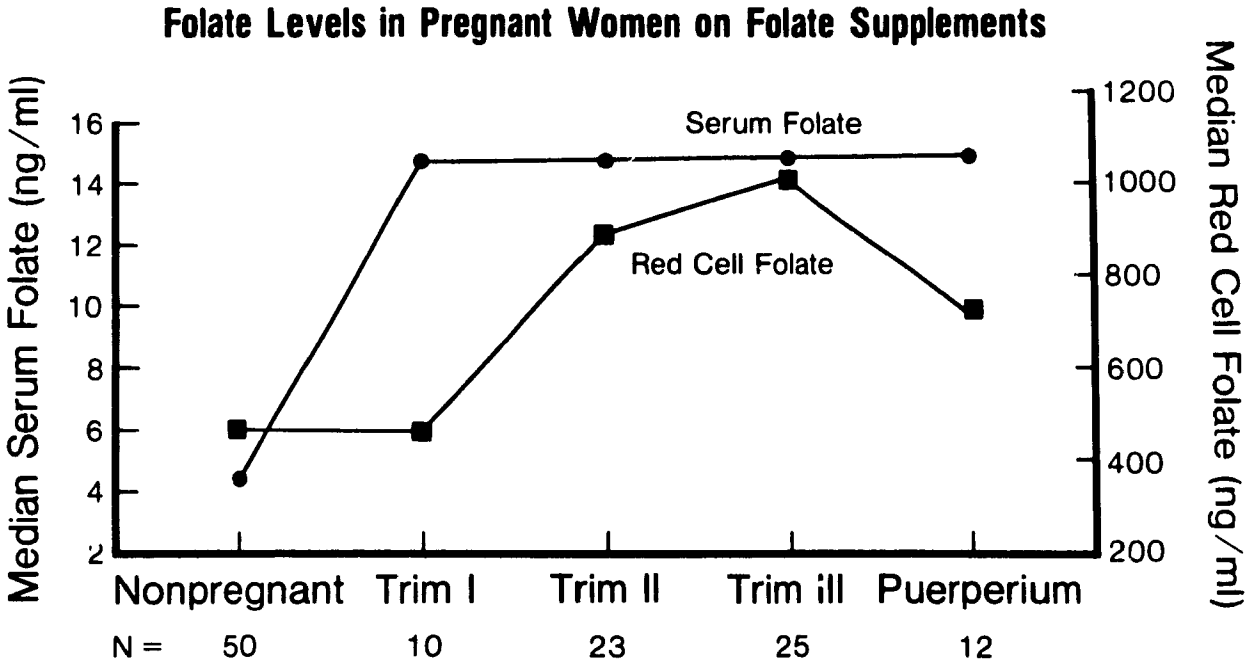


Figure V.5

Median folate levels in pregnant women on folate supplements as compared with those of nonpregnant women not taking such supplements. In the first trimester, serum folate levels rose from nonpregnant levels to the upper limit of the range reported by the laboratory. A progressive increase in red cell folate levels followed the rise in serum folate levels.

higher than those for nonpregnant women. Most of the women achieved SF levels above the normal range reported by the laboratory, and therefore the increase in SF levels after the first trimester, is not as apparent as that for RCF. Only one supplemented woman monitored serially during pregnancy had a single subnormal SF level in the second trimester.

In patients who were not taking folate supplements (see Table V.18), 46% of nonpregnant women had SF levels below 4, and 8% had RCF levels below 175. In the first trimester, the frequencies of low SF (52.4%) and RCF (4.8%) levels were similar to those for nonpregnant women. As the pregnancies advanced, the proportion of women with subnormal blood folate levels rose progressively to maximum values of 81.8% for SF and 27.3% for RCF in the third trimester. These frequencies tended to differ from those for nonpregnant women (SF, $p<0.05$; RCF, $p<0.10$).

(c) Outcome of pregnancy

Among 49 pregnancies in which folate levels were monitored, there were 9 children with major congenital anomalies (18.4%): 1 each with ventricular septal defect, hypertrophic cardiomyopathy with endocardial fibroelastosis and conduction defect, cleft lip and palate, hydrocephalus (Dandy-Walker cyst), hypospadias, and bilateral clubfoot; and 3 children with inguinal hernias and/or umbilical hernias. The malformed children were born to different women, except for 2 children with inguinal and/or umbilical hernias who were brothers. There were 4 early spontaneous abortions (8.2%) in 3 women and 1 ectopic pregnancy. Two pregnancies ended in therapeutic abortion; 1 of these was carried out at 19 weeks of gestation, following the prenatal diagnosis of a

Dandy-Walker cyst by ultrasound. The ectopic pregnancy and the therapeutic abortion with unknown embryopathology were excluded from further analyses.

(d) Folate and outcome of pregnancy

Median average SF and RCF levels (ng/ml) prior to and during pregnancy in relation to subsequent outcome are shown in Figures V.6 and V.7. RCF levels, in a small number of patients monitored prior to pregnancy, were significantly lower ($p < 0.10$) in women who had spontaneous abortions and in women who had children with major congenital anomalies than in those with normal outcomes. SF ($p < 0.10$) and RCF ($p < 0.02$) concentrations in these women were also significantly reduced for total abnormal outcomes. Median SF and/or RCF levels prior to pregnancy tended to be lower among women who gave birth to children with DA or PDH, but the differences were not statistically significant.

In the first trimester, women with spontaneous abortions tended to show lower SF and RCF levels than did women with normal outcomes Mothers of children with DA showed significantly lower SF levels ($p < 0.10$), while mothers of children with PDH showed significantly higher SF levels ($p < 0.10$), compared with mothers of normal children. In later trimesters and over all trimesters combined, relatively low SF and/or RCF concentrations persisted in women who had children with DA, while significantly higher SF concentrations were found for mothers of children with PDH.

SF levels plotted against RCF levels (ng/ml) for each pregnancy according to outcome are shown in Figure V.8. Prior to pregnancy, SF

Figure V.6

Median serum folate levels prior to pregnancy, and median average values of serum folate levels in each trimester and over all trimesters combined in relation to pregnancy outcome. Folate levels before pregnancy are the closest measurements to the last menstrual period (LMP) (1 to 272 days prior to LMP; median = 30 days). Numbers at bottom of bars are the number of pregnancies monitored. Asterisks refer to significance levels for Wilcoxon rank sum test comparing folate levels in pregnancies resulting in total abnormal outcomes ■, spontaneous abortions ⁘, total major congenital anomalies ▨, developmental anomalies ▥, isolated congenital postural deformities or hernias ▩ versus normal outcomes □: * $p \leq 0.10$, ' $p \leq 0.01$.

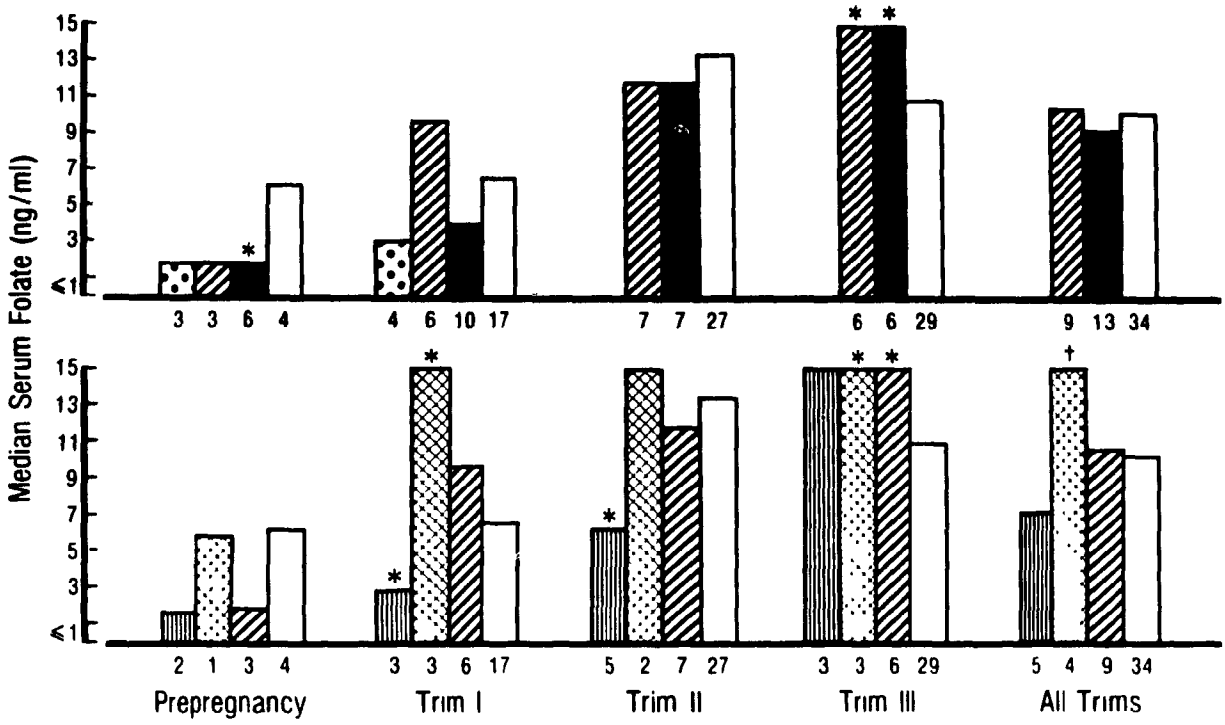
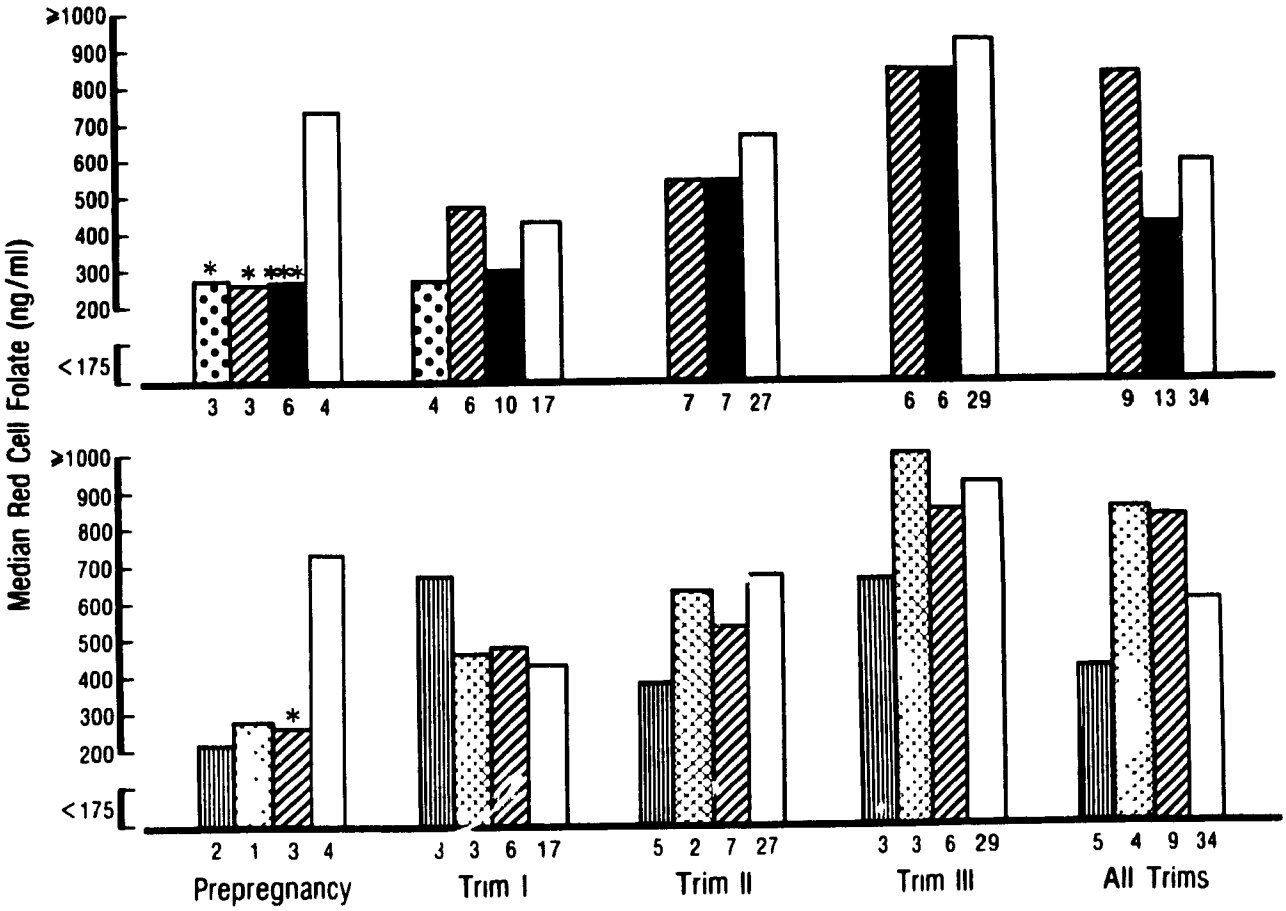

Median Serum Folate (ng/ml)
15
13
11
9
7
5
3
≤1
3 3 6 4
4 6 10 17
7 7 27
6 6 29
9 13 34
2 1 3 4
3 3 6 17
5 2 7 27
3 3 6 29
5 4 9 34
Prepregnancy
Trim I
Trim II
Trim III
All Trims

Figure V.7

Median red cell folate levels prior to pregnancy, and median average values of red cell folate levels in each trimester and over all trimesters combined in relation to pregnancy outcome. Folate levels before pregnancy are the closest measurements to the last menstrual period (LMP) (1 to 272 days prior to LMP; median = 30 days). Numbers at bottom of bars are the number of pregnancies monitored. Asterisks refer to significance levels for Wilcoxon rank sum test comparing folate levels in pregnancies resulting in total abnormal outcomes , spontaneous abortions , total major congenital anomalies , developmental anomalies , isolated congenital postural deformities or hernias versus normal outcomes : * $p \leq 0.10$, *** $p \leq 0.02$.


Median Red Cell Folate (ng/ml)
≥1000
900
800
700
600
500
400
300
200
<175
3 3 6 4
4 6 10 17
7 7 27
6 6 29
9 13 34
2 1 3 4
3 3 6 17
5 2 7 27
3 3 6 29
5 4 9 34
Prepregnancy
Trim I
Trim II
Trim III
All Trims

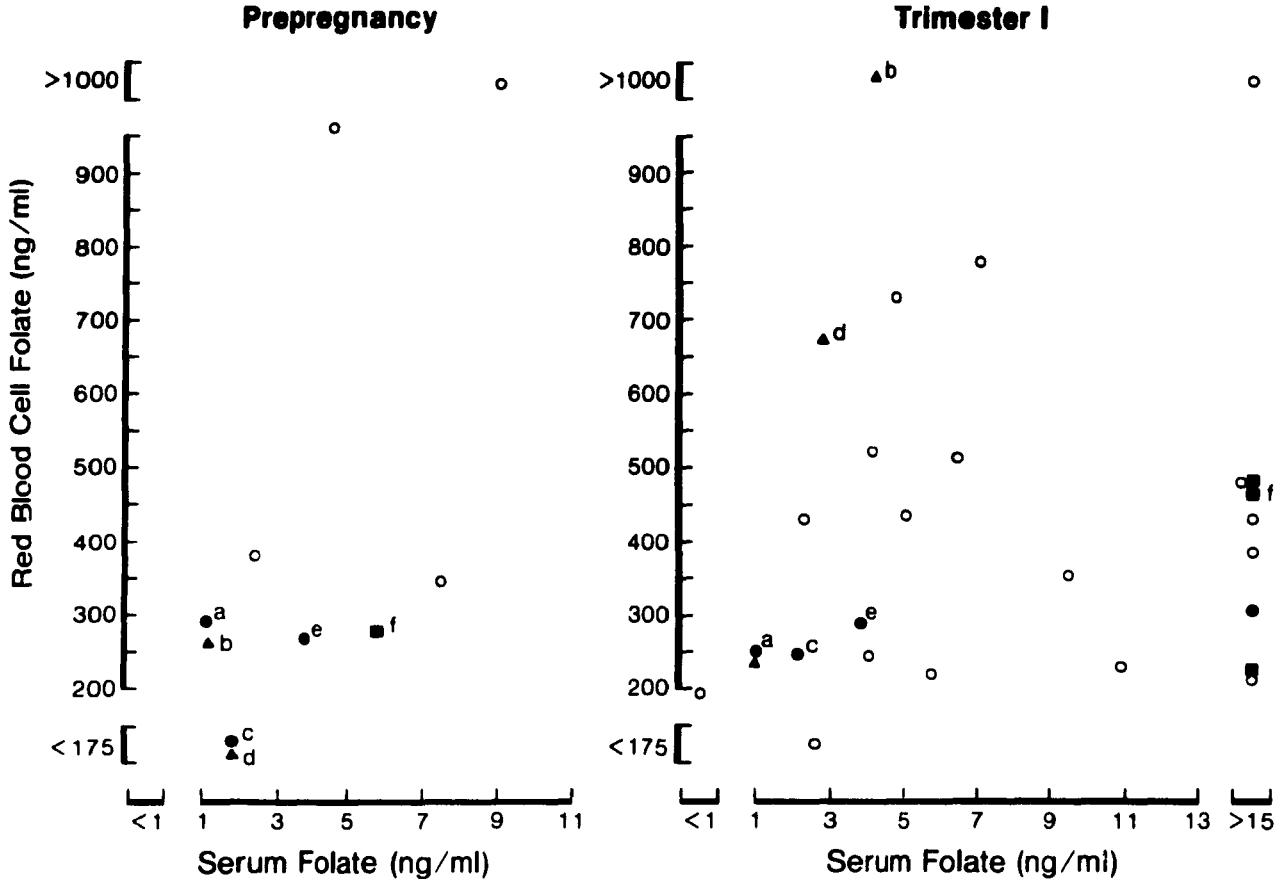


Figure V.8

Pregnancy outcome in relation to serum folate levels plotted against red cell folate levels before pregnancy and in the first trimester. Folate levels before pregnancy are the closest measurements to the last menstrual period (LMP) (1 to 272 days prior to LMP; median = 30 days) and those for the first trimester are mean values. o = normal outcome; ● = spontaneous abortion; ▲ = developmental anomaly; ■ = isolated congenital postural deformity, or hernia. a through f refer to pregnancies with abnormal outcome, in which folate levels were monitored both before pregnancy and in the first trimester.

and/or RCF levels in women who subsequently had a spontaneous abortion or a child with a developmental anomaly were low relative to those for women with normal outcomes. This was also apparent in the first trimester, although here abnormal pregnancy outcomes were not invariably associated with low values. In particular, women who gave birth to children with PDH showed high SF levels, while women who had spontaneous abortions or children with DA showed low or subnormal SF levels.

Prior to pregnancy, 2 women showed deficient RCF levels (<175 ng/ml): 1 of these pregnancies terminated in a spontaneous abortion and the other in the therapeutic abortion of a fetus with hydrocephalus. None of the women with abnormal pregnancy outcome showed deficient RCF concentrations in the first trimester, while 1 woman who had a normal child was folate-deficient (Figure V.8).

Spontaneous abortion and DA occurred more frequently when SF levels were less than 4 ng/ml prior to pregnancy, in the first trimester and/or second trimester (Figures V.8, V.9). Fifty percent of pregnancies with at least one subnormal SF level in the first or second trimester resulted in abnormal outcomes, compared with 19.2% of pregnancies with normal levels ($p<0.05$)(Figure V.9)

Evaluation of pregnancy outcome in relation to both SF and RCF levels (ng/ml) before pregnancy and in the first trimester showed certain trends (Figure V.10). The frequency of total adverse outcomes was highest in women with both SF less than 4 and RCF below the median level; intermediate when only one of the folate parameters, SF or RCF, was below these levels; and lowest in those with both measurements

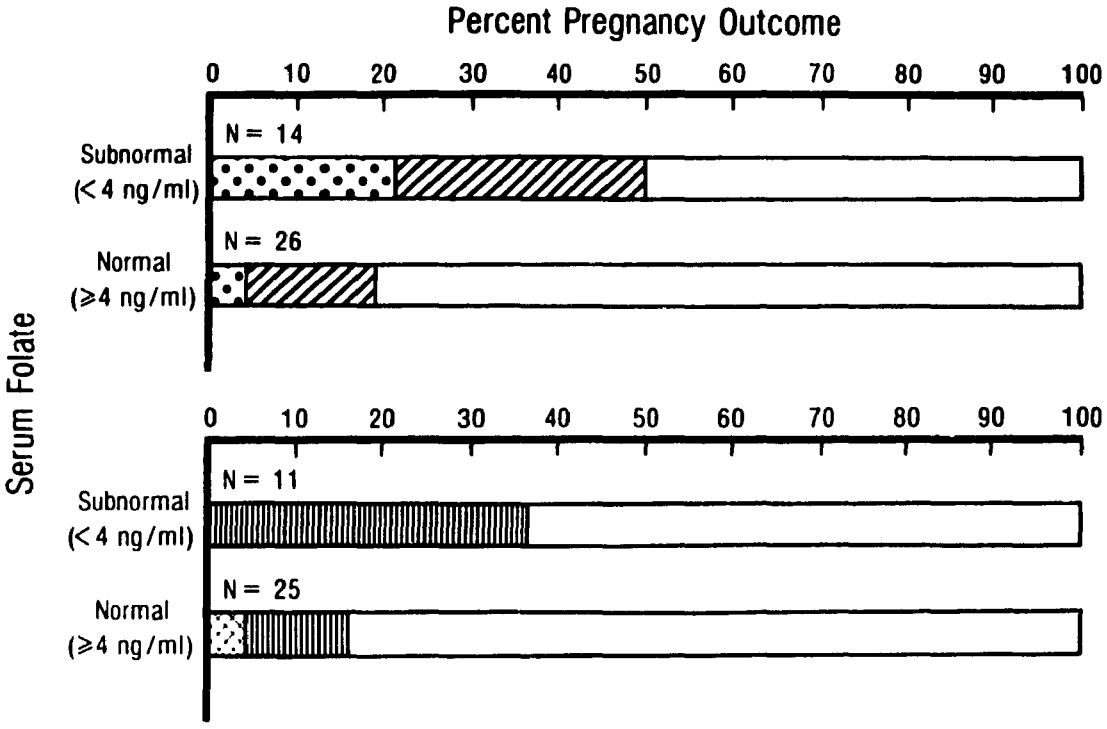


Figure V.9

Percent pregnancy outcome in relation to the presence of at least one subnormal serum folate level in the first and/or second trimesters dichotomized as less than 4 (subnormal) or at least 4 ng/ml. N = number of pregnancies with subnormal or normal serum folate levels. A significantly higher number of pregnancies with subnormal levels resulted in abnormal outcomes than did pregnancies with normal levels. (Using *chi*-square test , $p \leq 0.05$ for total abnormal pregnancy outcomes; $p \leq 0.10$ for spontaneous abortions; $p \leq 0.02$ for developmental anomalies)

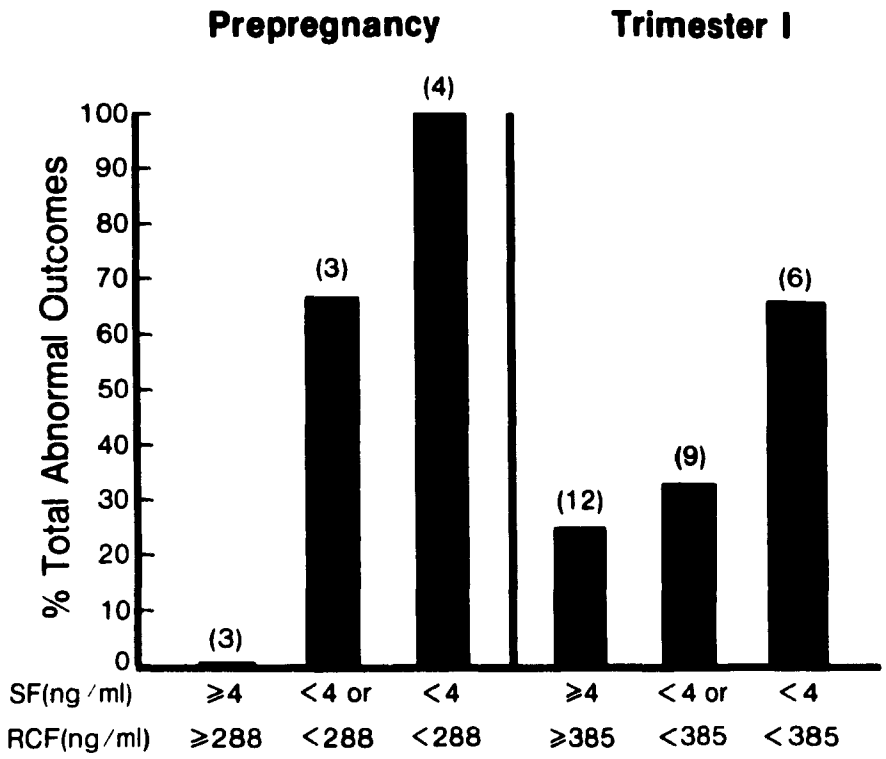


Figure V.10

Percent abnormal pregnancy outcome in relation to serum folate (SF) and red cell folate (RCF) levels before pregnancy and in the first trimester. Folate levels before pregnancy are the closest measurements to the last menstrual period (LMP) (1 to 272 days prior to the LMP; median = 30 days), and those for the first trimester are mean values. Numbers in parentheses = number of pregnancies monitored. SF levels are dichotomized as <4 and ≥4 and RCF levels as < or ≥ then the median level. Pregnancy outcome is analyzed in relation to relatively high values for both SF and RCF, low values in both measurements, and low levels of only one folate parameter. Significance prepregnancy : $p \leq 0.02$ for total abnormal pregnancy outcomes, *chi*-square test.

greater than or equal to these values (Prepregnancy: $p<0.025$). Similar statistically significant findings were also noted for DA in relation to both SF and RCF levels prior to pregnancy ($p<0.05$), and spontaneous abortion in relation to folate levels in the first trimester ($p<0.02$).

Among 94 pregnancies in which it was known whether or not vitamins were ingested in the first trimester, no vitamins were used in 43 pregnancies, folate supplements were taken in 50 pregnancies and vitamins of unknown type were taken in 1 pregnancy. Four spontaneous abortions occurred only among unsupplemented pregnancies (9.3%) ($p<0.05$).

(11) Detoxification defects of phenytoin arene oxide metabolites

There were twenty children and their parents in the present prospective study who had toxicity tests carried out on their lymphocytes (Table V.19). Five of the children had DA and 5 PDH according to the criteria of the present study, and were exposed to phenytoin, phenobarbital, primidone and/or other AED in utero .

Ten of the 20 children showed a positive response to challenge with phenytoin metabolites in vitro (cell death) (Table V.19). Each child with a positive assay also had at least one parent with a positive response, and there was no difference according to the sex of the parent. Forty percent of children with a positive response exhibited DA as compared to 10% of those who had a negative response. This difference was not statistically significant at the 0.10 level. The corresponding frequencies for PDH were 30% versus 20%. The results were similar when only those children exposed to PHT with or without other AED in utero (N=17) were considered.

Table V.19

Correlation of Major Congenital Anomalies with Response to In-Vitro Phenytoin Metabolite Challenge

Case No.[a]	Type of Anomaly	Positive Response (n=10)	Negative Response (n=10)
	Developmental Anomaly Group		
1.	VSD Hypospadias Umbilical hernia	+	
3.	VSD	+	
6.	Hypertrophic cardiomyopathy with endocardial fibroelastosis and conduction defect	+	
21.	Cleft lip		-
22.	Cleft lip + cleft palate	+	
	Isolated Congenital Postural Deformity or Hernia Group		
7.	Metatarsus adductus	+	
9.	Metatarsus varus	+	
10.	Talipes calcaneovalgus		-
12.	Talipes equinovarus Inguinal hernia Umbilical hernia	+	
14.	Inguinal hernia Umbilical hernia		-
	Total Major Congenital Anomalies (N=10)	7 +	3-
	Normal Group (N=10)	3 +	7-

[a] Case Numbers in Table V.2.

b. Multiple Logistic Regression Analysis

The results of multiple logistic regression analysis of total abnormal outcomes, total major congenital anomalies, and DA in relation to risk factors found to be statistically significantly associated with these outcomes by univariate analysis are given in sections VB2b(1) and VB2b(2) (Tables V.20 to V.23).

Section VB2b(1) and Table V. 20 present both the theoretical or proposed models and the models of risk factors found to be significantly associated with each type of pregnancy outcome following multiple logistic regression analysis. Section VB2b(2) and Tables V.21 to V.23 give the odds ratios and the 95% confidence intervals for the risk factors which remained significantly associated with each type of pregnancy outcome after multiple logistic regression analysis.

(1). Theoretical models and models of significant risk factors following multiple logistic regression analysis

In Table V.20 all factors which were entered into the theoretical models are shown in abbreviated form. In each theoretical model, factors in **BOLD TYPE** are those which were entered into that particular model, while factors in ***UNDERLINED BOLD ITALIC TYPE*** are those which maintained a significant association with adverse pregnancy outcome following multiple logistic regression analysis.

(a). Total abnormal outcomes

All the theoretical or proposed models for total abnormal outcomes included a history of one or more seizures in paternal third degree

Table V.20A: Multiple Logistic Regression Analysis of Risk Factors in Relation to Abnormal Pregnancy Outcome.

Risk factors entered into proposed model are in bold print. Risk factors significantly associated with abnormal pregnancy outcomes following multiple logistic regression analysis are in underlined bold italic print. N = total number of pregnancies contributing to model. Numbers in brackets refer to pregnancies resulting in abnormal/normal outcomes, respectively.

Total Abnormal Outcomes

1. **N=69 (20/49)**
   SZ3P; **PREGNORDER**; POLYA; ***PHTmgl***; PHTmgALL; PHTmg/kgALL; PHTPLALL; PBPLALL

2. **N=69 (21/48)**
   SZ3P; **PREGNORDER**; POLYA; PHTmgl; **PHTmgALL**; **PHTmg/kgALL**; ***PHTPLALL***; PBPLALL

3. **N=41 (17/24)**
   SZ3P; **PREGNORDER**; POLYA; PHTmgl; PHTmgALL; PHTmg/kgALL; PHTPLALL; ***PBPLALL***

4. **N=28 (12/16)**
   SZ3P; **PREGNORDER**; **POLYA**; PHTmgl; **PHTmgALL**; **PHTmg/kgALL**; **PHTPLALL**; ***PBPLALL***

Total Major Congenital Anomalies

1. **N=64 (15/49)**
   SZ3P; **MAJSZA**; ***PHTmgl***

Table V.20B: Multiple Logistic Regression Analysis of Risk Factors in Relation to Abnormal Pregnancy Outcome.

Risk factors entered into proposed model are in bold print. Risk factors significantly associated with abnormal pregnancy outcomes following multiple logistic regression analysis are in underlined bold italic print. N = total number of pregnancies contributing to model. Numbers in brackets refer to pregnancies resulting in abnormal/normal outcomes, respectively.

Developmental Anomalies

1. **N=60 (8/52)**
   SZ123P; **ALC**; **MINSZA**; PHTPBPRMMPA; ***PHTmgl***; PHTPLI; PHTmgALL; PHTmg/kgALL; PHTPLALL; PBPLALL

2. **N=27 (4/23)**
   SZ123P; **ALC**; **MINSZA**; PHTPBPRMMPA; **PHTmgl**; ***PHTPLI***; PHTmgALL; PHTmg/kgALL; PHTPLALL; PBPLALL

3. **N=60 (8/52)**
   SZ123P; **ALC**; **MINSZA**; PHTPBPRMMPA; PHTmgl; PHTPLI; **PHTmgALL**; **PHTmg/kgALL**; ***PHTPLALL***; PBPLALL

4. **N=20 (4/16)**
   SZ123P; **ALC**; **MINSZA**; **PHTPBPRMMPA**; PHTmgl; PHTPLI; **PHTmgALL**; **PHTmg/kgALL**; **PHTPLALL**; ***PBPLALL***

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relatives (**SZ3P**) and pregnancy order (**PREGNORDER**) as background factors (Table 20A). Other factors which were also entered in the models in various combinations were type of polytherapy at anytime during pregnancy (**POLYA**); mean daily dose (mg) of PHT in the first trimester (**PHTmgI**); mean daily dose (mg) of PHT over all trimesters combined (**PHTmgALL**); mean dose (mg/kg) of PHT over all trimesters combined (**PHTmg/kgALL**); mean plasma level (ug/ml) of PHT over all trimesters combined (**PHTPLALL**); and mean plasma level (ug/ml) of PB over all trimesters combined (**PBPLALL**).

The theoretical *model 1* included mean daily dose (mg) of PHT in the first trimester along with the background factors of family history of seizures and pregnancy order. There were 69 pregnancies in which full data was available on all three factors. The only risk factor which was significantly associated with total abnormal outcomes following multiple logistic regression analysis was mean daily dose (mg) of PHT in the first trimester (Table 20A). These results were not altered when the mean daily dose (mg) or plasma level (µg/ml) of PHT in the second trimester were also included along with factors in model 1.

The theoretical *model 2* included the background factors and mean dose (mg and mg/kg) and plasma levels of PHT over all trimesters. The mean plasma level (µg/ml) of PHT was the only factor significantly associated with total abnormal outcomes among 69 pregnancies analyzed by multiple logistic regression. (Table V.20A).

The proposed *model 3* included the background factors and mean plasma levels (µg/ml) of PB over all trimesters combined. In 41 pregnancies in which the relationship between these factors and total abnormal outcomes was analysed by multiple logistic regression

analysis, the plasma PB level was the only factor significantly associated with the outcome (Table V.20A).

When the number of AED in the first trimester or at anytime during pregnancy were analysed along with other factors in models 1 to 3, identical results to those above were obtained.

Analysis of the theoretical *model 4* among 28 pregnancies showed that the mean plasma level (µg/ml) of PB maintained a significant association with total abnormal outcomes, when this factor was entered with mean dose (mg and mg/kg) and plasma level (µg/ml) of PHT over all trimesters, and the type of polytherapy at anytime during pregnancy (PHT+PB vs PRM + other AED vs VPA + other AED vs CBZ + other AED excluding VPA or PRM vs other AED combinations) (Table V.20A).

Other risk factors in various combinations were analysed in relation to total abnormal outcomes by multiple logistic regression analysis, and were found not to be significantly associated with the outcome: these were the number of AED in the first trimester and at anytime during pregnancy; type of polytherapy in the first trimester (PHT + PB vs PRM + other AED vs VPA + other AED vs CBZ + other AED excluding VPA or PRM vs other AED combinations); mean daily dose (mg) and plasma level (ug/ml) of PB in the second trimester; mean dose (mg/kg) of PRM over all trimesters combined; and the presence of at least one subnormal serum folate level (ng/ml) in the first and/or second trimester(s). The data was not sufficient to evaluate the mean daily dose (mg) of PRM in the second trimester.

(b). Total major congenital anomalies

The risk factors entered into the proposed model for total major congenital anomalies were : a history of one or more seizures in paternal third degree relatives (**SZ3P**); the presence of one or more major seizures at anytime during pregnancy (**MAJSZA**); and mean daily dose (mg) of PHT in the first trimester (**PHTmgI**) (Table V.20A). Sixty-four pregnancies had complete data with respect to these factors. Mean daily dose (mg) of PHT in the first trimester was the only factor which was significantly associated with total major congenital anomalies following multiple logistic regression analysis. This association was maintained when the number of AED in the first trimester or at anytime during pregnancy, and mean daily dose (mg) and plasma level (µg/ml) of PHT in the second trimester were also entered into the same model.

Other risk factors, such as the type of polytherapy in the first trimester (PHT + PB vs PRM + other AED vs VPA + other AED vs CBZ + other AED excluding VPA or PRM vs other AED combinations); mean daily dose (mg) and plasma level (µg/ml) of PB in the second trimester; mean daily dose (mg) of PRM in the second trimester; and mean daily dose (mg) and plasma level (µg/ml) of PHT over all trimesters combined, were not found to be significantly correlated with total major congenital anomalies following multiple logistic regression analysis.

(c) Developmental anomalies

The background risk factors which were included in all the theoretical models for DA (Table V.20B) were a history of one or more seizures in paternal first to third degree relatives combined (**SZ123P**),

alcohol intake at anytime during pregnancy (**ALC**), and the presence of at least one minor seizure at anytime during pregnancy (**MINSZA**). Other risk factors entered in the theoretical models in different combinations were type of AED (PHT, PB, PRM vs PHT, PRM vs PHT, PB vs PHT alone vs PB alone) at anytime during pregnancy (**MPPHTPBPRMA**), mean daily dose (mg) of PHT in the first trimester (**PHTmgI**); mean plasma level (µg/ml) of PHT in the first trimester (**PHTPLI**); mean daily dose (mg) of PHT over all trimesters combined (**PHTmgALL**); mean dose (mg/kg) of PHT over all trimesters combined (**PHTmg/kgALL**); mean plasma level (µg/ml) of PHT over all trimesters (**PHTPLALL**); and mean plasma level (µg/ml) of PB over all trimesters (**PBPLALL**).

In the proposed *model 1* mean daily dose (mg) of PHT in the first trimester was included with the background factors history of seizures among paternal relatives; maternal alcohol use during pregnancy, and the presence of minor seizures at anytime during pregnancy. There were 60 pregnancies in which full data was available on all these factors. Mean daily dose (mg) of PHT was the only factor which remained significantly associated with DA following multiple logistic regression analysis (Table V.20B).

In the proposed *model 2*, mean plasma level (µg/ml) of PHT in the first trimester was entered along with the factors in model 1 (Table V.20B). There were 27 pregnancies which had complete data on these factors. Following multiple logistic regression analysis, mean plasma level (µg/ml) but not mean daily dose (mg) of PHT in the first trimester remained significantly associated with DA.

The significant associations were maintained in models 1 and 2 when the number of AED in the first trimester, the type of AED in the

first trimester (PHT, PB, PRM vs PHT, PRM vs PHT, PB vs PHT alone), and/or the mean daily dose (mg) of PHT in the second trimester were included together with factors in models 1 and 2.

In the theoretical *model 3*, mean dose (mg and mg/kg) and mean plasma level (µg/ml) of PHT over all trimesters combined were entered with the background factors In 60 pregnancies, mean plasma level (µg/ml) of PHT was the only factor to maintain a significant association with DA after multiple logistic regression analysis (Table V 20B). Adding the number of AED or the type of AED at anytime during pregnancy (PHT, PB, PRM vs PHT, PRM vs PHT, PB vs PHT alone) to the model did not change the results.

The theoretical *model 4* included all factors entered in model 3, together with the type of AED at anytime during pregnancy (PHT, PB, PRM vs PHT, PRM vs PHT, PB), and mean plasma level (µg/ml) of PB over all trimesters combined. Multiple logistic regression analysis of 20 pregnancies having complete data on all these factors revealed a single significant association between DA and mean plasma level (µg/ml) of PB over all trimesters combined (Table V.20B).

The type of polytherapy in the first trimester or at anytime during pregnancy (PHT + PB vs PRM + other AED vs VPA + other AED vs CBZ + other AED excluding VPA or PRM vs other AED combinations), and mean daily dose (mg) of PRM in the second trimester and over all trimesters combined, were not found to be significantly associated with DA after multiple logistic regression analysis. The data was insufficient to evaluate the contribution of mean plasma level (µg/ml) of PHT or PB in the second trimester, and the presence of at least one subnormal serum folate level (ng/ml) in the first and/or second trimester(s), or over the

entire pregnancy, to the occurrence of DA.

(d). Other pregnancy outcomes

Risk factors found to be significantly associated with spontaneous abortion and congenital postural deformities or hernias by univariate analysis were also analyzed in relation to these outcomes by multiple logistic regression analysis. There were no factors which remained significantly correlated with these outcomes following this analysis

(2). Relative risks of significant risk factors

As described in Methods (section IV.J2), the odds ratios (estimate of relative risk) were calculated for risk factors found to be significantly associated with adverse pregnancy outcome, according to the logistic model. These are presented in Tables V. 21 to 23. Because only one variable in each model was found to be significantly associated with the occurrence of abnormal pregnancy outcome by multiple logistic regression analysis, the odds ratio for this factor was not adjusted for any other variable in the model It was therefore thought more appropriate to refer back to the univariate stratified results in order to further examine the relationship between the occurrence of abnormal pregnancy outcome and increasing dose and plasma levels of PHT and PB

Relative risks and 95% confidence intervals for the risk factors found to be significantly associated with abnormal pregnancy outcomes following multiple regression analysis were calculated using the univariate stratified results (Tables V 12A, 13A,B, 15B) As can be seen, the relative risks using the univariate results (Tables V. 24 to 26) and those calculated according to the logistic model (Tables V 21 to 23) are quite different. This is due to the fact that the logistic model

Table V.21: Point Estimate of Relative Risk and 95% Confidence Intervals for Risk Factors Significantly Associated with Total Abnormal Outcomes Following Multiple Logistic Regression Analysis

Risk Factor (Abbreviation)	Relative Risk	95% Confidence Intervals
Mean daily dose (mg) PHT Trim I (PHTmg I)		
< 200.0 [a]	1.0	–
200.0–299.9	2.1	1.05[b] – 4.3
300.0–349.9	4.5	1.1 – 18.4
≥ 350.0	9.5	1.1 – 78.8
Mean plasma PHT level (µg/ml) all Trims (PHTPLALL)		
< 5.0[a]	1.0	–
5.0–9.9	2.2	1.1 – 4.6
≥ 10.0	5.1	1.2 – 20.7
Mean plasma PB level (µg/ml) all Trims (PBPLALL)		
< 10.0 [a]	1.0[c], 1.0[d]	–
10.0–19.9	4.3[c], 5.9[d]	1.6 – 11.6[c], 1.7 – 20.4[d]
≥ 20.0	18.2[c], 35.2[d]	2.5 – >100.0[c], 3.0–>100.0[d]

[a] Reference group in calculation of relative risk

[b] Shown at two decimal places to indicate that the lower limit of the confidence interval is greater than 1.0

[c] Model 3 for total abnormal outcomes, Table V.20A

[d] Model 4 for total abnormal outcomes, Table V.20A

Table V.22: Point Estimate of Relative Risk and 95% Confidence Intervals for Risk Factors Significantly Associated with Total Major Congenital Anomalies Following Multiple Logistic Regression Analysis

Risk Factor (Abbreviation)	Relative Risk	95% Confidence Intervals
Mean daily dose (mg) PHT Trim I (PHTmg I)		
< 200.0 [a]	1.0	-
200.0-299.9	2.3	1.05[b] - 5.0
300.0-349.9	5.3	1.1 - 25.2
≥350.0	12.1	1.1 - >100.0

[a] Reference group in calculation of relative risk

[b] Shown at two decimal places to indicate that the lower limit of the confidence interval is greater than 1.0

Table V. 23: Point Estimate of Relative Risk and 95% Confidence Intervals for Risk Factors Significantly Associated with Developmental Anomalies Following Multiple Logistic Regression Analysis

Risk Factor (Abbreviation)	Relative Risk	95% Confidence Intervals
Mean daily dose (mg) PHT Trim I (PHTmg I)		
< 200.0[a]	1.0	–
200.0-299.9	7.9	1.6 - 39.5
300.0-349.9	62.8	2.5 ->100.0
≥ 350.0	>100.0	4.0 ->100.0
Mean plasma PHT level (µg/ml) Trim I (PHTPLI)		
< 5.0[a]	1.0	–
5.0-9.9	15.8	1.4 - >100.0
≥ 10.0	>100.0	2.1 - >100.0
Mean plasma PHT level (µg/ml) all Trims (PHTPLALL)		
< 5.0[a]	1.0	–
5.0-9.9	4.0	1.4 - 11.8
≥ 10.0	16.1	1.9 ->100.0
Mean plasma PB level (µg/ml) all Trims (PBPLALL)		
< 10.0[a]	1.0	–
10.0-19.9	6.0	1.01[b] - 35.6
≥ 20.0	35.9	1.01 ->100.0

[a] Reference group in calculation of relative risk

[b] Shown at two decimal places to indicate that the lower limit of the confidence interval is greater than 1.0

Table V.24: Point Estimate of Relative Risk and 95% Confidence Intervals for Risk Factors Significantly Associated with Total Abnormal Outcomes - Calculated from Univariate Stratified Results

Risk Factor (Abbreviation)	Relative Risk	95% Confidence Intervals
Mean daily dose (mg) PHT Trim I (PHTmg I)		
< 200.0 [a]	1.0	–
200.0-299.9	1.3	0.3 - 6.3
300.0-349.9	2.4	0.6 - 9.5
≥ 350.0	3.5	0.8 -14.6
Mean plasma PHT level (ug/ml) all Trims (PHTPLALL)		
< 5.0[a]	1.0	–
5.0-9.9	1.5	0.7 - 3.3
≥ 10.0	2.0	0.9 - 4.4
Mean plasma PB level (ug/ml) all Trims (PBPLALL)		
< 10.0 [a]	1.0 [b], 1.0 [c]	–
10.0-19.9	0.9 [b], 0.7 [c]	0.3 - 2.4 [b], 0.2 - 2.9 [c]
≥ 20.0	3.3 [b], 3.4 [c]	1.8 - 6.1 [b], 1.7 - 7.0 [c]

[a] Reference group in calculation of relative risk

[b] Model 3 for total abnormal outcomes, Table V.20A

[c] Model 4 for total abnormal outcomes, Table V.20A

Table V.25: Point Estimate of Relative Risk and 95% Confidence Intervals for Risk Factors Significantly Associated with Total Major Congenital Anomalies - Calculated from Univariate Stratified Results.

Risk Factor (Abbreviation)	Relative Risk	95% Confidence Intervals
Mean daily dose (mg) PHT Trim I (PHTmg I)		
< 200.0 [a]	1.0	-
200.0-299.9	0.7	0.1 - 4.6
300.0-349.9	2.2	0.6 - 8.7
≥350.0	3.5	0.8 - 14.6

[a] Reference group in calculation of relative risk

Table V. 26: Point Estimate of Relative Risk and 95% Confidence Intervals for Risk Factors Significantly Associated with Developmental Anomalies - Calculated from Univariate Stratified Results

Risk Factor (Abbreviation)	Relative Risk	95% Confidence Intervals
Mean daily dose (mg) PHT Trim I (PHTmg I)		
< 200.0 — referent [a]	1.0	–
200.0-299.9 — referent [a]	1.0	–
300.0-349.9	6.1	0.8 - 46.9
≥ 350.0	9.9	1.2 - 85.0
OMean plasma PHT level (µg/ml) Trim I (PHTPLI)		
< 5.0 — referent [a]	1.0	–
5.0-9.9 — referent [a]	1.0	–
≥ 10.0	8.4	1.9 - 38.2
Mean plasma PHT level (µg/ml) all Trims (PHTPLALL)		
< 5.0[a]	1.0	–
5.0-9.9	3.5	0.7 - 16.6
≥ 10.0	5.7	1.2 - 27.8
Mean plasma PB level (µg/ml) all Trims (PBPLALL)		
< 10.0[a]	1.0[b], 1.0[c]	–
10.0-19.9	1.0[b], 0.6[c]	0.1 - 9 6[b], 0.1 - 5.3[c]
≥ 20.0	6.0[b], 6.4[c]	1.4 - 24.9[b], 1.9 - 21.7[c]

[a] Reference group in calculation of relative risk. [b] Relative risk calculated for plasma PB levels among patients taking PRM and/or PB, PHT ± other AED. See Model 4 for developmental anomalies, Table V. 20B. [c] Relative risk calculated for plasma PB levels in all patients on PB monotherapy or PB polytherapies.

assumes a log linear relationship between the risk factor and outcome and because the risk factors, dose and plasma levels of PB and PHT, were scaled variables in the logistic model. Furthermore, the odds ratio, an estimate of the relative risk, always provides a point estimate further from one than does the relative risk. Finally, patients that had data on all risk factors in the modelled analysis constituted a subset of the patients included in the univariate stratified results on dose and plasma levels of PHT and PB.

As indicated by the relative risk calculated from the univariate results, the risk of total abnormal pregnancy outcomes increased with increasing dose (mg) of PHT in the first trimester and mean plasma levels (µg/ml) of PHT over all trimesters combined (Table V.24). Similar findings were noted with respect to the occurrence of total major congenital anomalies in relation to increasing mean daily doses (mg) of PHT in the first trimester (Table V.25).

As shown in Table V.26, the associations between DA and mean daily dose (mg) or plasma levels (µg/ml) of PHT in the first trimester and over all trimesters combined were stronger than that for total abnormal outcomes or total major congenital anomalies: both the point estimates (ranging from 3.5 to 9.9) and the lower limits of the 95% confidence intervals of the relative risks of DA at different levels of these factors (ranging from 0.7 to 1.9) were larger than those for the broader categories of adverse pregnancy outcomes. The relative risk of DA increased with increasing dose and plasma levels of PHT.

A threshold effect was noted with respect to the occurrence of DA and total abnormal outcomes in relation to mean plasma PB levels over all trimesters combined: the relative risk of both these outcomes was

greater than one only in women who had mean plasma PB levels ≥ 20 ug/ml (Tables V. 24 and V. 26.). The point estimate of the risk of DA associated with PB levels ≥ 20µg/ml was strong (6.0) and higher than that associated with total abnormal outcomes.

C. Discussion

1. *Frequency of Major Congenital Anomalies*

This prospective study confirms previous investigations showing that major congenital anomalies occur more frequently in the offspring of women with epilepsy taking AED than in the general population. In the present series the overall risk of total major congenital anomalies (25.5%) was 6-13 times higher than the risk of 2-4% in the general population. This is higher than the 2-3 fold increased risk reported in many prospective as well as historical cohort studies (see Tables II.3 to II.6 in Literature Review). Several other studies, however, have shown relatively high malformation risks of 15 to 25% (Biale et al., 1975; Hill, 1979; Hill and Tennyson, 1982b; Hill et al., 1974; Majewski et al., 1981). In evaluating these findings, several factors should be considered. These include the make-up of the patient population such as severity of the epilepsy; type of AED regimen; genetic background; and methods including definition of major congenital anomalies and length of follow-up of the children. For example, many of the women followed in the 1970's in the present study were prescribed older drug regimens , particularly polytherapies, whereas the newer drug regimens (e.g., CBZ and VPA monotherapies) were less represented in the present series, as compared to other studies.

Previous studies have shown an increase in congenital heart disease and/or cleft anomalies in the offspring of women with epilepsy (see Table II.6, Literature Review), whereas an increase in major anomalies of other organ systems (central nervous system; musculoskeletal; urogenital; gastrointestinal) has been less consistently demonstrated. In our series, the most common major congenital anomalies were

congenital heart disease, clubfoot, hypospadias; and inguinal and/or umbilical hernias. Clubfoot is generally considered to represent a major congenital anomaly, while inguinal and umbilical hernias have previously been considered as either major (Myrianthopoulos and Chung, 1974) or minor anomalies (Heinonen et al., 1977; Shapiro et al., 1976). These anomalies are variable in degree and etiology, and at times may be easily corrected. Although in the present series not all cases of inguinal or umbilical hernias were severe enough to require surgical correction, it was felt that less severe to more severe forms of these particular anomalies all had a similar developmental basis, and were therefore included as major congenital anomalies. Minor degrees of clubfoot were excluded from the present series as these were felt to be most easily explained by intrauterine compression. Taken together, the 15 children with corrected clubfoot, inguinal and/or umbilical hernia, out of a total number of 28 children with major congenital anomalies (54%), contributed considerably to the high frequency of major congenital anomalies in the present series. Majewski et al. (1981) found that inguinal and/or umbilical hernias (without dysmorphic features) were the most frequent anomaly found among offspring of epileptic women as well as in offspring of epileptic men, and considered these anomalies to be not specific to maternal epilepsy.

Many congenital anomalies may be asymptomatic or missed at birth, and therefore the length of follow-up could influence the recognized prevalence of these anomalies. One of 6 children with congenital heart disease and 11 of 15 children with PDH were diagnosed after the neonatal period.

2. *Abnormal Pregnancy Outcome in Relation to Anticonvulsant Therapy*

All major congenital anomalies were studied as one group and were then further subdivided into those anomalies which were thought to represent a disturbance in embryological or fetal development ("Developmental" anomaly group; for example, closure of the ventricular septum) and into those anomalies in which mechanical factors may also play a role along with other genetic and/or environmental factors ("Isolated congenital postural deformity or hernia" group; for example, uterine constraint leading to congenital dislocation of the hip in an infant with inherited laxity of the joints). In this way, it was hoped to maximize the likelihood of detecting an association between specific AED and maldevelopment, and to determine whether anomalies observed in offspring of epileptic women may have heterogeneous etiologies.

The exact time of origin of some major congenital anomalies is not entirely clear. Although abnormalities such as cleft lip have defined critical periods early in pregnancy (Warkany, 1971), others such as ventricular septal defect (Mitchell et al.,1967), patent ductus arteriosus, anomalies of the valves of the heart and great vessels (Warkany,1971), and clubfoot (Fripp,1976) may be due to various disturbances in early or late prenatal life. Thus the occurrence of major congenital anomalies was examined in relation to the presence of possible risk factors not only in the first trimester, the critical period of embryogenesis, but also later in pregnancy.

A number of studies have shown that the risk of major congenital malformations and/or minor anomalies tends to be greater with the

combined as opposed to single use of AED. (Fedrick, 1973 ; Hill et al.,1982; Kallen 1986 ; Kaneko et al., 1988; Koch et al.,1983 ; Lindhout et al.,1982,1985b; Majewski et al.,1981; Miyakoshi and Seino, 1984; Nakane et al., 1980; Rating et al.,1982a,1985,1987a,b). The greater risk associated with polytherapy may be due to the number of drugs, larger doses of specific drugs taken in combination, and/or drug interactions (Kaneko et al.,1988; Lindhout et al., 1984; Rating et al 1982b)

In the present study, univariate analysis showed that overall there was no difference in the frequency of abnormal pregnancy outcome according to whether the woman took one or more AED. However, significant differences in pregnancy outcome were found among different polytherapies, and among the specific AED type(s) PHT,PB,PRM vs PHT,PRM vs PHT, PB vs PHT alone vs PB alone, with the highest frequency of abnormal outcome associated with PHT,PB,PRM. Abnormal pregnancy outcome was also associated with increased dose and/or plasma levels of PHT, PB, and PRM, among other epileptic and nonepileptic variables. Following multiple logistic regression analysis, the occurrence of abnormal pregnancy outcome was found to correlate consistently only with increased dose or plasma levels of PHT and plasma PB levels. Dose or plasma levels of specific AED appeared to be more highly correlated with adverse pregnancy outcome than the actual number or type(s) of AED. For example, in models where the number of AED, type of polytherapy or specific type(s) of AED, and dose or plasma levels of PHT and/or PB were entered, only the dose or plasma levels of AED remained significantly associated with adverse pregnancy outcome after multiple logistic regression analysis. These findings did not

exclude the fact that certain drug combinations appeared to contribute to high plasma levels of PB. (see discussion concerning abnormal pregnancy outcomes and the drug combination PHT, PB and PRM below).

In models of pregnancies of women who took PHT in monotherapy or polytherapy , total abnormal outcomes were significantly positively associated with increased mean daily dose (mg) of PHT in the first trimester or mean plasma PHT levels (µg/ml) over all trimesters combined. Among all women who took PB and/or PRM (which is metabolized to PB) with or without other AED, increased mean plasma PB levels (µg/ml) over the entire pregnancy were also significantly associated with total abnormal outcomes. For women who took any type of polytherapy involving PHT and PB (the latter taken orally or derived metabolically from PRM) at anytime during pregnancy, mean plasma PB levels over all trimesters combined was the only factor to maintain a significant association with abnormal pregnancy outcome above other factors entered into the model.

Multiple logistic regression analysis also showed that total major congenital anomalies were positively associated with the mean daily dose (mg) of PHT in the first trimester.

Following multiple regression analysis, the risk of DA in the offspring was significantly associated with the mean plasma level of PHT in the first trimester, among all women who took PHT in monotherapy or polytherapy in the first and second trimesters. This association was not altered by the inclusion of the number of AED, PHT dose in the first or second trimesters, or plasma PHT levels in the second trimester. This suggests that the level of PHT in maternal plasma at the critical time of embryonic development is the single most

important criterion associated with the occurrence of aberrant development. That the plasma level of PHT appeared to be more highly correlated with DA than the dose, suggests the possible influence of individual differences in handling of phenytoin, and of drug interactions

In women who took PHT in monotherapy or polytherapy at anytime during pregnancy, the occurrence of DA was also significantly associated with mean plasma PHT levels over all trimesters combined after multiple logistic regression analysis Among women who took PHT, PB, PRM versus PHT, PRM versus PHT, PB at anytime during pregnancy, the mean maternal plasma PB level (µg/ml) was the single most important factor associated with the occurrence of DA in their offspring (see model 4, Table V.20B). Thus both plasma levels of PHT and PB may be important with respect to the risk of DA.

Women who took PHT, PB, PRM had among the highest mean plasma PB levels over all trimesters combined (ranging from 21 0 to 49.2 µg/ml) of all women monitored for this drug. The mean daily dose (mg) of PRM over all trimesters combined also tended to be relatively high in these women ranging from 353 to 1000 mg/day (mean. 640 ± 212) Plasma PHT levels (µg/ml) ranged from 4.2 to 10 3 (median : 7.7). The high plasma PB levels associated with abnormal pregnancy outcome could have been due to the higher dose of PRM taken in combination with PHT and PB. The latter drugs may influence the disposition of PRM. It is known that PRM is metabolized to give rise to PB plus another metabolite with AED properties, phenylethylmalonic acid (PEMA) Patients taking PRM alone have especially high PB levels, as the ratio of PB to PRM plasma levels is in the order of 1 4/1 (Fincham and Schottelius, 1982). Phenobarbital, as metabolite or co-medication, may

stimulate the biotransformation of PRM. The conversion ratio is increased to 3.8/1 when PRM is employed in combination with PHT (Fincham and Schottelius, 1982). The latter may either promote the induction of an enzyme system responsible for the oxidation of PRM or inhibit PB metabolism and/or excretion (Fincham and Schottelius, 1982).

These pharmacological observations are consistent with the high risk of major malformations associated with the use of PHT and PRM during pregnancy, particularly when combined with PB, noted in this study and by Hill et al (1974). Craniofacial dysmorphic features have also been observed to be associated with the combination of hydantoins and barbiturates (Majewski et al , 1981 ; Rating et al., 1982b).

Univariate analysis of risk factors for spontaneous abortion showed that relatively high doses (mg) of PRM at the last menstrual period, high plasma levels of PB in the first trimester, and low serum folate levels prior to pregnancy and in the first trimester as well as other factors, such as elevated paternal age and family history of congenital malformations, were significantly associated with subsequent spontaneous abortion. Low serum folate levels have been associated with spontaneous abortion in the general population, and it is thought that inadequate levels may interfere with growth and development of the placenta and embryo (Hibbard, 1975). Phenobarbital induces the metabolism of endogenous steroids, and it is possible that alteration of hormone status may lead to interruption of pregnancy and spontaneous abortion (Fishman and Yanai, 1983).

No results were obtained for the multiple logistic regression analysis of spontaneous abortion in relation to risk factors, as the number of spontaneous abortions with complete data on all risk

factors was inadequate.

The number of pregnancies contributing to the proposed models for PDH were of sufficient size, but none of the factors found to be significantly associated with PDH anomalies by univariate analysis emerged as significant correlates of these anomalies (at the 0.05 significance level) following multiple logistic regression analysis. This could be due to the fact that the univariate analysis of risk factors in relation to PDH involved a different set of pregnancies from the set of pregnancies for which there were full data on all risk factors in a particular theoretical model Moreover, factors significant at the 0.10 level by univariate analysis, and which may have been significant at this level in a model analyzed by multiple logistic regression, would not have been maintained in the model, as the cut off point for retaining variables in the multiple regression model was chosen as 0 05.

Univariate analysis showed that relatively high plasma PHT levels (µg/ml) were not associated with PDH anomalies. In fact, women who had children with PDH anomalies showed significantly decreased plasma PHT levels in the first trimester, in contrast to the findings for DA.

Risk factors significantly associated with PDH by univariate analysis included increased mean daily dose (mg or mg/kg) of PB in the second trimester and over all trimesters combined, although not plasma PB levels It is of interest that previous workers have found an elevated frequency of clubfoot in infants exposed to PB in utero (Deblay et al.,1982; Desmond et al., 1972). Desmond and associates (1972) speculated that decreased fetal movements due to sedation may lead to persistence of the intra-uterine position after birth.

Univariately, it was noted that a family history of concordant anomalies appeared to be associated to some extent with the occurrence of PDH among the offspring, and this usually involved a sibling of the affected child. It is not known whether the concordant findings among sibs were due to the effects of maternal AED medication , other common environmental factors, and/or a genetic predisposition to specific congenital anomalies In contrast, there were no concordant anomalies among families of children with DA, suggesting that the effects of AED may operate independently of the genetic background. This was also confirmed by multiple logistic regression analysis which showed that family history of any type of major congenital anomaly or of seizures was not an important factor in the risk of DA.

In the univariate and multivariate results, a dose-response relationship was observed between the risk of total abormal outcomes, total major congenital anomalies, or developmental anomalies, and dose and plasma levels of PHT and/ or PB. However, the strength of the relative risks in the univariate results were smaller than those calculated according to the logistic model. This is due to the fact that 1) The odds ratio of the modelled analysis gives a point estimate further from one than does the relative risk 2) In the modelled analysis, the AED exposure is not treated as a categorical variable, but as a scaled variable in which the increase in the risk of abnormality associated with increments of the dose is log linear. 3) The patients comprising the modelled analysis are a subset of the patients included in the univariate analysis.

The point estimates in the modelled and univariate analyses were stronger for developmental anomalies than for total abnormal outcomes,

indicating a stronger association between AED exposure and the specific outcome DA than with the broader category of total abnormal outcomes (which included specific pregnancy outcomes which were not significantly associated with increased dose or plasma levels of PHT, such as the outcome PDH)

Due to the small numbers of pregnancies at different plasma levels or dosages of AED, the point estimates of the odds ratios in the modelled analysis and of the relative risks in the univariate analysis were unstable, as indicated by the wide confidence intervals Larger sample sizes for specific pregnancy outcomes would be required to provide more precise estimates of the relative risks associated with increasing plasma PHT and PB levels

Univariate analysis provided evidence for a dose-response relationship between PRM and the occurrence of abnormal pregnancy outcome, particularly spontaneous abortion and developmental anomalies, although this was not apparent following multiple regression analysis. Failure to detect such a relationship may have been due to small numbers. Plasma PRM levels were not as well correlated with adverse outcome as was dose administered Because of the short half-life of PRM, it would be necessary to monitor plasma levels at short constant intervals following administration of the drug, in order to adequately assess the relationship between plasma levels and the occurrence of malformations.

The present study did not detect a difference in the occurrence of malformations among different monotherapies, including VPA and CBZ However, the prevalence of malformations associated with each individual monotherapy was greater than the general population prevalence of 2-4% Therefore, a role for VPA and CBZ in the genesis of

major congenital malformations could not be excluded. An association between dose and plasma levels of VPA, CBZ or ETH and an increased risk of major congenital anomalies was not observed in the present study, and this could have been due to the small numbers of women taking these drugs in the present series. Most other studies have not found ETH or CBZ to be associated with an increased risk of major congenital anomalies, except for CBZ in certain drug combinations (Lindhout et al., 1984; Kaneko et al.,1988). Recently, however, Jones and associates (1989) have implicated CBZ monotherapy in the occurrence of an altered pattern of growth and development, but this is the only study to have done so. There is also data suggesting that CBZ monotherapy may also be associated with an increased risk of spina bifida (Lindhout 1988, Lindhout and Meinardi,1985). Valproic acid has been shown to be associated with spina bifida and possibly other major congenital malformations (Robert and Gibaud, 1982; Robert and Rosa, 1983)

Few previous studies have investigated a dose-response effect between AED and congenital malformations. Several studies found no relationship between the occurrence of congenital malformations and maternal daily AED dose (Biale et al.,1975; Cvetko, 1969; Kaneko et al ,1988; Koch et al.,1983; Martin,1978; Speidel and Meadow, 1972). In contrast Nakane et al. (1980) from the collaborative study in Japan, noted that the risk of malformations increased with the total amount of AED taken during the first or second trimester of pregnancy (total "drug score" based on both the number and dose of AED). Kaneko et al. (1988), in a later collaborative prospective study in Japan, also found a significant positive relationship between the "drug score" and the risk of congenital malformations, which he attributed to the adverse effects

of polytherapy, as women on polytherapy had a higher "drug score" than those on monotherapy. With respect to individual AED, major and/or minor congenital malformations have been found to be more frequent with higher doses of valproate (Rating et al., 1987a,b), phenobarbital (Béthenod and Fréderich, 1975; Fedrick, 1973, Nakane et al, 1980; Nelson and Forfar, 1971); mephophenobarbital (Nakane et al, 1980), primidone (Nakane et al, 1980, Rating et al., 1987a,b), and the metabolite/ primidone ratio (Rating et al., 1982b), but not phenytoin (Monson et al., 1973; Nakane et al., 1980).

The present study is the first clinical report of a significant positive correlation between plasma AED levels and the risk of spontaneous abortion or major congenital anomalies following univariate and /or multivariable analyses. Only a few other studies have attempted to correlate plasma AED levels with the occurrence of congenital malformations (using univariate analyses) Hiilesmaa et al. (1983), in Finland, did not find any relationship between plasma AED and the occurrence of major birth anomalies (n=10) among 133 pregnancies. According to the classification of the present study, the anomalies included 4 PDH and 6 DA . The lack of correlation may have been due in part to the timing of monitoring of plasma AED levels about one-quarter of the pregnancies were monitored by 12 weeks of gestation as compared with 49% of the pregnancies in the present study Oguni (1989) found a significant positive correlation between plasma VPA levels and the risk of major congenital malformations Similar findings have been reported by Rating et al, 1987a and Lindhout et al, 1985b Gaily and associates (1988a) showed that the occurrence of minor anomalies, such as digital hypoplasia, was positively correlated with

plasma PHT levels. Rating et al. (1987a,b) did not find a significant correlation between plasma levels of PHT, PB or PRM and minor birth anomalies, although these were significantly positively correlated with dose (mg/kg) of the latter drug.

Although there are many problems in extrapolating animal data to humans, experimental findings support some of the findings of the present study. In a mouse model of tonic-clonic convulsions (Finnell, 1980,1981, 1982), the frequency of defects correlated positively with plasma PHT levels within the mouse therapeutic range (2-10 µg/ml), but not with the frequency of maternal seizures. An increased frequency of defects was evident even at the lower limit of this range.

A few collaborative studies have attempted to examine the relative contribution of maternal AED therapy and other epileptic and nonepileptic variables to pregnancy outcome by more complex methods than univariate analysis. In the U.S. Collaborative Perinatal project, Monson and associates (1973) found that early regular use of PHT was significantly associated with the occurrence of major congenital malformatons, after taking into account potential confounding factors. However, a dose-response relationship was not established for this drug. In a collaborative Japanese study of 458 livebirths (54 with malformations) to epileptic women taking AED, ascertained retrospectively or prospectively (Nakane and Nakamura, 1982; Nakane et al.1984), multiple logistic regression analyses showed that the nonepileptic variables: presence of maternal malformation and previous pregnancy outcome (miscarriage, stillbirth), and the epileptic variables: absence of organic cause of maternal epilepsy; frequency of maternal seizures; maximum daily total drug "score" (based on the number and

doses of specific drugs); and the use of primidone, trimethadione, acetylpheneturide, or sulthiame (but not phenytoin), were the combination of variables which correlated with the occurrence of congenital malformations.

In the Japanese study, different results were obtained when only the pregnancies followed prospectively were studied (151 livebirths , 21 with malformations) (Nakane, 1982; Nakane et al.,1984) by step-wise multiple discriminant analysis. Maternal use of trimethadione, acetylpheneturide or phenytoin, as well as the maximum daily total drug "score", and previous pregnancy outcome (artifical abortion, stillbirth, miscarriage, livebirth), were the combination of variables which best discriminated between congenital malformations and normal pregnancy outcomes. In later studies (Kaneko et al., 1984a, 1988), the combination of valproic acid and carbamazepine and/or partial seizures were associated with the occurrence of congenital malformations. Thus the present findings of a statistically significant association between AED and congenital malformations following multiple risk factor analysis are supported by previous findings, although the exact type of AED implicated varies. This probably reflects variation in the patient population and their drug regimens at different time periods, and differences in methodology (logistic regression versus discriminant function analysis) (see section II.K7, Epidemiological Multivariable Studies) .

3. *Folate Studies*

Folate deficiency has been postulated as a possible mechanism for the teratogenic effects associated with maternal use of AED during

pregnancy. This hypothesis has been suggested by studies showing decreased folate levels in patients on AED therapy (Baylis et al., 1971; Dellaportas et al, 1982; del Ser et al.,1983; Edeh and Toone, 1985; Houben et al., 1971; Krause et al., 1982; Muñoz-Garcia et al., 1983; Reizenstein and Lund, 1973; Reynolds et al., 1972), and the suspected role of folate deficiency as a risk factor for congenital anomalies in the nonepileptic population (Hibbard, 1975; Meadow, 1979; Smithells et al., 1976). The present prospective study attempted to evaluate the relationship of folate status to AED, pregnancy, and pregnancy outcome in epileptic women.

a. Folate in Relation to Anticonvulsant Therapy

The present study showed evidence of mild folate deficiency in nonpregnant epileptic women receiving AED treatment, as indicated by subnormal serum folate (SF) levels in a high proportion of patients (46%), and less often subnormal red cell folate (RCF) levels (8%). The frequency of subnormal SF levels in previous studies of outpatient epileptics on AED therapy has varied from 8-76%, presumably due to the definition of deficiency, duration and type of AED regimen, and dietary background (Dellaportas et al., 1982; Klipstein, 1964; Krause et al., 1982; Muñoz-Garcia et al., 1983).

In agreement with the findings of other workers (Baylis et al., 1971; del Ser et al., 1983; Gatenby, 1960; Houben et al., 1971; Muñoz-Garcia et al., 1983; Reynolds et al., 1972), folate levels were negatively correlated with plasma PHT and PB levels, as well as with the number of AED. A higher proportion of women taking PHT showed suboptimal plasma levels than did women on PB, suggesting that PHT may have a

more significant effect on folate status. Similar findings were observed by Reynolds et al. (1972). In the present series, a combination of PHT taken together with PB or PRM, with or without other AED, was associated with the highest risk of folate deficiency. The frequency of subnormal SF levels in a small number of nonpregnant women who were taking CBZ or VPA in monotherapy, was similar to that for PHT monotherapy, however, blood folate levels were not significantly correlated with plasma VPA or CBZ levels. While experimental studies have demonstrated the antifolate effects of high doses of the latter drugs (Marden et al, 1981; Smith and Carl, 1982), previous human investigations of CBZ have shown equivocal results (Dellaportas et al, 1982; Hendel et al., 1984, Hiilesmaa,1982; Hiilesmaa et al., 1983; Reizenstein and Lund, 1973). Hendel et al. (1984) found evidence for the antifolate effects of VPA in humans.

b. Folate During Pregnancy

Pregnant women who did not take folate supplements were more likely than nonpregnant women to show deficient SF levels, particularly at the time of high fetal demand in the last trimester of pregnancy This is in accord with previous studies in nonepileptic (Ball and Giles, 1964; Chanarin, 1979; Chanarin et al, 1968; Ek and Magnus, 1981; Fleming et al., 1974b; Molina et al., 1974; Willoughby and Jewell, 1968) and epileptic (Ueda et al., 1984; Yamamoto et al., 1984) women, showing a decrease in SF levels in the course of pregnancy and/or in the puerperium. In our series, low SF levels tended to persist in serial measurements, indicating that the patients were in negative folate balance. Red cell folate levels also fell during pregnancy, but the

majority of women maintained normal levels, suggesting that initial tissue stores and dietary sources of folate were sufficient to maintain normal tissue folate levels during pregnancy. This is in agreement with previous studies reporting lower SF in pregnant epileptic women, as compared with pregnant nonepileptic controls (Ogawa et al., 1985; Pritchard et al.,1971; Ueda et al., 1984; Zachau-Christiansen et al., 1962).

The present findings showing lower folate levels in pregnant women taking AED in polypharmacy than in monotherapy further suggest that AED might increase the risk of folate deficiency during pregnancy. On the other hand, women in our study who took folate supplements during pregnancy (including relatively low doses of 100 to 500 µg) achieved normal or supranormal blood folate levels, despite the influence of AED. The Finnish prospective study (Hiilesmaa, 1982; Hiilesmaa et al., 1983) found similar results.

c. Folate and Pregnancy Outcome

Univariate analysis showed that decreased maternal SF and RCF levels before conception and in the embryonic period were significantly associated with the subsequent occurrence of spontaneous abortions or major congenital anomalies, particularly developmental anomalies. Folate levels obtained after this critical period appeared to have a weaker association with abnormal pregnancy outcome.

In contrast to the findings for DA, women who had children with PDH anomalies showed high SF levels (and low plasma PHT levels in the first trimester). Mothers of children with PDH and who had SF levels monitored were all taking folate supplements during pregnancy, and this

was shown to result in high SF levels. There may also have been an interaction between folate and PHT (Rivey and Schottelius,1984), with low doses and plasma levels of PHT permitting high SF levels, or high SF levels decreasing plasma PHT levels. Doses of PHT received by women who had children with PDH were low to average (100 to 300 mg per day), suggesting the first mechanism.

While subnormal SF levels were relatively frequent among women with adverse pregnancy outcome, only 2 of these women showed deficient RCF levels. This suggests that in epileptic women taking AED, mild folate deficiency, without tissue folate depletion, may contribute to abnormal embryonic development.

There were no cases of neural tube defects in the present series. A much larger study would be required to detect whether AED-induced folate deficiency is associated with this relatively rare defect.

In contrast to the findings in this study, Hiilesmaa and co-workers, in Finland, (Hiilesmaa, 1982; Hiilesmaa et al., 1983) and Ogawa and colleagues, in Japan, (1985) did not find an association between low SF levels during pregnancy in epileptic women taking AED, and congenital malformations in the offspring. Insufficient information is available regarding the protocol used by Ogawa and colleagues The methods of the Finnish study may not have detected folate deficiency in early pregnancy: pregnancies ending before 24 weeks were excluded and, on the average, folate supplementation was used more extensively (91% vs 63%) while folate levels were measured later in pregnancy than was done in our study. In particular, Hiilesmaa and colleagues did not measure folate levels before 8 weeks of gestation, the major period of organogenesis, and only one quarter of the patients were monitored by

12 weeks of gestation. The present folate study, in contrast, included both viable and non-viable pregnancies, of which 22% were monitored prior to 8 weeks, and 53% by 12 weeks of pregnancy.

The present study by univariate and/or multivariable analyses showed a significant positive correlation between maternal dose and plasma levels of AED during pregnancy and the occurrence of spontaneous abortions and major anomalies in the offspring, especially developmental anomalies. Evidence was also found, by univariate analysis, for an association between low blood folate levels and abnormal pregnancy outcome, as well as an inverse correlation between blood folate and plasma AED levels. Although SF levels were not found to be significantly associated with total abnormal outcomes by multiple logistic regression, the sample size of pregnancies may not have been large enough to detect an association between this variable and pregnancy outcome. Furthermore, a role for blood folate in the etiology of spontaneous abortion or DA cannot be ruled out, as the numbers of pregnancies in which blood folate levels were monitored were too small to assess the relative contribution of this factor to specific abnormal pregnancy outcomes.

Although the univariate association between low blood folates and abnormal pregnancy outcome does not imply they are causally related, the data suggest that the effects of AED on the fetus might be related to their influence on folate metabolism. Other possible effects of AED and their metabolites must also be considered (see Table II.10 in Literature Review on possible mechanisms of AED-induced congenital malformations).

4. Detoxification Defects of Arene Oxide Metabolites of Phenytoin

A report by Strickler, Dansky and colleagues (Dansky et al., 1987; Strickler et al.,1985) showed a significant association between major congenital anomalies and a genetically determined detoxification defect of arene oxide metabolites of PHT (see section II R1a, Toxic Metabolites of AED). This study included offspring and their parents ascertained both retrospectively and prospectively. Only those offspring exposed to PHT with or without other AED throughout pregnancy were included in this report (N=24).

The present study reexamined the role of detoxification defects in 17 of the offspring exposed to PHT throughout pregnancy, who had been ascertained prospectively in the previous report, and in 3 offspring exposed to PRM and/or PB in utero. The criteria and classification of major congenital malformations were also different from the previous study. For example, microcephaly was included as a major congenital malformation in the study by Strickler and colleagues, while in the present study, this was studied separately from other anomalies and along with other newborn growth parameters. Inguinal and umbilical hernias were counted as minor anomalies in the previous report, whereas these were included as major anomalies in the present series. Two of the children in the study by Stricker and colleagues had been ascertained from the Toronto Hospital for Sick Children, both of whom had severe major congenital malformations (congenital heart disease, cleft lip + palate), and these children were not included in the present report. Despite these differences, the present study found similar results, particularly with respect to developmental anomalies, but the

findings were not statistically significant. As in the report by Strickler et al. (1985), at least one of the parents of each malformed offspring had a detoxification defect, indicating that the defect is inherited. The extent of abnormal detoxification (cell death) in both the parents and the children was similar to that seen in parents of patients exhibiting phenytoin hepatotoxicity. This was an intermediate pattern between controls and patients with hepatotoxicity, compatible with an autosomal codominant mode of inheritance for the detoxification defect. An equal number of mothers and fathers showed a defect in detoxification, suggesting that the metabolism of the fetus may be more important than maternal metabolism in the etiology of birth defects.

It is of interest that among three children exposed to PB in the first trimester, one of two children with major anomalies had a positive response to in vitro challenge with PHT metabolites, while the third child without major anomalies showed a negative response. It has been postulated that the defect in detoxification may be a mutation in the epoxide hydrolase enzyme which alters its substrate specificity for structurally related compounds (Spielberg, 1987).

PHT and/or its arene oxide metabolite may decrease activated folates or act independently of folate on embryonic development. Tests for detoxification defects may provide a means for predicting which families may be genetically predisposed to having abnormal fetal outcomes. A larger number of children with toxicity tests and DA would be required in order to determine whether relatively high plasma PHT levels might result in elevated levels of arene oxide metabolites, which would interact with detoxification defects to produce fetal abnormality.

5. *Other Risk Factors*

An unexpected result was the higher frequency of DA in offspring of women who reported no alcohol consumption during pregnancy as compared with that among offspring of those who reported alcohol use. This may have been a chance finding or may have reflected underreporting among those who were alcohol users.

6. Summary

In summary, the present study showed significantly elevated risks of specific developmental anomalies (DA) and isolated congenital postural deformities and hernias (PDH) in the offspring of epileptic women taking AED during pregnancy, as compared with the general population. The relative contribution of AED and other genetic and environmental risk factors to the occurrence of abnormal pregnancy outcome was evaluated both by univariate analyses and multiple logistic regression analysis. Univariate analysis showed that one or more abnormal outcomes - spontaneous abortion, DA and PDH - were significantly associated with dose and/or plasma levels of PHT, PB or PRM, as well as other risk factors including maternal seizures, and family history of major congenital anomalies and seizures. The risk of total abnormal outcomes and DA increased with increasing mean dose and/or plasma levels of PHT and PB in the first trimester, and over all trimesters combined. Metabolic interactions involving certain combinations of drugs such as PHT, PB and PRM may have contributed in part to high plasma PB levels. Multiple logistic regression analysis showed that dose and/or plasma levels of PHT and PB were the only risk

factors which were consistently significantly associated with adverse pregnancy outcome. In contrast, no significant association between PDH and AED therapy was found by multiple logistic regression analysis. Family history of concordant anomalies was evident for offspring with PDH, while this was not the case for DA. This suggests that the occurrence of DA and PDH in the offspring of epileptic women may have heterogeneous etiologies. Multiple logistic regression analysis of spontaneous abortion in relation to risk factors could not be done due to small numbers.

Univariate analysis showed that SF and/or RCF levels were significantly negatively correlated with spontaneous abortion and DA, although their relative role in the etiology of abnormal pregnancy outcomes could not be adequately assessed by multiple logistic regression analysis. The data suggest that folate deficiency might be a possible mechanism for the teratogenic effects associated with AED therapy.

VI. PART II OF THE PRESENT INVESTIGATION: SOMATIC PARAMETERS AT BIRTH

A. Introduction

A number of studies have shown a decrease in newborn growth parameters, particularly of HC, in offspring of epileptic women taking AED during pregnancy. These findings have usually been investigated in relation to the possible teratogenic effects of AED, and only some studies have also looked at a few other potential risk factors.

The present investigation looks in detail at a number of factors which might explain the variation in growth parameters. In particular, the influence of maternal seizures and of AED, including dose and plasma levels, was studied after having controlled for the potential confounding effects of other risk factors.

B. Results

1. Outcome of Pregnancy

a. *Distributions of Head Circumference, Weight and Length at Birth*

Head circumference (HC), weight (WT), and length (LT) at birth were studied in 109 live-born children of 91 women with epilepsy, who were followed prospectively during their pregnancies.

Figures VI.1 to VI.3 show the distributions of HC, WT and LT in the newborns, in standard score form after correction for gestational age, relative to those expected if the distributions were normally distributed. The distribution of LT is relatively normal with approximately equal numbers of offspring falling into both halves of the curves. On the other hand, the distribution of HC is positively skewed

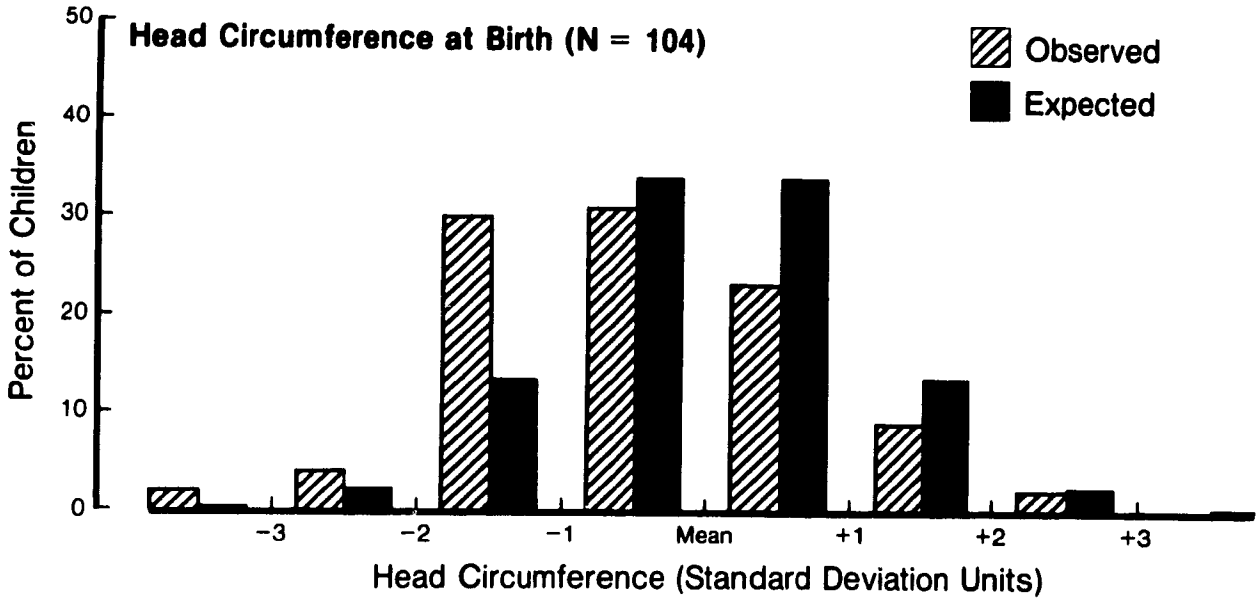

Figure VI.1

Distribution of standard scores of head circumference at birth among offspring of epileptic women.

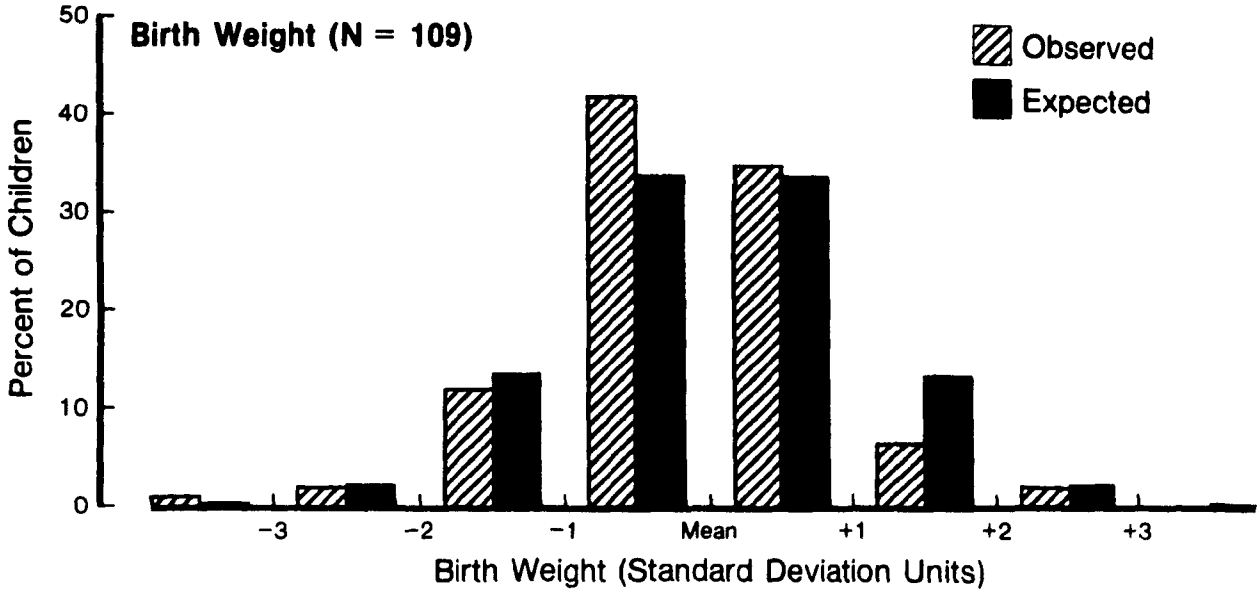

Figure VI.2

Distribution of standard scores of birth weight among offspring of epileptic women.

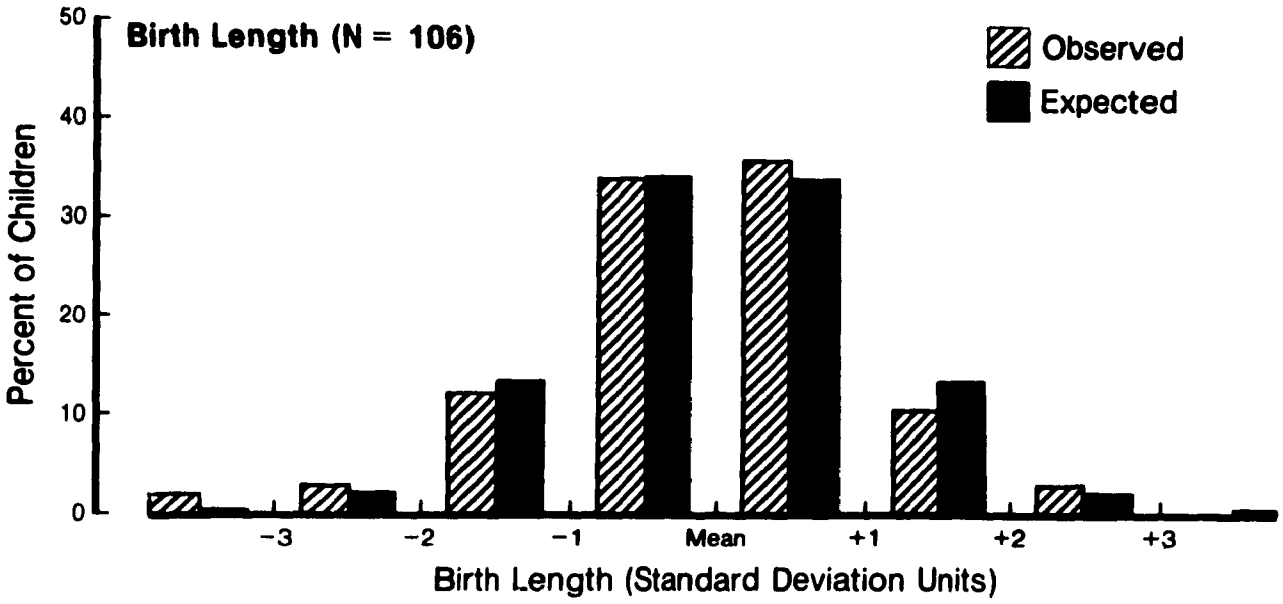

Figure V1.3

Distribution of standard scores of birth length among offspring of epileptic women.

with more of the offspring falling below than above the midpoint of the curve. The distribution of weight is also slightly positively skewed.

Table VI.1 presents the number of offspring whose HC, WT or LT at birth were at or below the 10th percentile for gestational age, as compared with the expected number for a normally distributed population. The observed number did not differ significantly from the expected number for WT or LT. In contrast, 27 of 104 (26.0%) offspring had HCs at or below the 10th percentile ($p<0.001$). Nine (8.7%) children were microcephalic with HCs at or below the 3rd percentile ($p<0.001$).

HC, WT, and LT at birth were strongly positively correlated (Table VI.2). However, the distribution of standard score of HC at birth minus the standard score of WT for each newborn (Figure VI.4) showed that HC was actually small for the child's weight. 66.3% of the children had smaller standard scores for head cirumference than for weight, while 33.7% of children had larger standard scores for HC than for WT ($p < 0.001$).

HC was also small relative to the newborn's LT (Figure VI.5): 59.6% of children showed a small HC for their LT as compared with 40.4% of children who showed a large HC for their LT ($p< 0.05$)

Of 27 children with small HCs ($\leq$ 10th percentile), only 8 children also had WT and/or LT measurements $\leq$ 10th percentile. Only one child had all three growth parameters at or below the 3rd percentile. Thus, HCs tended to be small for WT or LT.

The distribution of standard score of WT minus that for LT (Figure VI.6) showed that overall the newborns had slightly decreased WTs for their LTs.

Table VI.1: The Frequency of Growth Parameters at Birth Less Than or Equal to the 10TH Percentile as Compared to that Expected for a Normally Distributed Population

Head Circumference Percentile (N=104)	Observed No.	Observed %	Expected No.	P
>3 - ≤10	18	17.3	7.3	
≤3	9	8.7	3.1	<0.001
≤10	27	26.0	10.4	<0.001
Birth Weight Percentile (N=109)	**Observed No.**	**Observed %**	**Expected No.**	**P**
>3 - ≤10	6	5.5	7.6	
≤3	4	3.7	3.3	NS
≤10	10	9.2	10.9	NS
Birth Length Percentile (N=106)	**Observed No.**	**Observed %**	**Expected No.**	**P**
>3 - ≤10	6	5.5	7.4	
≤3	5	4.7	3.2	NS
≤10	11	10.4	10.6	NS

Table VI.2

Correlation between Growth Parameters at Birth

Growth Parameters	Correlation Coefficient	P
Head Circumference and Birth Weight	0.632	<0.0001
Head Circumference and Birth Length	0.318	<0.001
Birth Weight and Birth Length	0.562	<0.0001

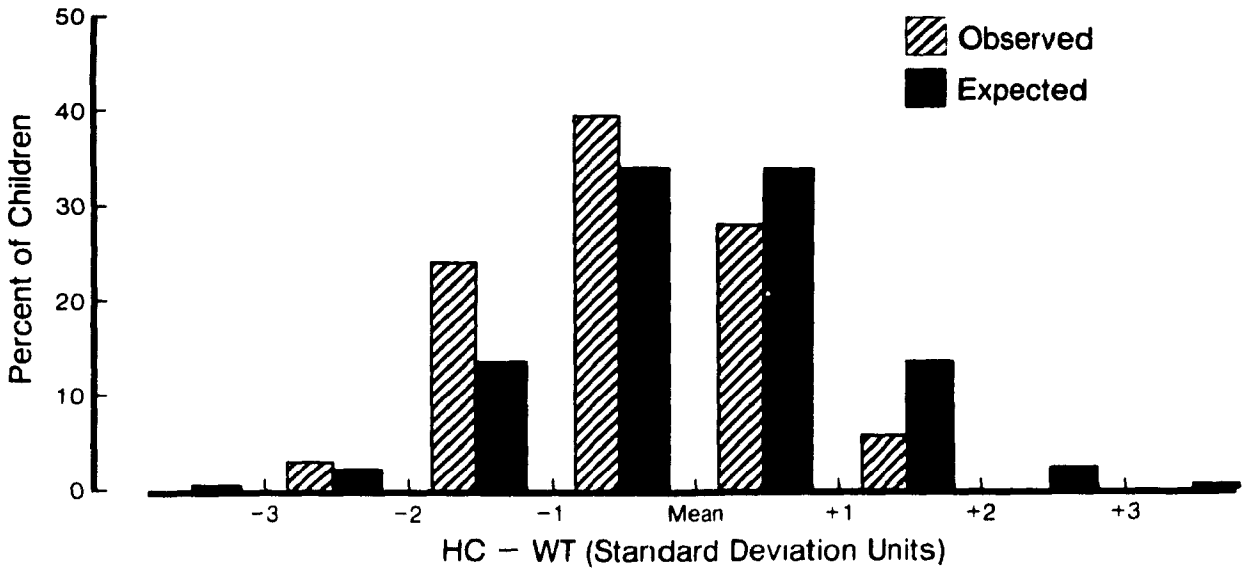

Figure VI.4

Distribution of standard scores of head circumference at birth minus standard scores of birth weight among offspring of epileptic women.

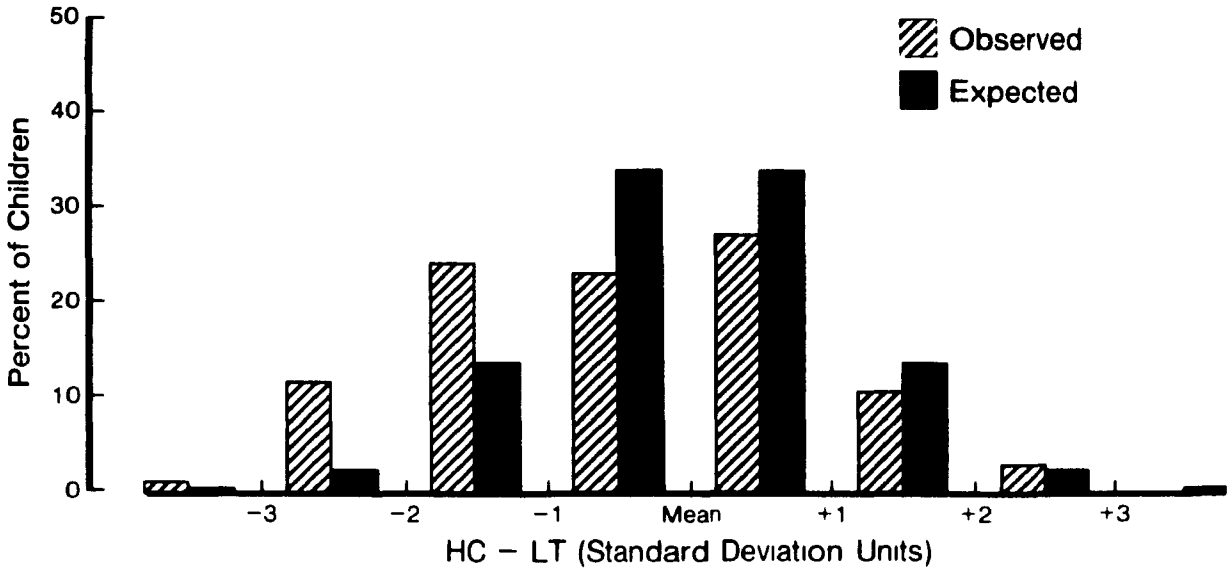

Figure VI.5

Distribution of standard scores of head circumference at birth minus standard scores of birth length among offspring of epileptic women.

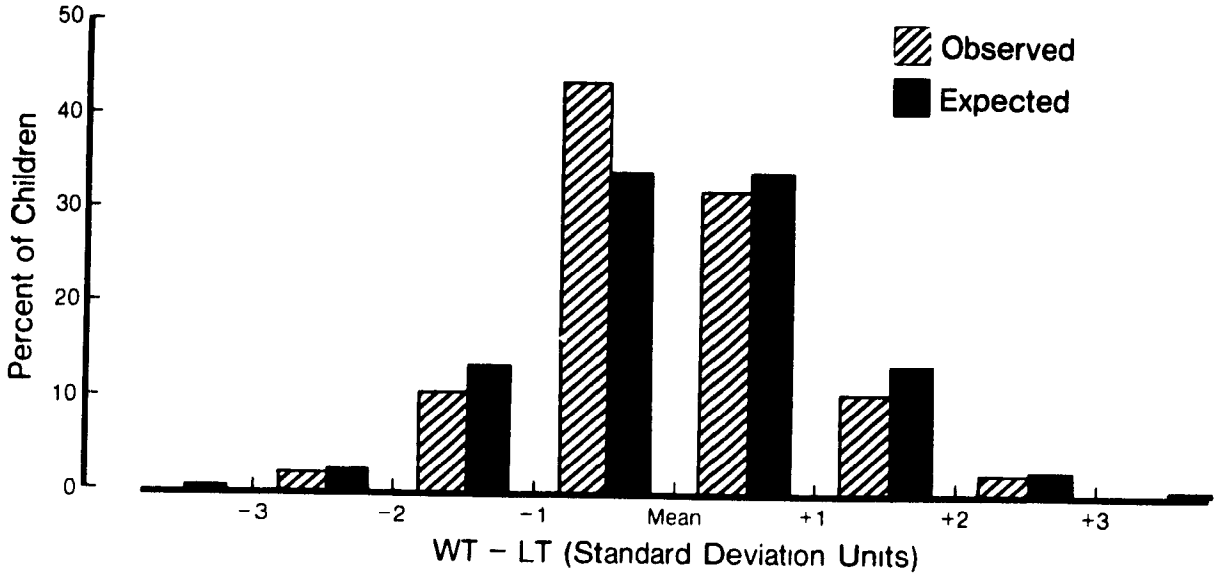

Figure VI.6

Distribution of standard scores of birth weight minus standard scores of birth length among offspring of epileptic women.

b. *Growth Parameters at Birth in Relation to Major Congenital Anomalies*

Children with or without major congenital anomalies did not differ significantly with respect to mean standard scores of growth parameters at birth. However, as shown in Table VI.3, children with "Developmental" anomalies (DA) or " Congenital postural deformities and hernia defects" (PDH), and children without such anomalies, tended to differ with respect to the proportion who were found to have growth parameters at ≤ 10th and ≤ 3rd percentiles.

Among children with DA, 45.5% had HC measurements ≤ 10th percentile, as compared with 20.0% of children with PDH and 24.4% of children without these defects. All three groups were found to have a significantly higher number of children with HCs at ≤ 10th and/or ≤ 3rd percentiles as compared with the expected number.

Children with DA were also shorter at birth than children with PDH or normal children (Table VI.3). Only children with DA had a significantly higher proportion of children with LT measurements ≤ 3rd percentile than expected ($p<0.01$).

Small WTs also tended to occur more frequently among children with DA than among other malformed or normal children (Table VI.3). However, the number of children with small WTs did not differ significantly from the expected numbers for malformed or nonmalformed children.

Table VI.3: The Frequency of Growth Parameters at Birth Less Than or Equal to the 10TH Percentile as Compared to that Expected for a Normally Distributed Population : Relation to Presence or Absence of Major Congenital Anomalies.

Head Circumference Percentile	Observed No.	%	Expected No.	P
Developmental Anomaly (N=11)				
≤ 10	5	45.5	1.10	<0.001
≤ 3	1	9.1	0.33	NS[a]
Congenital Postural Deformity, Hernia (N=15)				
≤ 10	3	20.0	1.50	NS
≤ 3	2	13.3	0.45	<0.05
Normal Outcome (N=78)				
≤ 10	19	24.4	7.80	<0.001
≤ 3	6	7.7	2.34	<0.02

Weight Percentile	Observed No.	%	Expected No.	P
Developmental Anomaly (N=12)				
≤ 10	2	16.7	1.20	NS[a]
≤ 3	1	8.3	0.36	NS
Congenital Postural Deformity, Hernia (N=15)				
≤ 10	0	0.0	1.50	NS
≤ 3	0	0.0	0.45	NS
Normal Outcome (N=82)				
≤ 10	8	9.8	8.20	NS
≤ 3	3	3.7	2.46	NS

[a] Not statistically significant

Table VI.3 cont'd: The Frequency of Growth Parameters at Birth Less Than or Equal to the 10TH Percentile as Compared to that Expected for a Normally Distributed Population: Relation to Presence or Absence of Major Congenital Anomalies.

Length Percentile	Observed No.	%	Expected No.	P
Developmental Anomaly (N=11)				
≤ 10	3	27.3	1.10	<0.10
≤ 3	2	18.2	0.33	<0.01
Congenital Postural Deformity, Hernia (N=15)				
≤ 10	1	6.7	1.50	NS
≤ 3	0	0.0	0.45	NS
Normal Outcome (N=80)				
≤ 10	7	8.8	8.00	NS
≤ 3	3	3.8	2.40	NS

[a] Not statistically significant

2. *General Description of Selected Risk Factors*

a. *Growth Parameters of Parents*

HC was measured in 61 epileptic mothers. Maternal HC tended to show a similar overall distribution to that of the offspring (Fig VI.7), but the proportion of mothers with small HCs was less than that in their offspring: 16.4 % of the mothers had HCs ≤ 10th percentile and 4.9% were microcephalic (≤ 3rd percentile). In contrast, head measurements tended to be large in 38 husbands in whom HC was measured (Fig VI.8).

The distributions of maternal and paternal height are shown in Table VI.4 Both epileptic women and their husbands showed a preponderance towards relatively short heights, and this was more marked for epileptic mothers.

The results of univariate correlation analyses of newborn growth parameters in relation to parental head circumferences and heights will be presented in sections VIB.3a(1), 4a(1), and 5a(1).

b. *Family History of Seizures and of Major Congenital Anomalies*

As outlined in Methods , section IV.I, a history of seizures or major congenital malformations in maternal and/or paternal relatives of the offspring was analyzed in separate degrees of relatives, and in 1st to third degree relatives combined. Family history was classified as both a dichotomous (yes/no variable) and as a continuous variable, involving the proportion of affected relatives for each offspring.

In general, the results concerning the dichotomous classification of family history of seizures or malformations in relation to growth parameters were similar to those for family history analyzed as a

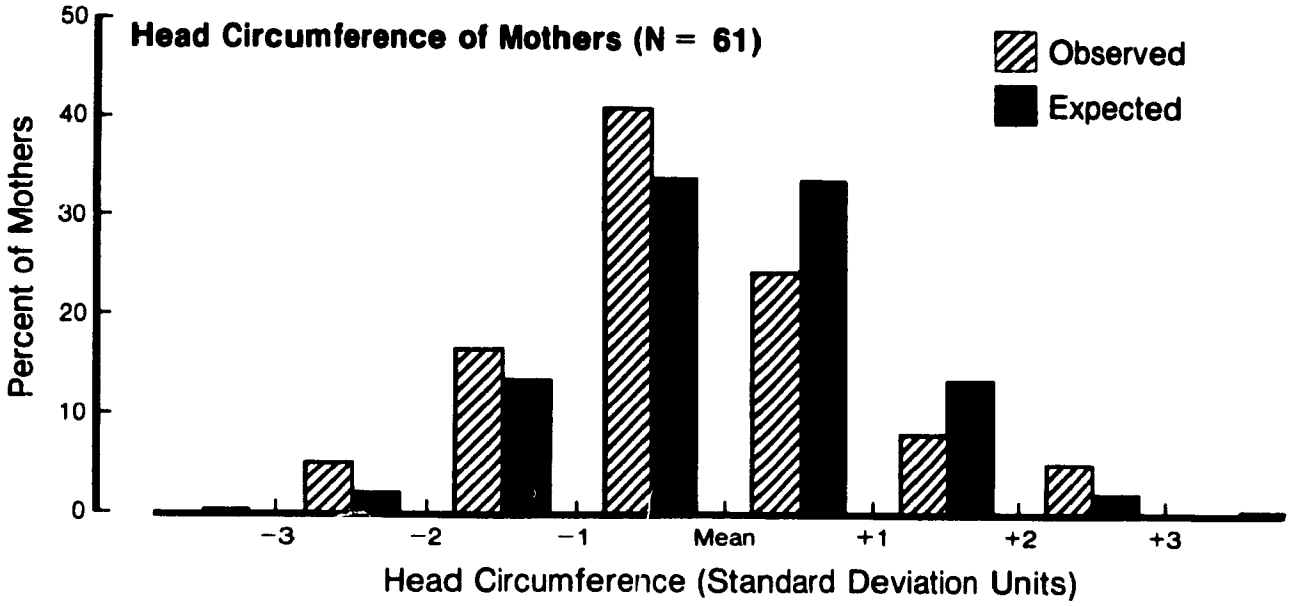

Figure VI.7

Distribution of standard scores of head circumference among epileptic mothers.

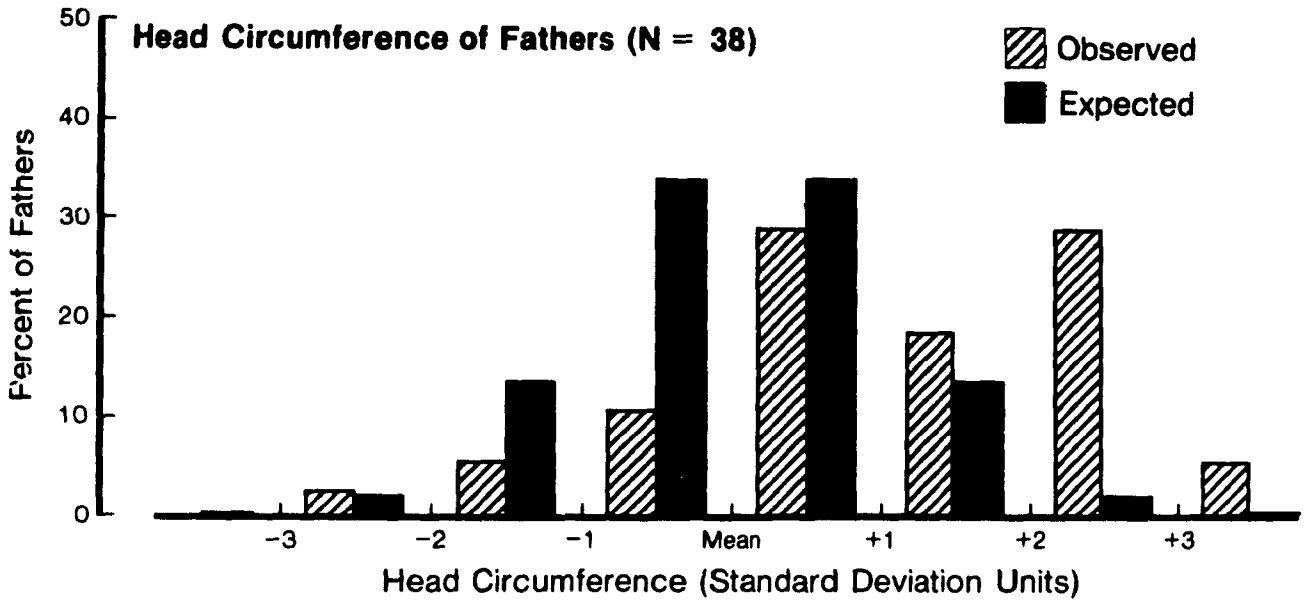

Figure VI.8

Distribution of standard scores of head circumference among fathers.

Table VI.4

Distributions of Maternal and Paternal Heights

Percentile	Mothers (N=87)		Fathers (N=79)	
	No.	%	No.	%
≤ 5	10	11.5	11	13.9
6-10	10	11.5	6	7.6
11-25	23	26.4	10	12.7
26-50	24	27.6	25	31.6
51-75	12	13.8	14	17.7
76-90	7	8.0	10	12.7
91-95	1	1.1	0	0.0
> 95	0	0.0	3	3.8

proportion, although occasionally results for particular degrees of relatives were significant using the proportion and not the dichotomy, and vice versa.

There was only one significant finding for family history of malformations in relation to growth parameters: a poor positive correlation (0.200, $p \leq 0.05$) was noted between WT and the proportion of individuals with major malformations among 1st and 2nd degree paternal relatives combined.

The only significant result for family history of seizures in separate degrees of relatives in relation to growth parameters was a negative correlation between history of seizures in paternal second degree relatives and LT (-0.212, $p \leq 0.05$). However, significant results were found for all three growth parameters when family history of seizures was analyzed with different degrees of maternal and/or paternal relatives combined. Tables VI.6 , VI.12, and VI.17 show the results for family history of seizures classified as a dichotomous variable.

A history of seizures in 1st and/or 2nd degree paternal relatives tended to be more often associated with decreased growth parameters than a history of seizures in maternal relatives. This is probably an artifact as maternal 1st degree relatives, i.e. epileptic mothers, had to be excluded by virtue of the fact that they were ascertained because of their epilepsy.

c. Consanguinity

As previously reported in section V.B.2.a.(3), cousin marriages were reported in only two instances. In the child whose parents were second degree cousins, measurements of WT and LT at birth were unremarkable,

while HC was 1.24 standard deviations below the mean . This child was also born with cardiomyopathy. Only LT and WT measurements were available for the child whose parents were third degree cousins, and her WT was somewhat small (1.08 standard deviations below the mean).

A more detailed account of newborn growth parameters in relation to parental growth measurements, family history of seizures and malformations,and other risk factors is given in the following sections.

3. Analysis of Head Circumference at Birth in Relation to Risk Factors

a. *Univariate Risk Factor Analysis*

(1). Correlations with continuous or ordinal independent variables

Table VI.5 shows the correlation between various groups of risk factors and HC at birth for those correlations which were found to be significant at less than or equal to the 0.05 significance level.

A number of background factors were found to have low (0.2 to 0.3) positive correlations with HC at birth. These included socioeconomic status as measured by father's occupation[a] and maternal but not paternal HC.

On the other hand, other background factors showed low negative correlations with HC. These included the proportions of maternal and paternal 1st to 3rd degree relatives combined with a history of seizures, and the number of cigarettes smoked during pregnancy.

Among the main variables of interest, the frequency of major seizures anytime during pregnancy, and the frequency of minor

[a]Coded as 1 to 5 for unskilled to professional,as noted in Appendix C,No.1

Table VI.5: Results of Univariate Correlation Analysis

Outcome Variable: Head Circumference at Birth

Risk Factor Abbreviation	Risk Factor	Correlation Coefficient	N	P
	Personal Characteristics			
OCCUPF	Father's occupation	0.238	101	0.02
HCM	Mother's head circumference	0.375	73	0.005
HCMIDP	Mid-parent head circumference	0.391	45	0.01
	Family History			
SZ12DEGMP	Seizures in maternal, paternal 1st,2nd degree relatives	-0.238	96	0.02
SZ123DEGMP	Seizures in maternal, paternal 1st,2nd,3rd degree relatives	-0.244	84	0.05
	Drugs Other Than AED			
SMOKING	Cigarette smoking	-0.328	104	0.001
	Maternal Seizures			
FMAJSZA	Frequency of major seizures Anytime	-0.221	103	0.05
FMINSZI	Frequency of minor seizures Trim I	-0.238	85	0.05
FMINSZA	Frequency of minor seizures Anytime	-0.243	84	0.05
	Maternal AED Therapy			
NAEDI	No. of AED Trim I	-0.260	104	0.01
NAEDA	No. of AED Anytime	-0.302	104	0.005
PHTmgII	Mean daily dose (mg) PHT Trim II	-0.277	71	0.02
PHTmg/kgLMP	Dose (mg/kg) PHT at LMP	-0.332	69	0.01
PHTmg/kgI	Mean dose (mg/kg) PHT Trim I	-0.539	32	0.005
PHTmg/kgALL	Mean dose (mg/kg) PHT All Trims	-0.304	76	0.01
PHTPLI	Mean plasma (µg/ml)PHT Trim I	-0.364	32	0.05
PHTPLII	Mean plasma (µg/ml) PHT Trim II	-0.301	44	0.05
PHTPLALL	Mean plasma (µg/ml) PHT All Trims	-0.310	77	0.01
PBmg/kgLMP	Dose (mg/kg) PB at LMP	-0.313	43	0.05
PBPLIII	Mean plasma (µg/ml) PB Trim III	-0.336	36	0.05
VPAmg/kgLMP	Dose (mg/kg) VPA at LMP	0.717	9	0.05
PRMmg/kgIII	Mean dose (mg/kg) PRM Trim III	-0.594	12	0.05
	Folate			
RCFIII	Mean red cell folate (ng/ml) Trim III	0.528	35	0.005

seizures in the first trimester and anytime were found to have low negative correlations with HC.

Within factors related to maternal AED therapy, the number of AED taken in the first trimester or at anytime during pregnancy, also showed low negative correlations with HC.

Dose and/ or plasma levels of PHT, PB, and PRM were significantly negatively correlated with HC. This was more consistently demonstrated through the pregnancy for PHT than for the other AED.

For PHT, dose (mg/kg) at the LMP, and both dose (mg or mg/kg) and plasma levels (µg/ml) in the first and second trimesters, and over all trimesters combined were significantly negatively correlated with HC. Most of the correlations tended to be low, except for mean dose (mg/kg) in the first trimester which showed a moderate negative correlation of -0.539.

Low negative correlations were found between HC and both the dose (mg/kg) of PB at the LMP and mean plasma level (µg/ml) of this drug in the third trimester.

In a small number of mother-child pairs, mean dose (mg/kg) of PRM in the third trimester was noted to have a moderate negative correlation (-0.594) with HC.

The only positive correlation within maternal AED therapy was noted in a small group (n=9) of women who took VPA during pregnancy There was a very high positive correlation (0.717) between the dose (mg/kg) of the drug taken at the LMP and HC in the offspring.

Mean red cell folate levels (ng/ml) only in the third trimester were noted to have a moderate positive correlation with HC (0.528)

2. Categorical (dichotomous) independent variables (t-tests)

Table VI.6 presents the results for various dichotomous risk factors, in which the mean standard score of HC was found to differ significantly according to the presence (1) or absence (0) of the factor (as described in detail in Appendix C) at ≤0.05 significance level. Generally the difference between the means for the two groups with and without the factor was less than one standard deviation unit (SD unit), except when specifically noted below.

Female children had significantly smaller HCs than male children ($p<0.05$).

There were 3 families in which the fathers also had a history of seizures, and their children showed significantly smaller HCs (by more than one SD unit) than children without a history of paternal seizures ($p=0.02$). HC was also noted to be significantly smaller ($p<0.05$) in offspring with a positive history of seizures in various combinations of paternal and/or maternal 1st to 3rd degree relatives combined. Here, the difference in mean standard score of HC was less than one SD unit.

Three women became pregnant while using birth control pills, and one woman used progesterone for bleeding in the first trimester. Children of these women had significantly smaller HCs (> one SD unit difference) than children not exposed to sex hormones ($p<0.05$).

The presence of at least one major seizure in the first trimester or at anytime in pregnancy was significantly associated with decreased HC in the offspring ($p<0.05$). The occurrence of at least one minor seizure in the first trimester and at anytime in pregnancy was highly significantly associated with decreased HC ($p<0.005$)

Offspring exposed to maternal AED polytherapy had significantly

Table VI.6

Results of Univariate t-tests

Outcome Variable: Head Circumference at Birth (HC)

Risk Factor Abbreviation	Risk Factor	Mean Standard Score of HC ± SD No (0) Group	Yes (1) Group	N_0 / N_1	P
	Personal Characteristics				
FEMOFFSPR	Female sex of child	-0.257±1.103	-0.761±1.143	56/48	0.05
	Family History				
SZF	Seizures in father	-0.431±1.121	-2.013±1.663	96/ 3	0.02
SZ12DEGP	Seizures in paternal 1st, 2nd degree relatives	-0.406±1.119	-1.205±1.446	89/ 9	0.05
SZ12DEGMP	Seizures in maternal, paternal 1st, 2nd degree relatives	-0.315±1.149	-0.877±1.152	66/30	0.05
SZ123DEGMP	Seizures in maternal, paternal 1st, 2nd,3rd degree relatives	-0.284±1.113	-0.836±1.229	53/39	0.05
	Drugs Other Than AED				
SEXHORM	Sex hormones Trim I, Anytime	-0.435±1.121	-1.668±1.451	98/ 4	0.05
	Maternal Seizures				
MAJSZI	Major seizures Trim I	-0.345±1.144	-0.890±1.078	78/24	0.05
MAJSZA	Major seizures Anytime	-0.293±1.150	-0.769±1.090	61/43	0.05
MINSZI	Minor seizures Trim I	-0.156±0.974	-0.875±1.234	51/37	0.005
MINSZA	Minor seizures Anytime	-0.115±0.985	-0.796±1.246	47/49	0.005
	Maternal AED Therapy				
POLYI	Polytherapy Trim I	-0.218±1.080	-0.728±1.467	41/60	0.05
POLYA	Polytherapy Anytime	-0.154±1.054	-0.738±1.140	41/62	0.02
DETOX	Detoxification defect	-0.122±1.007	-1.167±1.205	10/10	0.05
	Folate				
RCF< 175II	Red cell folate < 175 ng/ml Trim II (at least one value)	-0.217±1.155	-2.191±1.192	31/ 2	0.05
RCF <175A	Red cell folate < 175 ng/ml Anytime (at least one value)	-0.307±1.119	-1.166±1.112	36/ 6	0.05

smaller HCs than children exposed to maternal AED monotherapy. This was noted both for the first trimester ($p<0.05$) and at anytime in pregnancy ($p<0.02$).

A comparison of newborn head circumference among offspring with and without detoxification defects of PHT arene oxide metabolites revealed that those with detoxification defects showed significantly decreased HC as compared to those without such defects ($p< 0.05$). Offspring with detoxification defects had at least one parent who also showed this defect.

Of 42 women who had red cell folate levels (ng/ml) monitored at anytime in pregnancy, two women had at least one red cell folate level < 175 ng/ml in the second trimester, and a total of six women had such abnormal levels at anytime in pregnancy. HCs of their offspring were significantly smaller than those of children born to women with normal red cell folate levels ($p<0.05$). The difference in mean standard scores of HC for the second trimester was greater than one SD unit.

3. Categorical independent variables (> 2 subclasses)(anova analysis)

Table VI.7 presents the group mean standard scores of HC for categorical factors analyzed by analysis of variance, and the pairwise comparisons which were significant at $p \leq 0.05$.

In a comparison of mean standard scores of HC among newborns of mothers with the four main types of classified epilepsies, children born to mothers with partial simple seizures with or without secondary generalization showed significantly smaller mean standard scores than did children of mothers with either primary generalized epilepsy, or secondary generalized epilepsy with or without focalization. However,

Table VI.7

Results of Univariate Anova

Outcome Variable: Head Circumference at Birth

Risk Factor Abbreviation	Risk Factor	Group Means	N	P Anova (Sign. pair-wise comparisons p<.05)
	Type of			
TYPEEP	***Maternal Epilepsy***			
	1. Primary generalized	-0.244	41	0.05
	2. Secondary generalized ± focalization	-0.053	14	(Type4<1,2)
	3. Partial complex ± secondary generalization	-0.735	23	
	4. Partial simple ± secondary generalization	-1.002	16	
	Type of AED			
MPPHTPBPRMI	PHT,PB,PRM monotherapy or polytherapy Trim I			
	1. PHT,PB,PRM	-0.897	7	0.01
	2. PHT,PRM	-1.830	4	(Type2<4)
	3. PHT,PB	-0.403	24	
	4. PHT	-0.021	20	
	5. PB	-1.009	7	
MPPHTPBPRMA	PHT,PB,PRM monotherapy or polytherapy Anytime			
	1. PHT,PB,PRM	-1.099	11	0.02
	2. PHT,PRM	-1.974	2	(Type2<4)
	3. PHT,PB	-0.413	22	
	4. PHT	-0.021	20	
	5. PB	-0.859	6	

children of patients with partial complex seizures with or without secondary generalization did not differ significantly from children born to mothers with primary generalized epilepsy, secondary generalized epilepsy with or without focalization, or partial simple seizures.

Four women took PHT in combination with PRM in the first trimester of pregnancy, while two women were on this drug combination throughout the entire pregnancy. Both for the first trimester and the entire pregnancy, the mean standard score of HC in their offspring was significantly less than the score in offspring exposed to PHT alone. The difference in mean standard scores between those on PHT and PRM versus PHT alone was greater than one SD unit.

b. Multiple Regression Analysis

The results of multiple regression analysis of risk factors in relation to the outcome variable HC are given in section VI.B.3.b.(1) and (2) (Tables VI.8 to VI.10), and Appendix F. The different theoretical models and models of significant risk factors following regression analysis are described in detail in section VI.B.3.b.(1) (Table VI.8) and Appendix F. A summary of the consistency, statistical significance and clinical importance of the significant risk factors emerging from the different models is given in section VI.B.3.b.(2) (Tables VI.9 and VI.10).

(1). Theoretical models and models of significant risk factors following regression analysis

Multiple regression analysis of risk factors in relation to HC at birth yielded 13 sets of models, involving a total of 25 models with a sample size equal to or greater than two times the number of factors in each model, and with an R^2 of ≥ 0.40. The models of combinations of risk factors that maintained a significant correlation with HC after multiple regression analysis were comprised of different subsets of pregnancies, ranging in size from 22 to 66 pregnancies. As indicated by the R^2, the linear combination of significant risk factors in the models explained from 45% to 80% of the variation in HC (Table VI.8 and Appendix F).

Theoretical models 2, 2A; 5, 5A; 6, 6A; 7, 7A and 8, 8A dealt with different combinations of maternal seizure and AED risk factors involving the first trimester of pregnancy; theoretical models 3, 3A; 9, 9A; 10, 10A; 11, 11A; and 12,12A with risk factors at anytime during pregnancy; and theoretical models 1, 1A; 4 ; and 13, 13A with risk factors pertaining to different trimesters and the entire pregnancy (Table VI.8 and Appendix F).

To illustrate the findings in all these models, the results of the multiple regression analysis of 6 sets of models are shown in Table VI.8, and described in detail in the following paragraphs. The remaining 7 sets of models are shown in Appendix F.

In Table VI.8 all factors which were entered into any of the theoretical or proposed models are shown in abbreviated form. In each proposed model, those factors which are in bold type are those which were entered into that particular model. Factors in underlined bold

italic type are those which remained significantly correlated with the outcome following multiple regression analysis. The regression coefficients and standard errors with significance levels are shown for the significant risk factors.

All of the theoretical models included the background factors sex of offspring (SEXOFFSP); father's occupation (OCCUPF), mother's HC (HCM); one or several variables involving family history of seizures in the father (SZF) and in 1st to 3rd degree maternal (M) and/or paternal (P) relatives of the offspring combined (SZ12DEGP; SZ12DEGMP; SZ123DEGMP); maternal smoking (SMOKING); maternal ingestion of sex hormones in the first trimester or at anytime during pregnancy (SEXHORM); and type of maternal epilepsy (TYPEEP). Other risk factors included in the models in different combinations were the presence or absence of at least one major seizure in the first trimester (MAJSZ1); frequency of maternal major seizures at anytime in pregnancy (FMAJSZA); frequency of maternal minor seizures in the first trimester (FMINSZ1) and at anytime in pregnancy (FMINSZA); the presence or absence of at least one maternal minor seizure in the first trimester (MINSZ1) and at anytime in pregnancy (MINSZA); number of maternal AED taken in the first trimester (NAED1) and anytime in pregnancy (NAEDA); type of AED (PHT, PB, PRM vs PHT, PRM vs PHT, PB vs PHT alone vs PB alone) in the first trimester (MPPHTPBPRM1) and anytime (MPPHTPBPRMA); dose (mg/kg) of PHT at the last menstrual period (PHTmg/kgLMP); mean dose (mg/kg) of PHT in the first trimester (PHTmg/kg1); mean plasma level (ug/ml) of PHT in the first trimester (PHTPL1); mean daily dose (mg) of PHT in the second trimester (PHTmg11); mean plasma level (ug/ml) of PHT in the second

trimester **(PHTPLII)**; mean dose (mg/kg) of PHT over all trimesters combined **(PHTmg/kgALL)**; mean plasma level (µg/ml) of PHT over all trimesters combined **(PHTPLALL)**; and mean red cell folate level (ng/ml) in the third trimester **(RCFIII)**.

The proposed *model 1* included all of the family history variables among the background factors. Other risk factors in this model were frequency of maternal major and minor seizures and the number of maternal AED, both for the first trimester and at anytime in pregnancy.

Following multiple regression analysis, there were 55 pregnancies which contributed to the model, and the linear combination of significant risk factors in the model explained 53% of the variation in HC. Sex of the offspring; father's occupation; cigarette smoking; and the frequency of major and of minor seizures at anytime during pregnancy maintained a significant correlation with HC. The direction of the associations, indicated by the sign of the regression coefficients, are the following: Female offspring had smaller HCs than male offspring. Father's occupation coded as 1 to 5 for unskilled laborer to professional was found to have a positive correlation with HC, that is, the higher the occupation, the bigger the HC of the newborn. HC was negatively correlated with smoking and maternal seizures, that is HC decreased with an increase in the number of cigarettes smoked and with an increase in the frequency of major and minor seizures at anytime in pregnancy.

The proposed *model 1A* involved the same type of variables as in the proposed model 1, but here the presence or absence of minor seizures in the first trimester and at anytime in pregnancy were entered instead of the actual frequency of minor seizures as in model 1.

Table VI.8 : Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,†0.001< p≤0.005,††0.0001<p≤0.001

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1. **N=55, R^2=0.5271**

SEXOFFSP	***OCCUPF***	HCM; SZF; SZ12DE6P; SZ12DE6MP; SZ123DE6MP;	***SMOKING*** ;
-0.548*	0.273†		-0.398†
(0.241)	(0.085)		(0.123)

SEXHORM; TYPEEP; MAJSZI;	***FMAJSZA***;	FMINSZI;	***FMINSZA*** ;	NAEDI; NAEDA;
	-0.400†		-0.242**	
	(0.126)		(0.095)	

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

1A. **N=58, R^2=0.5059**

SEXOFFSP	***OCCUPF***	HCM; SZF; SZ12DE6P; SZ12DE6MP; SZ123DE6MP;	***SMOKING*** ;
-0.627**	0.209**		-0.314**
(0.236)	(0.083)		(0.122)

SEXHORM; TYPEEP; MAJSZI; FMAJSZA; MINSZI;	***MINSZA***;	NAEDI;	***NAEDA***;
	-0.767†		-0.444**
	(0.241)		(0.167)

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII

PHTmg/kgALL; PHTPLALL; RCFIII

Following regression analysis, there were 58 pregnancies which contributed to model 1A of significant risk factors, and the R^2 was 51%. All of the significant risk factors as in model 1 were maintained in model 1A, except for frequency of major seizures anytime which became insignificant, while the number of maternal AED taken anytime in pregnancy entered into model 1A as a highly significant negative correlate with HC. This may indicate that in this particular group of patients, frequency of major seizures and the number of AED were highly correlated, so that one variable replaced the other when the model was changed slightly.

The proposed *model 4*, involving the same background factors as models 1 and 1A, evaluated maternal seizures and the number of AED at anytime during pregnancy along with mean red cell folate levels (ng/ml) in the third trimester. Father's occupation; cigarette smoking; the occurrence of minor seizures at anytime in pregnancy; and the number of AED at anytime were highly significant linear correlates of HC. Mean red cell folate levels in the third trimester were also found to be significantly associated with newborn HC: HC increased with an increase in folate levels. The combination of these significant risk factors explained a high proportion of the variation (79%) in HC among 30 newborns.

Model 4 was also analyzed with the variable of at least one red cell folate level < 175 ng/ml at anytime in pregnancy, in place of mean folate levels in the third trimester (not shown in Table VI.8). However, following multiple regression analysis, the former variable was not maintained as a significant correlate of HC.

The proposed *models 6 and 6A* differed somewhat from previous

Table VI.8 cont'd: Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,†0.001< p≤0.005,††0.0001<p≤0.001

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4. **N=30, R^2=0.7913**

SEXOFFSP; ***OCCUPE***; **HCM**; **SZF**; **SZ12DE6P**; **SZ12DE6MP**; **SZ123DE6MP**; ***SMOKING***;
0.305†
(0.069)
-0.334†
(0.107)

SEXHORM; **TYPEEP**; MAJSZI; **FMAJSZA**; FMINSZI; ***MINSZA***; NAEDI; ***NAEDA***;
-0.558**
(0.208)
-0.440**
(0.159)

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; ***RCFIII***
0.0006*
(0.0002)

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models in that only one family history variable was included among the background factors in each model: a history of seizures among maternal and paternal 1st and 2nd degree relatives in model 6 and up to 3rd degree relatives in model 6A. The main variables of interest in models 6 and 6A were the occurrence of major seizures; the frequency of minor seizures; the number of AED; the type of AED (PHT, PB, PRM vs PHT, PRM vs PHT, PB vs PHT alone) in the first trimester only; and the dose (mg/kg) of PHT at the LMP.

Model 6 following regression analysis explained 65% of the variation in HC among 25 newborns. Sex of the offspring, father's occupation and the number of AED in the first trimester emerged as significant linear correlates of HC.

In model 6A, 24 pregnancies contributed to the model of significant risk factors following regression analysis, and the R^2 (58%) was somewhat lower than in model 6. Father's occupation and the type of AED in the first trimester were highly significant correlates of HC. For the latter risk factor, the positive sign of the regression coefficient indicated that PHT alone taken in the first trimester was associated with a larger HC in the newborn than PHT taken together with PRM

The proposed models *8 and 8A* included the occurrence of maternal major and minor seizures and the number of AED in the first trimester, as well as mean maternal plasma PHT levels (µg/ml) in the first trimester (model 8), or the mean dose (mg/kg) of PHT in the first trimester (model 8A). Among the background factors, model 8 also included a family history of seizures among maternal and paternal 1st and second degree relatives combined, while model 8A included a family history of seizures in only paternal 1st and 2nd degree relatives

Table VI.8 cont'd: Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,†0.001< p≤0.005,††0.0001<p≤0.001

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6. **N=25, R^2=0.6486**

SEXOFFSP; ***OCCUPF***; **HCM**; SZF; SZ12DEGP; **SZ12DEGMP**; SZ123DEGMP; **SMOKING**;

SEXOFFSP	***OCCUPF***
-0.568*	**0.316'**
(0.272)	**(0.084)**

SEXHORM; **TYPEEP**; **MAJSZI**; FMAJSZA; **FMINSZI**; FMINSZA; ***NAEDI***; NAEDA;

NAEDI
-0.769''
(0.196)

MPPHTPBPRM1; MPPHTPBPRMA; **PHTmg/kgLMP**; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

6A. **N=24, R^2=0.5779**

SEXOFFSP; ***OCCUPF***; **HCM**; SZF; SZ12DEGP; SZ12DEGMP; **SZ123DEGMP**; **SMOKING**;

OCCUPF
0.344'
(0.093)

SEXHORM; **TYPEEP**; **MAJSZI**; FMAJSZA; **FMINSZI**; FMINSZA; **NAEDI**; NAEDA;

MPPHTPBPRM1; MPPHTPBPRMA; **PHTmg/kgLMP**; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII

0.535'
(0.155)

PHTmg/kgALL; PHTPLALL; RCFIII

Table VI.8 cont'd : Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,†0.001< p≤0.005,††0.0001<p≤0.001

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8. **N=22, R^2=0.5348**

SEXOFFSP; **OCCUPF**; **HCM**; SZF; SZ12DEGP; **SZ12DEGMP**; SZ123DEGMP; **SMOKING**;

SEXHORM; **TYPEEP**; **MAJSZI**; FMAJSZA; ***MINSZI***; FMINSZA; **NAEDI**; NAEDA;
-0.982**
(0.381)

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; ***PHTPLI***; PHTmgII; PHTPLII;
-0.149**
(0.054)

PHTmg/kgALL; PHTPLALL; RCFIII

8A. **N=22, R^2=0.4735**

SEXOFFSP; **OCCUPF**; **HCM**; SZF; **SZ12DEGP**; SZ12DEGMP; SZ123DEGMP; **SMOKING**;

SEXHORM; **TYPEEP**; **MAJSZI**; FMAJSZA; ***MINSZI***; FMINSZA; ***NAEDI***; NAEDA;
-1.038** **-0.642***
(0.407) **(0.302)**

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; **PHTmg/kgI**; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

Following multiple regression analysis, 22 pregnancies contributed to each model of significant risk factors. Model 8 explained 53% of the variation in HC, while model 8A accounted for a lower proportion (47%) of the variance. The presence of minor seizures in the first trimester was highly significantly associated with decreased HC in both models. In model 8, an increase in mean plasma PHT levels (µg/ml) in the first trimester was also highly significantly correlated with decreased HC, while in model 8A, the dose (mg/kg) of PHT in the first trimester did not emerge as a significant risk factor. Instead in the latter model, the number of AED in the first trimester emerged as a significant negative correlate of HC.

The proposed *models 10 and 10A* evaluated major and minor seizures at anytime during pregnancy, the type of AED at anytime (PHT,PB,PRM vs PHT, PRM vs PHT, PB vs PHT alone); and the mean dose (mg/kg) and/or plasma levels (µg/ml) of PHT over all trimesters combined In model 10, only mean plasma levels (µg/ml) of PHT over all trimesters was assessed, while model 10A also included the mean dose (mg/kg) of PHT for the entire pregnancy . As well, in models 10 and 10A, only one family history of seizures variable was entered into each model. model 10 included a history of seizures in paternal 1st and 2nd degree relatives combined, while model 10A included maternal and paternal 1st to 3rd degree relatives combined.

Following regression analysis, both models consisting of 24 to 28 pregnancies showed a very high R^2 of 80% , but the results of the two models were quite different In model 10, father's occupation maintained a highly significant positive association with HC. Female sex of offspring, maternal smoking, the number of AED at anytime

Table VI.8 cont'd: Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,†0.001< p≤0.005,††0.0001<p≤0.001

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10. **N=24, R^2=0.8000**

SEXOFFSP ***OCCUPF*** **HCM**; SZF; **SZ12DEGP**; SZ12DEGMP; SZ123DEGMP; ***SMOKING***;
-0.541* **0.213*****
(0.246) **(0.073)**
-0.367**
(0.135)

SEXHORM; **TYPEEP**; MAJSZI; **FMAJSZA** ; FMINSZI; **FMINSZA**; NAEDI; ***NAEDA***;
-0.542***
(0.171)

MPPHTPBPRMI; **MPPHTPBPRMA**; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; ***PHTPLALL***; RCFIII
-0.139***
(0.048)

10A. **N=28, R^2=0.7991**

SEXOFFSP **OCCUPF**; ***HCM***; SZF; SZ12DEGP; SZ12DEGMP; ***SZ123DEGMP***; **SMOKING**;
-0.988** **0.199***** **-1.254****
(0.252) **(0. 069)** **(0.234)**

SEXHORM; ***TYPEEP***; MAJSZI; ***FMAJSZA***; FMINSZI; **MINSZA**; NAEDI; **NAEDA**;
-0.169* **-0.797****
(0.080) **(0.144)**

MPPHTPBPRMI; **MPPHTPBPRMA**; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; **PHTPLALL**; RCFIII

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during pregnancy , and mean plasma PHT levels (µg/ml) over all trimesters combined emerged as significant negative correlates of HC.

When the mean dose (mg/kg) of PHT over all trimesters was entered as an additional factor in the theoretical model 10 (not shown in Table VI.8), the results of multiple regression analysis were identical to those above, but the number of pregnancies contributing to the model was less than twice the number of factors in the model.

In model 10A, after regression analysis, sex of offspring was maintained as a highly significant correlate of HC as in model 10. Other factors which emerged as significant correlates of HC in model 10A were the frequency of maternal major seizures at anytime in pregnancy; mother's HC; a positive family history of seizures in maternal and paternal 1st, 2nd and 3rd degree relatives combined; and type of maternal epilepsy. The regression coefficients with standard errors for the latter three variables indicated that increased HC of the mother was highly significantly correlated with increased HC in the offspring, while a positive family history of seizures was highly significantly associated with decreased HC. Mothers with partial simple seizures with or without secondary generalization gave birth to children with significantly smaller HCs, c than did mothers with either primary generalized epilepsy, or secondary generalized epilepsy with or without focalization.

The theoretical *models 13 and 13A* included a single family history variable - family history of seizures in maternal and paternal 1st to 3rd degree relatives combined - among the background factors; major and minor seizures; the number of AED; dose (mg/kg) of PHT at the LMP; mean daily dose (mg) of PHT in the second trimester; and

Table VI.8 cont'd: Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,†0.001<p≤0.005,††0.0001<p≤0.001

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13. **N=30, R^2=0.6814**

SEXOFFSP; **OCCUPF**; **HCM**; SZF; SZ12DE6P; SZ12DE6MP; **SZ123DE6MP**; ***SMOKING***;
-0.765**
(0.276)
-0.455†
(0.143)

SEXHORM; **TYPEEP**; MAJSZI; ***FMAJSZA***; FMINSZI; **FMINSZA**; NAEDI; **NAEDA**;
-0.547†
(0.167)

MPPHTPBPRMI; MPPHTPBPRMA; **PHTmg/kgLMP**; PHTmg/kgI; PHTPLI; **PHTmgII**; PHTPLII;

PHTmg/kgALL; ***PHTPLALL***; RCFIII
-0.176†
(0.050)

13A. **N=34, R^2=0.6528**

SEXOFFSP; **OCCUPF**; **HCM**; SZF; SZ12DE6P; SZ12DE6MP; ***SZ123DE6MP***; **SMOKING**;
-0.971††
(0.258)
-0.979††
(0.268)

SEXHORM; **TYPEEP**; **MAJSZI**; ***FMAJSZA***; **MINSZI**; **MINSZA**; NAEDI; ***NAEDA***;
-0.552†
(0.166)
-0.424*
(0.207)

MPPHTPBPRMI; MPPHTPBPRMA; **PHTmg/kgLMP**; PHTmg/kgI; PHTPLI; **PHTmgII**; PHTPLII;

PHTmg/kgALL; **PHTPLALL**; RCFIII

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mean dose (mg/kg) and plasma levels (µg/ml) of PHT over all trimesters combined. Model 13 included only the frequency of major and minor seizures and the number of AED at anytime in pregnancy, while model 13A evaluated these factors both for the first trimester of pregnancy and at anytime in pregnancy.

After multiple regression analysis, model 13 was comprised of 30 pregnancies and showed a high R^2 of 68%. Female sex of offspring; cigarette smoking; an increase in major seizures at anytime in pregnancy; and an increase in mean plasma PHT levels (µg/ml) over all trimesters combined emerged as highly significant factors associated with decreased HC.

When the frequency of maternal seizures and the number of AED in the first trimester were entered into multiple regression analysis along with other risk factors in model 13 (not shown in Table VI.8), the results were identical to those for model 13, but the number of pregnancies contributing to the model was less than twice the number of factors in the model.

Following multiple regression analysis, model 13A was comprised of 34 pregnancies and also showed a high R^2 of 65%. Female sex of offspring; positive family history of seizures in maternal and paternal 1st to 3rd degree relatives combined; increased frequency of major seizures at anytime during pregnancy; and an increase in the number of AED at anytime in pregnancy were significantly associated with decreased HC.

(2). Evaluation of consistency, statistical significance, and clinical importance of risk factors

Table VI.9 lists the risk factors assessed by multiple regression analysis according to the consistency of their emergence as significant correlates of HC, and the level of statistical significance.

For each significant risk factor, the proportion of models in which each risk factor was evaluated and emerged as statistically significant at ≤ 0.05 level was calculated. Then the proportion of all significant associations that were highly significant ($p \leq 0.02$) was also calculated.

The findings in Table VI.8 and VI.9 and Appendix F are summarized in Table VI.10 as categories of consistent and inconsistent risk factors, according to the general level of statistical significance and the direction of the significant association with HC.

There were a number of significant risk factors associated with decreased HC (Table VI.9, VI.10). The factors which were consistently significant in the models, that is in at least 40% of the models tested with these factors, and which were highly significant were: female sex of offspring (appearing in 72% of models); low socioeconomic class as measured by father's occupation (52%); an increase in the number of cigarettes smoked (48%); an increase in the frequency of major seizures anytime during pregnancy (66.7%); an increase in the number of AED anytime (46.7%); and an increase in mean dose (mg/kg) or plasma level (µg/ml) of PHT over all trimesters combined (62.5%). Mean dose or plasma levels of PHT in the first trimester was also a highly significant risk factor in one of two models.

→ Of these variables, female sex of the offspring was associated with the smallest reduction in HC of not more than one SD unit, while other

Table VI.9 : Evaluation of Consistency and Statistical Significance of Risk Factors Associated with Head Circumference at Birth Following Multiple Regression Analysis. x = Consistent risk factors appearing in at least 40% of models tested with the factor

Significant Risk Factors	Proportion of models in which risk factor is significant	Proportion of all significant associations in which P≤ 0.02
Background Factors		
x Sex of offspring	18/25 = 72.0%	12/18 = 66.7%
x Father's occupation	13/25 = 52.0%	12/13 = 92.3%
Mother's head circumference	3/25 = 12.0%	3/ 3 = 100.0%
Family history of seizures in maternal and paternal 1st to 3rd degree relatives combined	6/19 = 31.6%	6/ 6 = 100.0%
x Smoking	12/25 = 48.0%	10/12 = 83.3%
Sex hormones	0/25 = 0.0%	-
Type of maternal epilepsy	6/25 = 24.0%	3/ 6 = 50.0%
Maternal Seizures		
Major seizures Trim I	1/13 = 7.7%	0/ 1 = 0.0
x Major seizures Anytime	10/15 = 66.7%	10/10 = 100.0%
Minor seizures Trim I	4/13 = 30.8%	4/ 4 = 100.0%
Minor seizures Anytime	4/15 = 26.7%	4/ 4 = 100.0%
Major and/or minor seizures Trim I	5/13 = 38.5%	4/ 5 = 80.0%
x Major and/or minor seizures Anytime	12/15 = 80.0%	12/12 = 100.0%

Table VI.9 cont'd: Evaluation of Consistency and Statistical Significance of Risk Factors Associated with Head Circumference at Birth Following Multiple Regression Analysis. x = Consistent risk factors appearing in at least 40% of models tested with the factor. [a] Proportion of models in which both maternal seizures and AED were significant at p≤ 0.02.

Significant Risk Factors	Proportion of models in which risk factor is significant	Proportion of all significant associations in which P≤ 0.02
Maternal AED		
No. AED Trim I	5/13 = 38.5%	2/5 = 40.0%
x No. AED Anytime	7/15 = 46.7%	5/7 = 71.4%
PHT PRM vs. PHT alone Trim I	1/ 4 = 25.0%	1/ 1 = 100.0%
PHT PRM vs PHT alone Anytime	1/ 4 = 25.0%	0/ 1 = 0.0%
Dose (mg/kg) PHT LMP	0/ 6 = 0.0%	-
Mean daily dose (mg/kg) or plasma level (µg/ml) PHT Trim I	1/ 2 = 50.0%	1/ 1 = 100.0%
Mean daily dose (mg) or plasma level (µg/ml) PHT Trim II	0/ 3 = 0.0%	-
x Mean dose (mg/kg) or plasma level(µg/ml) PHT Trim ALL	5/ 8 = 62.5%	4/ 5 = 80.0%
x Any AED risk factor Trim I	7/13 = 53.8%	4/ 7 = 57.1%
x Any AED risk factor Anytime	10/15 = 66.7%	7/10 = 70.0%
Combination of Maternal Seizures and AED		
Major and/or minor seizures and any AED risk factor Trim I	4/13 = 30.8%	1/ 4 = 25.0%[a]
x Major and/or minor seizures and any AED risk factor Anytime	7/15 = 46.7%	4/ 7 = 57.1%[a]
Folate		
Mean red cell folate (ng/ml) Trim III	1/ 1 = 100.0%	0/ 1 = 0.0%

Table VI.10

Conclusions of Multiple Regression Analysis of Risk Factors in Relation to the Outcome Variable Head Circumference at Birth

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-Models explained 45-80% of the variation in head circumference

-Significant risk factors associated with decreased head circumference:

Consistent (47-72% of models), highly significant

Female sex of offspring

Low socioeconomic class (as measured by father's occupation)

Increase in no. of cigarettes smoked

Increase in frequency of major seizures Anytime

Increase in no. of AED Anytime

Increase in mean dose or plasma level of PHT Trim I or Trim All

Inconsistent (12-39% of models), highly significant

Decrease in mother's head circumference

Positive family history of seizures

Type of maternal epilepsy (HC associated with partial simple < generalized epilepsies)

Occurrence (yes/no), or an increase in frequency of minor seizures Trim I or Anytime

Increase in no. of AED Trim I

Type of AED (HC associated with PHT PRM < PHT alone) Trim I or Anytime

In one model, a decrease in mean red cell folate Trim III was significantly associated with a decrease in newborn head circumference at $0.02 < p \leq 0.05$.

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factors were associated with larger changes.

Other risk factors associated with a decrease in HC were inconsistent (< 40% of the models in which the factors were tested) (Table VI.9,VI.10), but usually highly significant. These were small HC of the mother (appearing in 12% of models); a positive family history of seizures in maternal and paternal 1st to third degree relatives combined (31.6%); maternal partial simple seizures with or without secondary generalization as compared with the generalized epilepsies (24%), the presence of minor seizures or an increase in the frequency of these seizures in the first trimester (30.8%) and at anytime during pregnancy (26.7%); and a combination of the drugs PHT and PRM versus PHT alone in the first trimester (25%) or anytime during pregnancy (25%).

An increase in the number of AED in the first trimester appeared as a significant risk factor for decreased HC in 38.5% of the models, with the associations highly significant in 40% of the cases This factor was therefore a borderline highly significant inconsistent factor.

Major seizures in the first trimester emerged as a significant risk factor associated with decreased HC in only 1 of 13 models (7 7%), and the association was not highly significant

A decrease in mean red cell folate levels (ng/ml) in the third trimester, evaluated in one model, was associated with a decrease in HC at $0.02 < p \leq 0.05$

Among these variables, mother's HC; family history of seizures, number of AED in the first trimester; and PHT PRM versus PHT alone in the first trimester and at anytime were associated with a greater than one SD unit change in HC, while type of maternal epilepsy, major seizures in the first trimester; minor seizures in the first trimester

and anytime in pregnancy, and mean red cell folate levels (ng/ml) in the third trimester were associated with smaller changes.

Overall, as seen in Table VI.9, major and/or minor seizures in the first trimester appeared as significant risk factors for decreased HC in 38.5% of the models, whereas these factors at anytime in pregnancy emerged as significant correlates of HC in 80% of the models. Any one of the AED risk factors in the first trimester appeared as a significant risk factor for decreased HC in 53.8% of the models tested, while any of these factors at anytime during pregnancy emerged as a significant correlate in 66.7% of the models A combination of major and/ or minor seizures and any AED risk factor in the first trimester was significantly correlated with decreased HC in 30.8% of the models, whereas a combination of these factors at anytime in pregnancy appeared in 46.7% of the models

4. Analysis of Birth Weight in Relation to Risk Factors

a. *Univariate Risk Factor Analysis*

(1). Correlations with continuous or ordinal independent variables

Table VI.11 shows the results of univariate correlation analysis for the outcome WT.

A low positive correlation was found between maternal height and WT in the offspring.

WT also showed a low positive correlation with the proportion of 1st and 2nd degree paternal relatives who had a history of major congenital malformations. In contrast, low negative correlations were observed between WT and the proportion of maternal and paternal 1st to 3rd degree relatives with a history of seizures.

Table VI.11

Results of Univariate Correlation Analysis
Outcome Variable: Birth Weight

Risk Factor Abbreviation	Risk Factor	Correlation Coefficient	N	P
	Personal Characteristics			
HEIGHTM	Mother's height	0.267	103	0.01
	Family History			
SZ12DEGP	Seizures in paternal			
	1st,2nd degree relatives	-0.213	101	0.05
SZ123DEGMP	Seizures in maternal, paternal			
	1st,2nd,3rd degree relatives	-0.228	86	0.05
CM12DEGP	Major congenital malfs in paternal			
	1st, 2nd degree relatives	0.200	102	0.05
	Drugs Other Than AED			
SMOKING	Cigarette smoking	-0.343	108	0.001
	Maternal Seizures			
FMAJSZI	Frequency of major seizures Trim I	-0.201	106	0.05
FMAJSZA	Frequency of major seizures Anytime	-0.264	107	0.01
FMINSZA	Frequency of minor seizures Anytime	-0.210	88	0.05
	Maternal AED Therapy			
PHTmgI	Mean daily dose (mg) PHT Trim I	-0.290	80	0.01
PHTmgII	Mean daily dose (mg) PHT Trim II	-0.279	76	0.02
PHTmgALL	Mean daily dose (mg) PHT All Trim	-0.263	83	0.02
PHTmg/kgLMP	Dose (mg/kg) PHT at LMP	-0.353	74	0.005
PHTmg/kgI	Mean dose (mg/kg) PHT Trim I	-0.595	32	0.001
PHTmg/kgII	Mean dose (mg/kg) PHT Trim II	-0.385	47	0.01
PHTmg/kgIII	Mean dose (mg/kg) PHT Trim III	-0.255	62	0.05
PHTmg/kgALL	Mean dose (mg/kg) PHT All Trims	-0.340	81	0.005
PBmg/kgLMP	Dose (mg/kg) PB at LMP	-0.295	45	0.05
VPAmg/kgLMP	Dose (mg/kg) VPA at LMP	0.739	9	0.05
VPAmgI	Mean daily dose (mg) VPA Trim I	0.701	9	0.05

Low negative correlations were noted for such factors as maternal cigarette smoking, and the frequency of major and minor seizures in the first trimester or at anytime during pregnancy.

With respect to maternal AED therapy, significant negative correlations were found for the dose (mg or mg/kg) of PHT at the LMP , in all three trimesters of pregnancy, and over all trimesters combined. The correlation coefficients were generally low, with dose in mg/kg showing higher correlations (-0.255 to -0.595) than mean daily dose in mg (-0.263 to -0.290) . The highest correlation between WT and dose (mg/kg) was in the first trimester (-0.595), followed by a decrease in the correlation from the first to third trimester. Surprisingly, significant negative correlations were not noted between plasma levels of phenytoin (µg/ml) and WT.

A low negative correlation was found for dose (mg/kg) of phenobarbital at the LMP only, with no other significant findings during pregnancy.

In 9 women who took valproic acid during pregnancy, there were very high positive correlations between the dose (mg/kg) at the LMP (0.739) or the mean daily dose (mg) in the first trimester (0.701) and WT of the offspring.

(2). Categorical (dichotomous) independent variables (t-tests)

Several dichotomous variables were shown to be significantly associated with WT (Table VI.12).

A highly significant decrease ($p < 0.01$) in WT was noted for female children as compared to male children.

WT was significantly decreased in children with a positive history

Table VI.12

Results of Univariate t-tests

Outcome Variable: Birth Weight (WT)

Risk Factor Abbreviation	Risk Factor	Mean Standard Score of WT ± SD No (0) Group	Yes (1) Group	N_0 / N_1	P
	Personal Characteristics				
FEMOFFSPR	Female sex of child	**0.066** ± 0.816	**-0.396** ± 0.926	56/53	0.01
	Family History				
SZ12DEGP	Seizures in paternal 1st, 2nd degree relatives	**-0.080** ± 0.867	**-0.832** ± 1.109	92/ 9	0.02
	Pregnancy Characteristics and Complications				
NAUSEA	Nausea +/- vomiting, treated	**-0.065** ± 0.924	**-0.518** ± 0.717	86/22	0.05
BLOODSENS	Blood group sensitization	**-0.107** ± 0.880	**-1.374** ± 0.522	104/ 4	0.01
	Drugs Other Than AED				
OTHHORMA	Other (not sex) hormones Anytime	**-0.218** ± 0.849	**0.522** ± 1.342	99/ 7	0.05
	Maternal Seizures				
MAJSZI	Major seizures Trim I	**-0.054** ± 0.905	**-0.513** ± 0.848	81/25	0.05
MAJSZA	Major seizures Anytime	**0.020** ± 0.948	**-0.406** ± 0.777	63/45	0.02
MINSZI	Minor seizures Trim I	**-0.028** ± 0.824	**-0.450** ± 0.938	54/38	0.05

of seizures in paternal 1st and 2nd degree relatives combined, as compared to children who had a negative history ($p<0.02$).

Within the group of factors involving pregnancy complications, nausea and/or vomiting treated by antinauseants or antiemetics was associated with a significant decrease in WT ($p<0.05$). There were two women each with 2 children who exhibited maternal-infant blood group incompatibilities (positive antibody tests). The first woman and her two infants showed mild ABO blood group incompatibility, while the second woman and her two infants showed mild Rh (Group E) blood group incompatibility. Overall, blood group sensitization was associated with a highly significant decrease in WT ($p<0.01$), there being a difference of greater than one SD unit in mean standard score of WT in mother-child pairs with and without this factor.

There were six women with seven children who took hormones other than sex hormones during pregnancy. These included one woman with two children (standard scores of WT= 0.223 and 1.586), two other women each with one child (standard scores of WT= -1.516, 0.994) who took synthroid throughout pregnancy for the treatment of hypothyroidism. Two women took steroids - one woman, subsequent to the onset of premature contractions in the fourth month of pregnancy, took corticosteroids from the fourth month onwards to accelerate maturation of the fetal lungs (standard score of newborn's WT = 0.903), and the other was prescribed topical corticosteroids in the second trimester for the treatment of allergic rhinitis (standard score of WT = -0.847). One woman became pregnant while being treated with clomiphene citrate for infertility (standard score of WT=2.308). The seven children of these women showed significantly larger WT than

children unexposed to such hormones ($p<0.05$).

A significant decrease in WT was associated with the presence of major seizures in the first trimester ($p<0.05$) or at anytime during pregnancy ($p<0.02$), and the presence of minor seizures in the first trimester ($p<0.05$).

3. Categorical independent variables (> 2 subclasses) (anova analysis)

Categorical risk factors with more than two subclasses [e.g, type of epilepsy, type(s) of AED] were not found to be significantly associated with birth weight.

b. Multiple Regression Analysis

The proposed models of risk factors in relation to WT and the detailed results of multiple regression analysis are given in section VI.B.4.b.(Table VI.13). Section VI.B.4.b. (Tables VI.14 and VI.15) summarizes the risk factors maintaining a significant association with WT following regression analysis with respect to their consistency, level of statistical significance and clinical importance.

(1). Theoretical models and models of significant risk factors following regression analysis

The theoretical models of risk factors in relation to the outcome WT, and the significant risk factors emerging from multiple regression analysis are shown with their regression coefficients and standard errors in Table VI.13.

There were 6 models following regression analysis with adequate sample size and R^2. The numbers of pregnancies contributing to these

models ranged from 25 to 54, and the R^2 ranged from 42% to 64%.

Theoretical models 1 to 3 included risk factors pertaining to the first trimester; models 4 and 4A involved factors related to the entire pregnancy; and model 5 included factors concerning the first and second trimesters and the entire pregnancy.

The factors which were entered into all the models (background factors) were: sex of offspring (SEXOFFSP); mother's height (HEIGHTM); family history of major congenital malformations in paternal 1st and 2nd degree relatives combined (CM12DEGP); family history of seizures in paternal 1st and 2nd degree relatives combined (SZ12DEGP) and/or in maternal and paternal 1st to 3rd degree relatives combined (SZ123DEGMP); blood group sensitization (BLOODSENS); nausea ± vomiting (treated) (NAUSEA); maternal smoking (SMOKING); and maternal ingestion of other (not sex) hormones at anytime during pregnancy (OTHHORMA). Other risk factors included in the models in different combinations were the frequency of maternal major seizures in the first trimester (FMAJSZ1) and at anytime in pregnancy (FMAJSZA); the presence or absence of at least one maternal minor seizure in the first trimester (MINSZ1); frequency of maternal minor seizures at anytime in pregnancy (FMINSZA); dose (mg/kg) of PHT at the last menstrual period (PHTmg/kgLMP); mean daily dose (mg) of PHT in the first trimester (PHTmg1), second trimester (PHTmg11), and over all trimesters combined (PHTmgALL); mean dose (mg/kg) of PHT in the first trimester (PHTmg/kg1) and over all trimesters combined (PHTmg/kgALL); and dose (mg/kg) of PB at the last menstrual period (PBmg/kgLMP).

The theoretical *model 1* was comprised of all background factors

Table VI.13: Multiple Regression Analysis of Risk Factors in Relation to Birth Weight. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print), *0.02<p≤0.05, **0.01<p≤0.02, ***0.005<p≤0.01,† 0.001<p≤0.005, ††0.0001< p≤0.001.

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1. **N=46, R^2=0.5584**

SEXOFFSPR **HEIGHTM; CM12DEGP;** ***SZ12DEGP***; **SZ123DEGMP; BLOODSENS; NAUSEA;**
-0.550* ** **-7.579††**
(0.197) **(2.079)**

SMOKING OTHHORMA; **FMAJSZI**; FMAJSZA; ***MINSZI***; FMINSZA;
-0.220* **-0.473***
(0.100) **(0.200)**

PHTmg/kgLMP; **PHTmgI**; PHTmg/kgI; PHTmgII; PHTmgALL; PHTmg/kgALL; PBmg/kgLMP

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2. **N=25, R^2=0.6290**

SEXOFFSPR; HEIGHTM; CM12DEGP; ***SZ12DEGP***; SZ123DEGMP; ***BLOODSENS***; **NAUSEA;**
-10.962†† **-1.183†**
(1.977) **(0.364)**

SMOKING; OTHHORMA; **FMAJSZI**; FMAJSZA; **MINSZI**; FMINSZA;

PHTmg/kgLMP; **PHTmgI**; **PHTmg/kgI**; PHTmgII; PHTmgALL; PHTmg/kgALL; PBmg/kgLMP

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and the frequency of major seizures in the first trimester; the presence or absence of minor seizures in the first trimester; dose (mg/kg) of PHT at the LMP ; and mean daily dose (mg) of PHT in the first trimester.

Following multiple regression analysis, decreased WT was highly significantly associated with female sex of offspring and a positive family history of seizures among paternal 1st and 2nd degree relatives combined. WT also significantly decreased with an increase in the number of cigarettes smoked, and with the occurrence of minor seizures in the first trimester of pregnancy. The combination of these significant risk factors explained 56% of the variation in WT among 46 children.

The proposed *model 2* differed from model 1 in that only one of the family history of seizure variables was included among the background factors, and the mean dose (mg/kg) of PHT in the first trimester was also entered along with other parameters of PHT dose at this stage of pregnancy.

The model of significant risk factors following regression analysis explained 63% of the variation in WT among 25 children. Again a positive family history of seizures among paternal 1st and second degree relatives combined emerged as a highly significant factor associated with decreased WT. The only other factor that was highly significantly associated with decreased WT was blood group sensitization. This factor involved only two women who each had two pregnancies with this particular complication.

The proposed *model 3* was designed to examine the possible influence of dose (mg/kg) of PB at the LMP along with major and minor seizures in the first trimester and all background factors.

Table VI.13: Multiple Regression Analysis of Risk Factors in Relation to Birth Weight. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print), *0.02<p≤0.05, **0.01<p≤0.02, ***0.005<p≤0.01, † 0.001<p≤0.005, ††0.0001< p≤0.001.

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3. N=26, R^2=0.5228

SEXOFFSPR; **HEIGHTM**; **CM12DEGP**; **SZ12DEGP**; **SZ123DEGMP**; **BLOODSENS**; ***NAUSEA***;
-0.605***
(0.200)
-0.497*
(0.237)

SMOKING; OTHHORMA; **FMAJSZI**; FMAJSZA; ***MINSZI***; FMINSZA;
-0.468*
(0.193)

PHTmg/kgLMP; PHTmgI; PHTmg/kgI; PHTmgII; PHTmgALL; PHTmg/kgALL; ***PHmg/kgLMP***
-0.311*
(0.125)

In this model, after regression analysis, female sex of offspring; the occurrence of nausea, with or without vomiting and requiring drug treatment; and the presence of minor seizures in the first trimester were significantly associated with decreased WT. An increase in the dose of PB at the LMP was also significantly correlated with decreased WT. This combination of factors explained 52% of the variation in WT among 26 children.

The theoretical models *4 and 4A* examined the interrelationship among mean dose of PHT (mg/kg and mg) over all trimesters combined, the frequency of major and minor seizures at anytime in pregnancy, other risk factors, and the outcome WT. Model 4 included only one of the family history of seizures variables among its background factors, while model 4A included both family history variables.

The results of multiple regression analysis were somewhat different for the two models. In model 4, female sex of offspring, a positive family history of seizures among paternal 1st and 2nd degree relatives combined, and an increase in cigarette smoking were highly significantly associated with decreased WT. An increase in maternal height was also highly significantly associated with an increase in WT of the newborn. The combination of significant risk factors in model 4 accounted for 53% of the variation in WT among 54 children.

In model 4A, female sex of offspring and maternal height were maintained as highly significant correlates of WT, while other factors were not maintained. Instead, in model 4A, the ingestion of other (not sex) hormones anytime during pregnancy emerged as a significant correlate of increased WT. The linear combination of risk factors in this model explained only 42% of the variation in WT among 44 children.

Table VI.13 cont'd: Multiple Regression Analysis of Risk Factors in Relation to Birth Weight. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05, **0.01<p≤0.02, ***0.005<p≤0.01, † 0.001< p≤0.005, ††0.0001< p≤0.001.

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4. **N=54, R^2=0.5289**

SEXOFFSPR ***HEIGHTM*** **CM12DE6P**; ***SZ12DE6P***; SZ123DE6MP; **BLOODSENS**; **NAUSEA**;

−0.557†	0.052***	−6.824†	
(0.179)	(0.019)	(1.988)	

SMOKING **OTHHORMA**; FMAJSZI; **FMAJSZA**; MINSZI; **FMINSZA**;

−0.227***
(0.080)

PHTmg/kgLMP; PHTmgI; PHTmg/kgI; PHTmgII; **PHTmgALL**; **PHTmg/kgALL**; PBmg/kgLMP

4A. **N=44, R^2=0.4206**

SEXOFFSPR ***HEIGHTM*** **CM12DE6P**; **SZ12DE6P**; **SZ123DE6MP**; **BLOODSENS**; **NAUSEA**;

−0.696†	0.079†
(0.234)	(0.024)

SMOKING; ***OTHHORMA***; FMAJSZI; **FMAJSZA**; MINSZI; **FMINSZA**;

1.005*
(0.466)

PHTmg/kgLMP; PHTmgI; PHTmg/kgI; PHTmgII; **PHTmgALL**; **PHTmg/kgALL**; PBmg/kgLMP

The theoretical *model 5* included one family history of seizures variable among its background factors, a number of measures of PHT dose for the first and second trimesters of pregnancy and over all trimesters combined, and major and minor seizures in the first trimester and at anytime during pregnancy.

Regression analysis resulted in a model of significant risk factors which accounted for a high proportion of the variation in WT (64%) among 46 children. Female sex of offspring, a positive family history of seizures in paternal 1st and second degree relatives combined, an increase in cigarette smoking, and an increase in the frequency of major seizures in the first trimester were highly significantly associated with decreased WT. The presence of minor seizures in the first trimester also emerged as a significant correlate of decreased WT.

(2). Evaluation of consistency, statistical significance, and clinical importance of risk factors

The proportion of models in which each of the risk factors were significantly associated with WT , and the proportion of significant associations which were ≤ 0.02 are shown in Table VI.14.

The consistency with which risk factors emerged as significant correlates of WT in all models, and the direction and extent of the significant associations with WT are summarized in Table VI.15.

Female sex of offspring (appearing in 83.3% of models), positive family history of seizures (66.7%), and cigarette smoking (50.0%) were consistently and highly significantly associated with decreased WT.

The presence of minor seizures in the first trimester (75%) was consistently associated with decreased WT at $0.02 < p \leq 0.05$.

Table VI.13 cont'd: Multiple Regression Analysis of Risk Factors in Relation to Birth Weight. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print), *0.02<p≤0.05, **0.01<p≤0.02, ***0.005<p≤0.01,† 0.001<p≤0.005, ††0.0001< p≤0.001.

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5. **N=46, R^2=0.6449**

SEXOFFSPR	**HEIGHTM**;	**CM12DEGP**;	***SZ12DEGP***	SZ123DEGMP;	**BLOODSENS**;	**NAUSEA**;
−0.473**			**−7.391††**			
(0.189)			**(1.924)**			

SMOKING	**OTHHORMA**;	***FMAJSZL***;	**FMAJSZA**;	***MINSZL***;	**FMINSZA**;
−0.318††		**−0.586†**		**−0.398***	
(0.081)		**(0.197)**		**(0.192)**	

PHTmg/kgLMP; **PHTmgI**; PHTmg/kgI; **PHTmgII**; **PHTmgALL**; **PHTmg/kgALL**; PBmg/kgLMP

Table VI.14 : Evaluation of Consistency and Statistical Significance of Risk Factors Associated with Birth Weight Following Multiple Regression Analysis.
x = Consistent risk factors appearing in at least 40% of models tested with the factor.

Significant Risk Factors	Proportion of models in which risk factor is significant	Proportion of all significant associations in which $P \leq 0.02$
Background Factors		
x Sex of offspring	5/ 6 = 83.3%	5/ 5 = 100.0%
Mother's height	2/ 6 = 33.3%	2/ 2 = 100.0%
Family history of major congenital malfs. in 1st and 2nd degree paternal relatives	0/ 6 = 0.0%	-
x Family history of seizures in 1st and 2nd degree paternal relatives	4/ 6 = 66.7%	4/ 4 = 100.0%
Blood group sensitization	1/ 6 = 16.7%	1/ 1 = 100.0%
Nausea ± vomiting, treated	1/ 6 = 16.7%	0/ 1 = 0.0%
x Smoking	3/ 6 = 50.0%	2/ 3 = 66.7%
Other (not sex) hormones Anytime	1/ 3 = 33.3%	0/ 1 = 0.0%
Maternal Seizures		
Major seizures Trim I	1/ 4 = 25.0%	1/ 1 = 100.0%
Major seizures Anytime	0/ 3 = 0.0%	-
x Minor seizures Trim I	3/ 4 = 75.0%	0/ 3 = 0.0%
Minor seizures Anytime	0/ 3 = 0.0%	-
Maternal AED		
Dose (mg/kg) PHT LMP	0/ 3 = 0.0%	-
Mean dose (mg/kg) or mean daily dose (mg) PHT Trim I	0/ 3 = 0.0%	-
Mean daily dose (mg) PHT Trim II	0/ 1 = 0.0%	-
Mean dose (mg/kg) or mean daily dose (mg) PHT Trim ALL	0/ 3 = 0.0%	-
Dose (mg/kg) PB LMP	1/ 1 = 100.0%	0/ 1 = 0.0%

Table VI.15

Conclusions of Multiple Regression Analysis of Risk Factors in Relation to the Outcome Variable Birth Weight

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-Models explained 52-64% of the variation in birth weight.

-Significant risk factors associated with <u>decreased</u> birth weight.

<u>Consistent (50-83%), highly significant</u>

Female sex of offspring

Positive family history of seizures in paternal 1st and 2nd degree relatives

Increase in no. of cigarettes smoked

<u>Consistent (75%), $0.02 < p \leq 0.05$</u>

Occurrence(yes/no) of minor seizures Trim I

<u>Inconsistent (17-33%), highly significant</u>

Decrease in maternal height

Blood group sensitization

Increase in frequency of major seizures Trim I

<u>Inconsistent (17%), $0.02 < p \leq 0.05$</u>

Nausea ± vomiting (treated)

In one model, an increase in dose PB at LMP was also significantly associated with <u>decreased</u> birth weight at $0.02 < p \leq 0.05$.

-The association of other (not sex) hormones Anytime with <u>increased</u> birth weight was inconsistent at the significance level of $0.02 < p \leq 0.05$.

Of these variables, family history of seizures was associated with the greatest reduction in WT, of more than one SD unit, while other factors were associated with a reduction in WT of less than one SD unit.

A number of factors were inconsistently but highly significantly associated with decreased WT. These were the occurrence of blood group sensitization (appearing in 16.7% of models) and an increased frequency of major seizures in the first trimester (25%). Mother's height was inconsistently (33.3%), but highly significantly positively associated with an increase in WT All of these factors were associated with a greater than one SD unit change in WT.

Other factors were inconsistently associated with WT at $0.02 < p \leq 0.05$. The occurrence of nausea with or without vomiting and requiring drug therapy (appearing in 16.7% of models) was associated with decreased WT, while the ingestion of other (not sex hormones) at anytime in pregnancy (33.3% of models) was associated with increased WT.

In the one model in which the dose (mg/kg) of PB at the LMP was evaluated, an increase in this factor was associated with a significant reduction in WT at $0.02 < p \leq 0.05$. The change in WT associated with these variables was one SD unit or less.

5. Analysis of Birth Length in Relation to Risk Factors

a. *Univariate Risk Factor Analysis*

(1). Correlations with continuous or ordinal independent variables

Very few factors were shown to be correlated with LT in the offspring (Table VI.16).

A history of seizures in various combinations of paternal 1st to

Table VI.16

Results of Univariate Correlation Analysis

Outcome Variable: Birth Length

Risk Factor Abbreviation	Risk Factor	Correlation Coefficient	N	P
	Family History			
SZ2DEGP	Seizures in paternal 2nd degree relatives	-0.212	100	0.05
SZ12DEGP	Seizures in paternal 1st,2nd degree relatives	-0.254	99	0.02
SZ123DEGP	Seizures in paternal 1st,2nd,3rd degree relatives	-0.232	89	0.05
	Maternal AED Therapy			
PHTmgII	Mean daily dose (mg) PHT Trim II	-0.251	73	0.05
VPAmg/kgLMP	Dose (mg/kg) VPA at LMP	0.667	9	0.05

3rd degree relatives of the child showed low negative correlations with LT.

Only mean daily dose (mg) of phenytoin in the second trimester was significantly negatively associated with LT, and this showed a low correlation. A high positive correlation (0.667) was found between dose (mg/kg) of valproic acid at the LMP and LT in 9 mother-child pairs, as was observed for the other growth parameters.

(2.) Categorical (dichotomous) independent variables (t-tests)

Female children were significantly shorter at birth than males ($p=0.05$)(Table VI.17).

LT was significantly smaller in children with a positive history of seizures among paternal 1st and second degree relatives combined, than in children with a negative history ($p<0.05$). The difference was greater than one SD unit.

Seven women had premature contractions, of whom 6 were treated by uterine relaxants. Newborns of these 7 women were significantly longer than babies of other mothers ($p<0.05$).

(3).Categorical independent variables (> 2 subclasses) (anova analysis)

Table VI.18 shows that two women who took PHT and PRM in combination at anytime during pregnancy had significantly shorter children than women who took PHT, PB, PRM; PHT, PB; PHT alone or PB alone. In all comparisons, the differences in LT were greater than one SD unit.

Table VI.17

Results of Univariate t-tests

Outcome Variable: Birth Length (LT)

Risk Factor Abbreviation	Risk Factor	Mean Standard Score of LT ± SD No (0) Group	Yes (1) Group	N_0 / N_1	P
	Personal Characteristics				
FEMOFFSPR	Female sex of child	**0.113** ± 0.980	**-0.305** ± 1.137	56/50	0.05
	Family History				
SZ12DEGP	Seizures in paternal 1st,2nd degree relatives	**0.049** ± 0.981	**-1.189** ± 1.553	90/9	0.05
	Pregnancy Characteristics and Complications				
PREMCONTR	Premature contractions, treated	**-0.146** ± 1.058	**0.788** ± 0.943	99/7	0.05

Table VI.18

Results of Univariate Anova
Outcome Variable: Birth Length

Risk Factor Abbreviation	Risk Factor	Group Means	N	P Anova (Sign. pair-wise comparisons p<05)
	Type of AED			
MPPHTPBPRMA	PHT,PB,PRM monotherapy or polytherapy Anytime			
	1. PHT,PB,PRM	0.094	11	0.01
	2. PHT,PRM	-2.665	2	(Type2<1,3,4,5)
	3. PHT,PB	-0.023	23	
	4. PHT	-0.187	21	
	5. PB	0.374	6	

b. *Multiple Regression Analysis*

(1). Theoretical models and models of significant risk factors following regression analysis

Although several theoretical model sets were proposed for the evaluation of risk factors in relation to LT, only one model set was of sufficient sample size and R^2. Table VI.19 shows the model which best represents the results of multiple regression analysis in this model set.

Factors entered into the model were sex of offspring **(SEXOFFSP)** ; family history of seizures in paternal 1st and 2nd degree relatives combined **(SZ12DEGP)** and in paternal 1st to 3rd degree relatives combined **(SZ123DEGP)** , the occurrence of premature contractions at anytime during pregnancy, requiring drug treatment in the majority of cases **(PREMCONTR)** ; type of AED (PHT, PB, PRM vs PHT, PRM vs PHT, PB vs PHT alone) at anytime during pregnancy **(MPPHTPBPRMA)** ; and mean daily dose (mg) of PHT in the second trimester **(PHTmgII)** .

The model of significant risk factors which emerged from multiple regression analysis explained only 47% of the variation in LT among 44 children A positive family history of seizures in paternal 1st and 2nd degree relatives was highly significantly associated with decreased LT. An increase in the mean daily dose (mg) of PHT in the second trimester was also significantly negatively associated with LT at $0.02 < p \leq 0.05$ (Table VI.19, V.20). No other factors evaluated were significant.

A positive family history of seizures in paternal relatives was associated with the larger reduction in LT of more than 2 SD units, while dose of PHT was associated with a maximum decrease of 2 SD units.

Table VI.19

Multiple Regression Analysis of Risk Factors in Relation to Birth Length. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print), *0.02<p≤0.05, **0.01<p≤0.02, ***0.005<p≤0.01, † 0.001< p≤0.005, ††0.0001< p≤0.001.

N=44; R^2=0.4690

SEXOFFSPR;	***SZ120E6P***;	SZ123DE6P;	PREMCONTR;	MPPHTPBPRMA;	***PHTmall***
	-2.698††				**-0.004***
	(0.516)				**(0.002)**

Table VI.20

Conclusions of Multiple Regression Analysis of Risk Factors in Relation to the Outcome Variable Birth Length

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-Model explained 47% of the variation in birth length

-Positive family history of seizures in paternal 1st and 2nd degree relatives was highly significantly associated with decreased birth length.

-Increase in mean daily dose (mg) PHT Trim II was also significantly negatively associated with birth length at $0.02 < p \leq 0.05$.

-No other factors evaluated were significant

C. Discussion

A number of previous studies have shown a decrease of HC in newborns of epileptic women, while WT or LT may or may not be reduced (see section II M, Studies of Somatic Parameters at Birth in Epileptic Patients and Table II 9). Few studies have investigated a possible dose-response relationship between maternal AED treatment or frequency of seizures and newborn growth parameters As well, only some studies have evaluated the role of other potential risk factors.

In a preliminary prospective study of 52 live births in epileptic women using British newborn weight measurements[a] as the standard population, Andermann, Dansky and Kinch (1982b) found that WT tended to be small for gestational age, particularly in female children A reanalysis of the data employing the Montreal standards of Usher and McLean (1969) confirmed a decrease in WT and showed a decrease in HC, but not in LT (Andermann et al, 1985) A significant relationship was found between AED polytherapy and a decrease in HC and WT The aims of the present prospective investigation were to evaluate the distribution of growth parameters among a larger series of newborns of epileptic women as compared with the standard population of Usher and McLean, and to assess which factors might account for the variation in growth parameters The independent associations of maternal seizures; number, type, dose and plasma levels of AED, as well as other potential risk factors with growth parameters were

[a] Tanner and Whitehouse, University of London Institute of Child Health

studied by multiple regression analysis.

The present study showed that, among the offspring of epileptic women taking AED, HC was small for length and weight at birth, in agreement with the findings of Hiilesmaa and colleagues (1981, 1982b). Twenty-six percent of the children had HCs at ≤10th percentile, while 8.7% were microcephalic with measurements ≤ 3rd percentile. The latter figure is at the lower range of that reported in the literature (8.0-27.3%). Small HCs were found in both malformed and nonmalformed children, and were particularly frequent in those with "Developmental" anomalies

For the group as a whole, weight and length were not significantly decreased, although children with DA were significantly shorter than expected. This suggests that reduced HC is a relatively frequent finding among offspring of epileptic women and that, in a small subgroup of offspring, there is also a generalized abnormality of growth and morphogenesis. This is in accord with previous reports of a constellation of anomalies, involving prenatal growth retardation and major congenital anomalies among other abnormalities, in some offspring of epileptic women taking AED (Béthenod and Fréderich, 1975 Hanson et al , 1976)

The present findings agree with the majority of studies which did not find an increased risk of low WT in the offspring of epileptic women (Deblay et al.,1982; Fedrick, 1973; Higgins and Comerford, 1974; Hiilesmaa et al ,1981, Hiilesmaa, 1983; Hill and Tennyson,1982a; Majewski and Steger,1984; Nelson and Ellenberg,1982; Neri et al., 1983; Nomura et al., 1984; Pritchard et al., 1971; Robert et al., 1986; Robertson et al., 1986; Speidel and Meadow, 1972; Svigos, 1984; Weber

et al.,1977; Vert et al.,1982). However, a few studies have found decreased WT (Akhtar and Millac, 1987; Bjerkedal and Bahna,1973; Bjerkedal,1982a; Bertollini et al., 1987; Göpfert-Geyer et al, 1982, Kallen, 1986; Klepel et al., 1986; Koch et al., 1983; Monson et al, 1973, Ogawa et al., 1982,1984; Yerby et al., 1985). In the latter studies, it is not clear to what extent selection bias (for example, towards more severe epilepsies) or factors associated with maternal epilepsy and predisposing to low WT, such as low socioeconomic class or toxemia, may have contributed to the observed difference in WT between the epileptic group and controls.

In the comparison of growth parameters in the present series with those reported by Usher and McLean (1969), there was no adjustment for sex as growth parameters by sex were not published for the standard population. Thus in the present study, sex had to be taken into account as a potential confounder. It is not known whether the distribution of WT and LT in the present series may have differed from those of Usher and McLean, had a comparison been made for each sex.

In the multiple regression analysis of risk factors in relation to growth parameters, a number of background variables were found to be significantly associated with one or more growth parameters, as might be expected in the general population. Decreased growth measurements were associated with female sex of offspring (HC, WT, LT), lower socioeconomic status (HC), and an increase in cigarette smoking during pregnancy (HC,WT). WT was inconsistently negatively correlated with maternal height.

Other factors which were inconsistently associated with decreased WT were nausea ± vomiting, treated with drugs, and blood group

sensitization. The latter was an interesting finding in view of the fact that ABO blood group incompatibility may be associated with a smaller placenta (Ounsted, 1978). However, this factor only involved four children born to two women. Sex hormone use during pregnancy was significantly associated with decreased HC univariately, but not after multiple regression analysis in any of the models tested. Estrogen has been shown to inhibit growth in rabbits (Abdul-Karim et al., 1971). Other hormones such as glucocorticoids, which have been associated with growth retardation in animals and man (D'Ercole and Underwood, 1986), were inconsistently associated with increased WT at only the 0.05 level after multiple regression analysis, and this probably represents a spurious finding.

Although the overall distribution of WT in offspring of epileptic women did not differ significantly from that observed for newborns in the general population, one could not definitely rule out the possibility that factors associated with maternal epilepsy, such as specific drug regimens, might contribute to a decrease in WT and explain in part the variability in WT observed in the present series. In the univariate analysis, the dose of PHT was consistently negatively correlated with WT; however, this did not emerge as a significant factor following multiple regression analysis. This may have been due to the fact that WT was unrelated to AED in the particular subset of patients analyzed by multiple regression analysis. [a] Another possibility is that dose of

[a] Due to the fact that not all women had full data on all risk factors, only a subset of women analyzed by univariate analysis could also be analyzed by multiple regression analysis (See General Discussion).

PHT was a proxy for some other risk factor, and therefore did not maintain an independent association with WT following multiple regression.

The only AED besides PHT that could be adequately evaluated by multiple regression analysis because of limitations in the number of women taking specific AED was PB. The dose of PB at the LMP was significantly associated with WT after controlling for the effects of other factors by multiple regression analysis. However, the dose of PB during pregnancy was not significantly associated with WT. Previous studies of epileptic women have not found PB use during pregnancy to be associated with decreased WT (Bertollini et al., 1987; Deblay et al., 1982; Vert et al., 1982).

Previous studies have shown a significant relationship between decreased WT and polytherapy, such as PHT taken together with barbiturates, as well as with specific monotherapies such as PRM or CBZ (Andermann et al., 1985; Göpfert-Geyer et al., 1982; Koch et al., 1983; Rating et al.,1982b; Majewski and Steger, 1984) Bertollini and colleagues (1987), however, reporting the results of a collaborative study, did not find that WT was decreased in newborns of mothers on PHT monotherapy.

Although the occurrence of minor seizures in the first trimester emerged as a consistent significant correlate of decreased WT after multiple regression analysis, the level of significance was only at the 0.05 level. If minor seizures were important, one would have also expected minor seizures at anytime during pregnancy to have occurred as a significant correlate of WT in the univariate analysis, but this was not the case.

Main variables of interest such as maternal AED therapy or maternal seizures were generally not significantly correlated with LT by univariate or multivariate analysis. Some previous studies have noted an inverse relationship between crown-heel length and total AED dose (Ogawa et al., 1982), CBZ monotherapy (Bertollini et al., 1987), and AED polytherapy (Koch et al., 1983), while other studies have not noted a relationship between AED treatment and decreased LT (Deblay et al., 1982; Hiilesmaa et al., 1981; Hill et al., 1974; Hill and Tennyson, 1982a; Klepel et al., 1986; Majewski and Steger,1984; Neri et al., 1983; Nomura et al., 1984; Robertson et al., 1986; Vert et al., 1982).

It is not clear why a family history of seizures in paternal relatives should have been specifically and consistently associated with decreased WT and LT at birth. One possibility is that a family history of seizures is related to a propensity for the child to develop seizures, and that those who develop seizures also have poor somatic growth beginning prenatally. In other words, the development of seizures and poor growth may have a common genetic and/or environmental liability. Several studies have looked at the postnatal growth of children after they have developed epilepsy and have found a reduced growth rate, which has been ascribed to the epileptic drugs and/ or the epilepsy itself (McGowan, 1983; Round,1981). Moreover, several authors have shown an excess of children with low WT at term among those who later develop epilepsy (Dunn et al., 1986).

It is not known why low WT and LT were associated with a positive family history of seizures only on the paternal side in the multivariable analysis. This may have been due to the fact that epileptic mothers were excluded from the analysis by virtue of the fact that they were

ascertained on the basis of their epilepsy. Another possibility is that family history of seizures is a proxy for some other unknown variable influencing WT and LT in the offspring of epileptic women.

Major seizures were found to be a highly consistent and statistically significant factor associated with decreased HC. Only one previous study analyzed the relationship between the number, type and distribution of maternal seizures and growth measurements in newborns of epileptic women (Hiilesmaa, 1982; Hiilesmaa et al., 1981), and found no significant relationship.

The number of AED and the dose or plasma levels of PHT each emerged as consistent highly significant factors associated with decreased HC. In the majority of cases where the number of AED and dose of PHT were evaluated in the same model, one or the other factor was the only one that emerged as a significant correlate of HC after multiple regression analysis, suggesting that these factors are highly correlated.

Previous studies have demonstrated a significant association between AED polytherapy (Hiilesmaa et al., 1981; Koch et al., 1983) or specifically the use of PHT with or without other AED (Hanson et al., 1976; Majewski and Steger, 1984) and decreased HC. However, the relationship between PHT dose and HC has not previously been studied. Although Hiilesmaa and colleagues (1981) reported no association between plasma AED levels in general and HC, it is not clear how they studied plasma AED levels. Ogawa and coworkers (1982) showed an inverse relationship between a number of head size parameters and the total drug score, based on the number of drugs and their doses. It is not known in this study which specific AED and in what doses may have been

associated with decreased head parameters.

In the present study, maternal seizures or AED each emerged as consistent highly significant correlates of HC in separate models. This suggests that in these cases the variables were highly correlated, but that one or the other of the variables had independent associations with HC. In other models , however, these variables were both significant . This indicates that in the group of patients involved in these models, seizures and AED were both independently linearly correlated with HC.

Possible interaction effects of seizures and AED were also examined in models where both these factors appeared as significant correlates of HC. The interaction of seizures and AED did not emerge as a significant factor , indicating that the combined effects of seizures and AED are additive rather than multiplicative

HC at birth in relation to maternal seizures, AED and other risk factors was assessed for the first trimester or at anytime during pregnancy in separate theoretical models. In both cases, maternal seizures and/or AED maintained a significant association with HC following multiple regression analysis. Some of the theoretical models were also designed to assess the importance of risk factors over the entire pregnancy relative to those for a specific trimester of pregnancy. For example, models *1, 1A* (Table VI. 8) evaluated the number of AED and maternal seizures simultaneously for the first trimester and at anytime during pregnancy. Following multiple regression, only factors anytime during pregnancy maintained a significant association with HC. This suggests that HC is susceptible to the growth-retarding effects of AED and/or seizures over the entire pregnancy, and not just in the first trimester.

PB was evaluated in relation to HC by multiple regression analysis, but the resulting models were not of adequate sample size. In these models, PB did not emerge as a significant factor associated with HC The role of PB in decreased HC cannot be excluded because of the small numbers in the present series, and in view of previous studies showing an association between PB monotherapy or polytherapy and a decrease of HC in newborns of epileptic women (Deblay et al., 1982, Hiilesmaa et al., 1981, 1982b; Majewski and Steger, 1984). One of these studies also demonstrated a significant negative correlation between plasma PB levels in cord blood and HC in the newborns of epileptic women (Deblay et al., 1982).

Although dose of PRM in a small number of women was found to be negatively associated with HC by univariate analysis, numbers were inadequate to evaluate this drug independently of other risk factors Several studies have implicated this AED alone or in combination in the reduction of HC, WT, or LT (Hiilesmaa et al., 1981; Klepel et al., 1986; Majewski and Steger, 1984; Neri et al., 1983; Rating et al.,1982b), or in proportionate growth retardation (Jacob et al., 1985; Rating et al., 1982b).

Treatment with CBZ therapy was not found to be significantly associated with decreased growth parameters by univariate analysis in the present study, but the number of children exposed to this drug in utero was small. Hiilesmaa and colleagues (1981, 1982b) demonstrated a significant association between this drug taken in monotherapy and decreased HC, but not WT or LT in the offspring, but later analyses revealed that their findings may have been confounded by the fact that

fathers of children in their series exposed to this drug had smaller HCs than controls (Granström, 1988). Subsequent studies have also noted a relationship between treatment with CBZ ± other AED and reduction of HC, WT and LT (Bertollini et al., 1987; Kuhnz et al., 1983) ; however, no information was given on potential confounders in these studies.

While maternal HC was significantly associated with HC by univariate analysis, this factor did not appear as a consistent correlate of HC in the offspring, following multiple regression analysis. Father's HC was also evaluated univariately and was not found to be significantly associated with HC in the offspring. However, the numbers were not large and an association between these parameters may have been missed.

Type of maternal epilepsy emerged as an inconsistent factor associated with decreased HC after multiple regression analysis. Other studies (Hiilesmaa et al., 1981; Jacob et al., 1985) have also not found this factor to be important in the occurrence of decreased growth parameters.

There may be several possible mechanisms for the effects of maternal seizures or AED on HC. Seizure-induced hypoxia and/or the effects of epileptic seizures on the hypothalamic-pituitary axis, causing alterations in hormone levels, may interfere with head growth at vulnerable periods of development.

Another possible mechanism might be through the effects of AED on thyroid levels. It has been shown that AED decrease thyroid levels, although the patient remains clinically euthyroid. Kaneko et al. (1983a) found a significant positive relationship between HC at birth in offspring of epileptic women taking AED and thyroid hormone levels in

maternal serum during pregnancy, and in cord blood.

Since folates are necessary for normal cell multiplication in all tissues, it is possible that a deficiency in folates might result in decreased growth rate. Folate levels have been found to be lowered in epileptic patients taking AED. Folate levels also tend to fall with advancing pregnancy and the increased demands for folate utilized in growth of the fetus, placenta, and maternal tissues (Chanarin, 1979).

Only one previous study of a small number of epileptic women examined the relationship between folate and growth parameters, and found no correlation between LT, WT, and HC and maternal serum folate levels during pregnancy (Ueda et al., 1984).

In the present study, a significant relationship was observed between decreased red cell folate levels and a decrease in HC only. The relationship was maintained after multiple regression analysis, although the clinical importance of this variable was somewhat less than other variables, such as AED or maternal seizures. This finding suggests that decreased folate levels contribute to a reduction in HC over and above the effects of AED. It does not exclude the possibility that the effects of AED may also be operating through a decrease of folate levels.

On the other hand, the exclusive association between folate levels and HC was somewhat surprising as a reduction in the growth rate of all tissues would be expected to occur in association with folate deficiency. This would suggest that folate deficiency may not be the direct cause of decreased HC associated with AED. There is evidence to indicate that PHT may decrease blood folate levels by decreasing the activity of folate-metabolizing enzymes (See section II.R2e, Folate

Metabolism) and that this effect is not a direct one (Billings and Hansen, 1984; Hansen and Billings, 1985). As low thyroid hormone also results in a reduction in folate-metabolizing enzymes (Stokstad, et al., 1980), perhaps the correlation between red cell folate levels and HC is due to the confounding effects of thyroid hormone. The selective inhibition of head growth would then actually be due to an AED-induced reduction in thyroid hormone. Alternatively, the reduction in folate-metabolizing enzyme activity induced by AED may not occur in all embryonic tissues, thereby leading to a localized inhibition of growth.

The possible teratogenic effects of arene oxide metabolites of PHT must also be considered. In the present study, a significant association was found between a genetically determined detoxification defect of PHT arene oxide metabolites and decreased HC at birth, but not birth weight or length. This is in accord with the previous reports of Strickler et al (1985) and Dansky et al. (1987b), which showed a significantly increased frequency of microcephaly among those with detoxification defects. In the present series, however, numbers of offspring were too small to study the relative contribution of this factor along with other risk factors shown to be associated with decreased HC.

As previously described (see section IV.G , Methods, and section V.C, Discussion), 3 of 20 children with lymphocyte testing in the present series had been exposed to PB and/or PRM during the first trimester. One of these children was also exposed to PHT after the first trimester, and she had both a small HC (standard score=-1.910) and a positive response to in vitro challenge with PHT metabolites, while the other two children, exposed to barbiturates alone, had relatively normal HCs

(standard score=-0.2247) and showed negative responses.

It is of interest that cigarette smoking and/or AED appeared as significant factors associated with decreased head circumference and birth weight following multivariable analyses. Smoking induces placental cytochrome P-450 monooxygenase and it is not known to what extent this alters placental metabolism and hormone status (Goodman et al.,1982), as well as activates other drugs such as AED to potential toxins (Challis, 1978).

In the univariate analyses, an increase in the dose of VPA was the only AED to be significantly associated with an increase in HC, WT and LT. It is interesting to note that some patients treated with VPA show excessive weight gain (Dinesen et al.,1984.) According to the present author's knowledge, there have been no previous reports of increased growth measurements among offspring exposed to VPA in utero. In the present study, there was a non-significant trend towards a positive correlation between dose of VPA at the last menstrual period and maternal weight gain during pregnancy (r=0.43), as well as with paternal height (rho=0.26), which may have explained at least in part the association between dose of VPA and increased growth measurements in the offspring.

In summary, our data suggest that maternal seizures and/or AED in addition to other factors are highly significantly associated with decreased HC, while these do not appear to be as strongly associated (in terms of level of significance and/or magnitude of clinical change) with other growth parameters. These findings suggest that maternal seizures and AED may have a selective effect on head size and underlying brain development. On the other hand, family history of

seizures in paternal relatives appears to be an important variable associated with a decrease in WT or LT.

VII. GENERAL DISCUSSION

As in all observational non-experimental studies carried out in humans in which the study design is not entirely under the control of the investigator, the present study is subject to a number of limitations and potential biases.

A. Data Collection and Possible Sources of Bias

The present study was a prospective cohort study in which most of the data on possible risk factors were collected prior to the outcome of pregnancy. Therefore, pregnancies resulting in normal or abnormal pregnancy outcome were treated similarly with respect to the collection of data, thus limiting possible information bias in the evaluation of risk factors associated with abnormal pregnancy outcome.

Some women had more than one pregnancy (see Methods, Section A), and the question of how the inclusion of more than one pregnancy per subject might affect the frequency of abnormal outcomes as compared with that in the general population, and the relationship between risk factors and pregnancy outcome should be addressed. Women with more than one pregnancy in the present study may have confounded the observed relationship between risk factors and pregnancy outcome in some unknown way. In order to have some indication of possible confounding, certain variables that were rough measures of multiparity were evaluated in relation to pregnancy outcome: these were pregnancy order, birth order and previous fetal loss. None of these variables were significantly associated with abnormal pregnancy outcome by univariate and/or multivariate analyses. All pregnancies in women with more than one pregnancy were therefore retained in this study. It was also

felt that excluding pregnancies other than the first one ascertained for each woman would have entailed too great a loss of data (23 of 116 pregnancies = 19.8%; 17 of 110 viable pregnancies= 15.5%). Future studies with larger numbers of pregnancies may choose to include only one pregnancy of each woman entered in the study.

Differential follow-up of children for abnormalities could be a possible source of bias in a cohort study such as this. This would not be a problem with somatic parameters at birth but could be a potential source of bias in the assessment of major congenital anomalies, particularly those which may be asymptomatic at birth. Not all children in this study were followed for the same length of time. This was due to the fact that some of the families were lost to follow-up after the neonatal period. Every effort was made to follow all children irrespective of their risk factors. There was also no significant difference in the length of follow-up according to whether or not the child had a major congenital anomaly, and therefore it is not likely that differential follow-up is an important source of bias in this study.

Patients in this study were mostly referred by private physicians and less frequently by physicians at the Seizure Clinic of the Montreal Neurological Hospital (See Methods , Section B). There may be certain unknown characteristics about a sample of women that were willing to participate in the present study, that may be different from a randomly chosen sample from the epileptic population. Furthermore, the Seizure Clinic population tends to have greater than average medical and socioeconomic problems. Although possible selection bias may have influenced to some extent the prevalence of birth defects and of particular risk factors among epileptic women, as compared with the

general population, all other comparisons were internal and therefore valid with respect to the series of patients in this study Multivariable analysis provided an explanation of the variation in newborn growth parameters and of the presence or absence of congenital anomalies in the present series, however, it is not known to what extent the explanations can be generalized to the epileptic population at large

B. Statistical Analysis

There were limitations in the methods of the present study The study was carried out over a long period, and initially the main interest was in the prevalence of birth defects in the offspring of epileptic women. When there were indications in the literature that growth and development may be abnormal in offspring of epileptic women, the study was expanded to incorporate these parameters. Thus, some of the risk factors which were important to study in relation to growth such as growth parameters of the parents, although collected in a relatively large proportion , were not available for all parents.

In addition, due to the nature of the clinical population of women, the women were taking different types and combinations of anticonvulsant drugs. Therefore, a homogeneous sample of women taking the same AED and with no missing data was not available on which to carry out either the univariate or the multivariable analyses

For example, in the univariate analyses of risk factors and growth outcomes, the analysis of the relationship between type of maternal epilepsy and growth outcome was based on a maximum of 109 viable pregnancies. When the independent effect of this risk factor was assessed along with other risk factors by multiple regression analysis,

the sample size decreased because not all women had full data on all risk factors. As a consequence, the relationship between type of maternal epilepsy and growth outcomes found in the multiple regression was not always the same as that obtained in the univariate analyses.

Moreover, the different models analyzed by multiple regression or multiple logistic regression analyses were often based on different subsets of pregnancies. In other words, when a particular risk factor was added to a model, the resulting sample of pregnancies on which the model was based was usually different from the sample of pregnancies which comprised the previous model. Thus, it was not always possible to evaluate a particular risk factor in relation to others within the same subset of subjects.

Because of these limitations, it was necessary to adopt a procedure which simultaneously assessed the consistency, statistical significance and clinical importance of risk factors over the different models generated by multivariable analyses.

The above comments are best illustrated by an examination of the relationship between a factor known to be associated with decreased growth in the general population, such as cigarette smoking, and decreased growth in the present series. Cigarette smoking was shown to be highly significantly correlated with decreased WT and HC by univariate analysis and, following multiple regression analysis , this factor was a highly significant independent correlate of decreased growth parameters in 48-50% of the models. Thus even with a factor known to affect growth, the association did not emerge in all models, or in all subsets of patients. However, the relationship was evident in at least 40% of the models which was the cut-off point used a priori to

distinguish between risk factors that may or may not be important in the occurrence of decreased growth parameters.

C. Association versus Causation

It should be cautioned that even strong associations (in terms of consistency and level of statistical significance) between the risk factors and particular pregnancy outcomes, as found in this study, do not prove that the risk factors are causes of abnormal pregnancy outcome. The risk factor may be a proxy for some unknown highly correlated measure. In the same vein, it is not known to what extent a particular risk factor may have been found to be significantly related to more than one pregnancy outcome, only because of the colinearity amongst the different pregnancy outcomes, and not because of any specific causal relationship between the risk factor and particular pregnancy outcomes. Nevertheless, risk factors in this study which were found to be consistently and highly significantly related to specific pregnancy outcomes should be suspected of specific adverse effects on fetal development. This would be particularly true for the association between maternal seizures and/or AED and HC which did not occur as strongly for other growth parameters, and for the relationship between AED and total abnormal outcomes or developmental anomalies. The point estimates of the relative risks of adverse pregnancy outcomes with increasing dose or plasma levels of PHT and PB, ranging up to 9.9 (see Tables V.24 to V.26) also indicate a a strong association between AED and abnormal pregnancy outcome.

The finding of dose-response relationships in the present study are also suggestive of causal relationships. The association of

developmental anomalies with dose or plasma levels of PHT in the first trimester at the critical time of embryonic development, when most of these anomalies would be expected to arise, strongly suggests that PHT is a causal factor in the occurrence of developmental anomalies. Similarly, the negative correlations between the frequency of maternal seizures and/or plasma PHT levels, as well as with the number of cigarettes smoked, and decreased head circumference in the offspring suggest that the associations are causal.

Although specificity of an association is not a necessary prerequisite for the establishment of a causal relationship, the finding of such associations may suggest that there is a causal relationship. In the present study, the specificity of the association of PHT use with developmental anomalies and not with PDH may be an argument in favor of PHT as the causal factor. Similarly developmental anomalies were found to be significantly correlated with PHT and not maternal seizures, while decreased head circumference was found to be correlated with maternal seizures and /or AED. Thus, specific abnormalites found in offspring of epileptic women, may have different etiologies.

D. Future Studies

In view of the limitations of the present study, future prospective multivariable studies of the outcome of pregnancy in epileptic women should include larger numbers of epileptic women taking specific monotherapies as well as polytherapies. As well, the study should also include a group of untreated epileptic women, so that an estimate might be calculated for the risk of abnormal pregnancy outcome at various doses of AED relative to an unexposed group, after having controlled for

possible differences between the untreated and treated groups (e.g., severity of epilepsy, etc.). In the present study, the relative risk of abnormal pregnancy outcome with increasing dose of PHT was calculated relative to a low exposure group. Since the risk associated with low exposure was greater than that for the general population, the relative risks associated with higher doses of PHT are probably underestimates of the relative risks that might be obtained by employing an unexposed group as the reference group.

Large numbers of pregnancies required for such analyses might best be obtained through collaborative studies, although much care would have to be exercised in the establishment of a uniform protocol for the different centres that would be involved in such a study Regional-based studies would be preferable to studies based at special centres, in order to assess the relative role of risk factors associated with abnormal pregnancy outcome in the epileptic population at large.

While this study has provided an explanation of the variation in somatic parameters at birth and the occurrence of major congenital malformations in the offspring of eileptic women, it has not provided a means of predicting for an individual, the probability of specific abnormal outcomes given for example a specific dose of PHT Future studies might attempt to predict the risk of such abnormal outcomes, based on risk variables found to be important by multivariable analysis in the present study

Attempts should also be made in future prospective studies to monitor metabolite levels of AED, particularly those involving an arene oxide intermediate, in order to further elucidate what role individual differences in the metabolism of AED may play in the determination of

abnormal fetal outcome.

Epileptic patients and their spouses should be tested in vitro for their capacity to detoxify arene oxide metabolites. Hopefully this might lead to predictive testing by amniocentesis in families where one or both parents have a defect in detoxification.

Free plasma levels during pregnancy should be determined, particularly for highly protein-bound drugs.

Other possible mechanisms, such as zinc deficiency should also be explored.

Attention might also be directed at determining whether there are sex differences in the occurrence of physical or developmental abnormalities in the offspring (as shown in some experimental studies), as any differences may provide clues to the mechanisms (e.g. sex steroids) causing these abnormalities. As well, more attention should be given to the pattern of growth retardation (e.g. proportionate growth retardation versus small head relative to body length or weight), as the timing, etiology, and clinical significance of these patterns may be different.

Future studies should also correlate the mental and neurological development of children exposed to AED in utero with head circumference at birth, in order to determine the clinical significance of a reduction in head size and in order to ascertain whether there is any risk for developmental or neurological abnormality in those who do not show any abnormalities in physical growth parameters. This would be important in view of the fact that animal models of the effects of AED have shown functional abnormalities at doses which do not necessarily produce overt effects on growth or morphogenesis.

E. CLINICAL MANAGEMENT OF THE EPILEPTIC WOMAN

In view of the findings in the present study and in previous studies, certain suggestions can be made regarding the use of AED during pregnancy (Andermann et al., 1981; Dansky et al., 1982b).

Ideally, a couple planning to have children should be seen prior to pregnancy and counselled about several important areas of concern, such as the risk of malformation and of epilepsy in their offspring, taking into account their specific medical and genetic background.

If at all possible, the patient should be followed for some time prior to pregnancy and the anticonvulsant regimen adjusted if necessary. In some patients where there is reason to believe that the epilepsy has remitted, it may be possible to discontinue antiepileptic medication. Since there is no single anticonvulsant that can with confidence be considered safe during pregnancy, including the newer drugs carbamazepine and valproic acid, the number and dosage of the drugs should be limited as far as possible, while still maintaining "optimal seizure control".

The present study provides evidence that major seizures are deleterious with respect to head circumference at birth. Minor seizures may also represent a risk Thus "optimal control of seizures" may involve a consideration of both major and minor attacks.

The choice of type and plasma level of medications continues to be subject to clinical judgement in each case. However, knowledge of the duration, type and severity of the epilepsy, previous response to medication, and the effect of prior pregnancies on seizure control, should help in making an appropriate decision (Rémillard et al., 1982) Although antiepileptic drug levels fall during pregnancy and are

frequently below the "therapeutic" range, the dosage should not be automatically increased, unless there is reason to believe that seizures are likely to recur.

Patients taking valproic acid should be offered prenatal diagnosis for neural tube defects based on serum alpha-fetoprotein (AFP) determinations together with ultrasound at about 15 weeks of gestation. If any abnormality is suspected, then amniocentesis with AFP determination should be carried out.

Following the birth of a child, the birth record should be reviewed and the child examined. Should the child be malformed or have seizures, the recurrence risks of these disorders will be increased in future offspring, since both congenital malformations and epilepsy are inherited in a multifactorial fashion (Andermann 1982a, Carter, 1965). The couple should then be counselled accordingly. The management of future pregnancies should also be discussed including the choice of anticonvulsants and prenatal monitoring.

It is recommended that women with epilepsy receive folate supplements prior to conception and early in pregnancy, in view of the association between low blood folate levels and neural tube defects (Smithells et al., 1976), as well as other adverse pregnancy outcomes (Dansky et al., 1987a), and in view of the benefits shown with the early prenatal use of folate supplements in the prevention of neural tube defects (Milunsky et al., 1989; Smithells et al., 1989).

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I-PROPOLITUS

Appendix A

MAIDEN NAME _____

Surname _____ First Name _____ Ascertainment _____

Birthdate _____ Sex _____ Religion _____ Occupation _____

Address _____ Phone _____

_____ Message _____

Admitted _____ Hospital _____ Ward _____ Number _____

Diagnosis _____

Medical History _____

_____

_____

_____

II PARENTS and GRANDPARENTS

Racial Origin: P G.F. _____ P.G.M. _____ M.G.F. _____ M G.M. _____

Birth Place P G F. _____ P.G.M. _____ M.G.F. _____ M.G.M. _____

Relig: Father _____ Mother _____ Occupation: Father _____ Mother _____

Maiden Name. Mother _____ P.G.M. _____ M.G.M. _____

Consanguinity. Parents _____ P G Ps. _____ M G Ps. _____ Date Married _____

Date	Person (s) interviewed	By whom	Summary of interview (counselling disposal, etc.)

Pro and born at _____

Appendix A

Contraceptives proband's pregnancy

Pill _____ Stopped _____ Other _____

before? _____ when? _____ method? _____

Was pregnancy _____ Pregnancy _____

planned? _____ test? _____

Attempted abortion _____ Other _____

Prob preg _____ Method _____ preg _____

Maternal pelvic or abdominal

X rays before pregnancy _____

Paternal pelvic or abdominal

X rays before pregnancy _____

Drugs during pregnancy (record stage dose Ask about antibiotics sleeping pills antidepressants antinauseants salicylates diuretics non medical drugs, other) _____

Mat wt Pre/Term/Post	Birth Presentation Month	B W.	Check – or - record details in last column				
			Bleeding	Fever	Rash	Adequate diet	

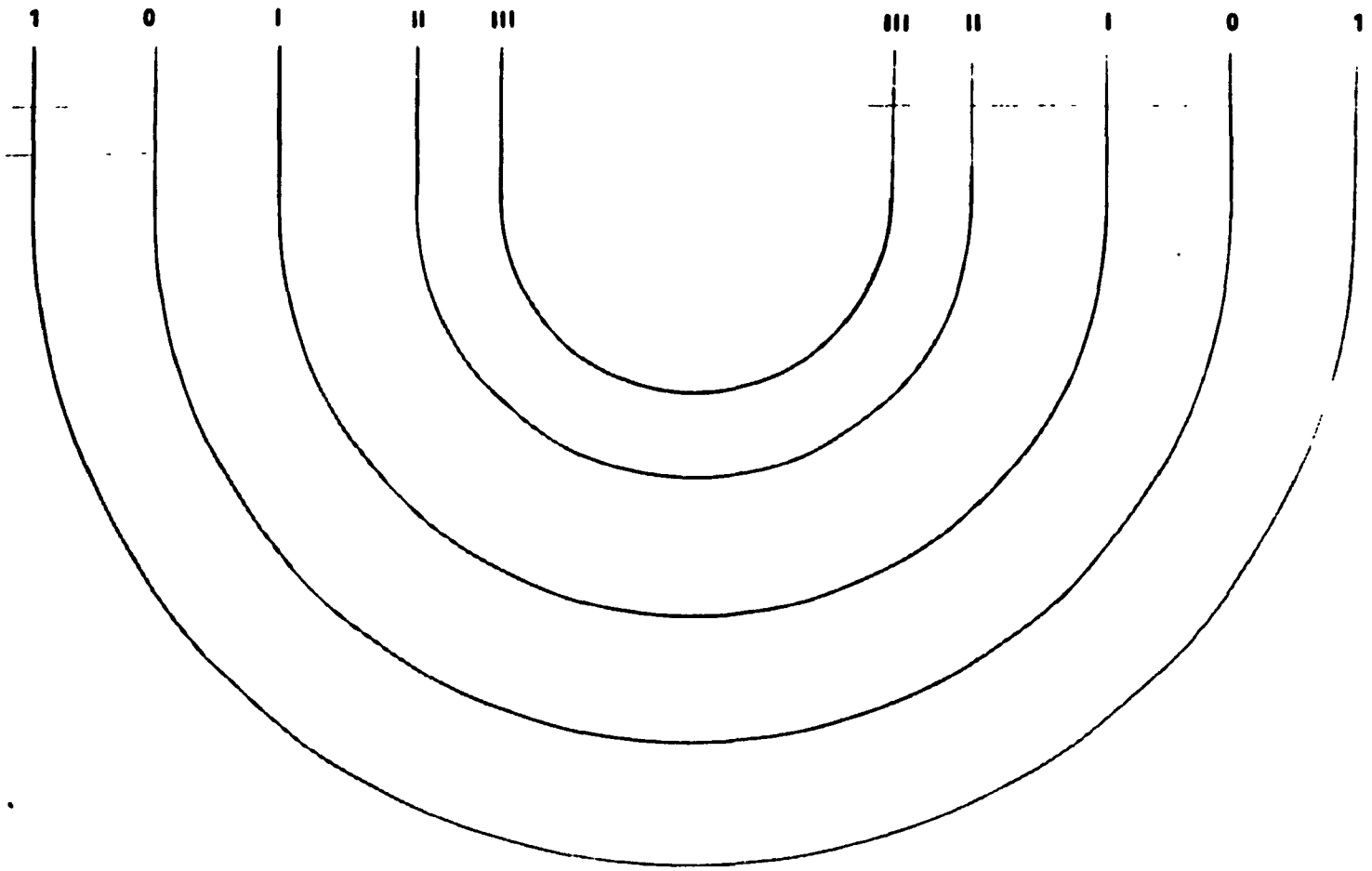
Appendix A

IV PARENTS and SIBLINGS (including propositus, still births, abortions, miscarriages)

Sex	Name	Birth Date	R/L		Congenital anomalies, serious illnesses, etc
Father					
Mother					
Children					

Appendix A

VI. PEDIGREE



ecord – Allergy, cancer, cong. defects, diabetes, epilepsy, rheumatic fever, twins, mental retardation, conditions like proband's

Pedigree Number	Name	Address, hospital, diagnosis, confirmation of diagnosis, etc.

Pregnancy History Supplementary Form. N.B. Record month of pregnancy for all findings.

Excessive Vomiting Medication?	Smoking Alcohol	Non-medical drugs (LSD, Speed, Marihuana, Heroin, etc).	X-rays Chest Abdomen	Operations	Exposure of mother to infectious diseases.	Serious emotional upset (death, family troubles, illness). Medication?	Miscellaneous

Appendix A

APPENDIX B

Mean or Median Daily Dose of AED (mg)

DRUG	TRIMESTER I NO. PREGN.	TRIMESTER I MEAN or MEDIAN (S.D.or RANGE)	TRIMESTER II NO. PREGN.	TRIMESTER II MEAN or MEDIAN (S.D.or RANGE)	TRIMESTER III NO. PREGN.	TRIMESTER III MEAN or MEDIAN (S.D.or RANGE)	ALL TRIMESTERS NO. PREGN.	ALL TRIMESTERS MEAN or MEDIAN (S.D.or RANGE)
PHT	85	300.0 (9.6-500.0)	77	300.0 (25.8-500.0)	77	300.0 (46.2-500.0)	88	300.0 (3.1-500.0)
PB	49	60.0 (1.8-123.9)	43	61.0 (30.0-120.0)	46	60.0 (0.6-223.4)	52	60.9 (0.2-125.2)
CBZ	22	655.4 (218.5)	22	659.9 (230.3)	22	679.3 (230.9)	24	629.8 (252.6)
PRM	19	467.4 (254.4)	18	416.9 (279.5)	18	479.5 (253.1)	23	402.7 (276.5)
VPA	9	824.7 (349.8)	8	940.7 (270.9)	8	874.8 (378.5)	9	816.5 (390.0)
ETH	8	1000.0 (411.4)	8	932.5 (375.2)	8	906.3 (399.5)	8	947.7 (377.5)

PHT=phenytoin; PB=phenobarbital; CBZ=carbamazepine; PRM=primidone;
VPA=valproic acid; ETH=ethosuximide

APPENDIX B

Mean or Median Dose (mg/kg) at Time of Monitoring of Plasma AED Levels

	TRIMESTER I		TRIMESTER II		TRIMESTER III		ALL TRIMESTERS	
DRUG	NO PREGN.	MEAN or MEDIAN (S.D.or RANGE)	NO PREGN.	MEAN or MEDIAN (S.D.or RANGE)	NO PREGN.	MEAN or MEDIAN (S.D.or RANGE)	NO PREGN.	MEAN or MEDIAN (S.D.or RANGE)
PHT	37	5.07 (1.65-7.04)	48	4.91 (1.35-8.05)	62	4.17 (1.48-7.10)	86	4.48 (1.62-7.98)
PB	24	1.41 (0.33-2.81)	30	1.38 (0.45-2.56)	36	1.05 (0.36-4.18)	51	1.18 (0.33-4.18)
CBZ	16	11.47 (4.06)	17	10.74 (3.63)	17	9.74 (3.08)	24	10.55 (3.20)
PRM	7	8.71 (4.41)	9	7.26 (4.45)	13	7.31 (2.80)	21	8.47 (3.79)
VPA	7	15.11 (5.50)	8	15.12 (4.11)	6	14.05 (4.64)	8	14.92 (4.34)
ETH	2	23.27 (4.02)	3	18.39 (4.47)	7	11.85 (5.42)	7	13.73 (5.58)

PHT=phenytoin; PB=phenobarbital; CBZ=carbamazepine; PRM=primidone;
VPA=valproic acid; ETH=ethosuximide

APPENDIX B

Mean or Median Dose (mg/kg) of AED at LMP

DRUG	NO. PREGNANCIES	MEAN or MEDIAN	S.D. or RANGE
PHT	79	5.1	1.5
PB	48	1.2	0.3-3.5
CBZ	21	11.9	3.7
PRM	18	9.5	4.0
VPA	9	15.7	5.4
ETH	6	18.1	8.1

PHT=phenytoin; PB=phenobarbital; CBZ=carbamazepine;
PRM=primidone; VPA=valproic acid; ETH=ethosuximide

APPENDIX B

Mean or Median Plasma AED Levels (µg/ml)

DRUG	TRIMESTER I NO. PREGN.	TRIMESTER I MEAN or MEDIAN (S.D. or RANGE)	TRIMESTER II NO. PREGN.	TRIMESTER II MEAN or MEDIAN (S.D. or RANGE)	TRIMESTER III NO. PREGN.	TRIMESTER III MEAN or MEDIAN (S.D. or RANGE)	ALL TRIMESTERS NO. PREGN.	ALL TRIMESTERS MEAN or MEDIAN (S.D. or RANGE)
PHT	37	6.6 (1.1-29.0)	48	4.5 (0-19.2)	63	4.6 (1.3-17.3)	87	5.0 (1.3-29.0)
PB	28	11.9 (2.7-49.2)	30	10.1 (0-29.7)	38	9.0 (2.7-42.1)	56	9.6 (0-49.2)
CBZ	16	6.5 (2.0)	17	6.6 (1.9)	16	6.2 (2.1)	23	6.1 (1.7)
PRM	7	6.5 (3.1)	8	4.2 (2.0)	11	5.8 (3.8)	19	6.0 (3.4)
VPA	7	39.2 (10.6)	8	37.3 (6.0)	6	40.8 (14.9)	8	39.4 (7.7)
ETH	2	64.0 (38.2)	3	43.0 (19.7)	8	36.7 (12.6)	8	39.0 (16.0)

PHT=phenytoin; PB=phenobarbital; CBZ=carbamazepine; PRM=primidone;
VPA=valproic acid; ETH=ethosuximide

APPENDIX B

Median Serum and Red Blood Cell Folate Levels (ng/ml)

	TRIMESTER I		TRIMESTER II		TRIMESTER III		ALL TRIMESTERS	
FOLATE	NO. PREGN.	MEDIAN (RANGE)	NO. PREGN.	MEDIAN (RANGE)	NO. PREGN.	MEDIAN (RANGE)	NO. PREGN	MEDIAN (RANGE)
SERUM	27	5.8 (1.0-15.0)	34	12.8 (1.6-15.0)	35	11.9 (1.0-15.0)	47	10.1 (1.0-15.0)
RED CELL	27	385 (175-1376)	34	652 (237-1763)	35	864 (175-1763)	47	531 (175-1763)

APPENDIX C

Description and Coding of Risk Factors

Variable No	Risk Factors	Description
Social Data		
1	Father's occupation	1 = unskilled, unemployed; 2 = craftsman, machine operator, 3 = clerical, technical, sales; 4 = proprietor, managerial; 5 = professional
2	Mother's education	1 = elementary; 2 = highschool incomplete, 3 = highschool completed, 4 = CEGEP or post-secondary training, 5 = university, 6 = professional
3	Father's education	1 = elementary; 2 = highschool incomplete; 3 = highschool completed; 4 = CEGEP or post-secondary training; 5 = university; 6 = professional
Personal Characteristics		
4	Sex of child in study	0 = female, 1= male
5	Length of follow-up of child in study	Age in years at last examination
6	Maternal age	Age in years at LMP
7	Paternal age	Age in years at spouse's LMP
8.	Mother's height	Height in cm.
9	Father's height	Height in cm
10	Mid-parent height	Mean of mother's and father's height in cm
11	Mother's prepregnancy weight	Weight in kg.
12	Mother's ponderal index	Weight (gm)/height^2 (m^2)
13	Mother's head circumference	Maximum circumference in cm

14	Father's head circumference	Maximum circumference in cm.
15	Mid-parent head circumference	Mean of mother's and father's maximum head circumferences in cm.

Family History

16	mother with major congenital anomaly	0 = no, 1 = yes
17	History of major congenital anomalies in maternal second degree relatives	Proportion affected of child's relatives
18	History of major congenital anomalies in maternal third degree relatives	Proportion affected of child's relatives
19	History of major congenital anomalies in 16 and 17 combined	Proportion affected of child's relatives; 0 = no, 1= yes
20	History of major congenital anomalies in 16 to 18 combined	Proportion affected of child's relatives; 0 = no, 1= yes.
21	Father with major congenital anomaly	0 = no, 1 = yes
22	History of major congenital anomalies in paternal second degree relatives	Proportion affected of child's relatives
23	History of major congenital anomalies in paternal third degree relatives	Proportion affected of child's relatives
24	History of major congenital anomalies in 21 and 22 combined	Proportion affected of child's relatives; 0 = no, 1= yes

25	History of major congenital anomalies in 21 to 23 combined	Proportion affected of child's relatives; 0 = no, 1 = yes
26	History of major congenital anomalies in mother or father	0 = no, 1 = yes
27	History of major congenital anomalies in maternal or paternal second degree relatives	Proportion affected of child's relatives
28	History of major congenital anomalies in maternal or paternal third degree relatives	Proportion affected of child's relatives
29	History of major congenital anomalies in 26 and 27 combined	Proportion affected of child's relatives, 0 = no, 1 = yes
30	History of major congenital anomalies in 26 to 28 combined	Proportion affected of child's relatives; 0 = no, 1 = yes
31	History of one or more seizures in maternal second degree relatives	Proportion affected of child's relatives
32	History of one or more seizures in maternal third degree relatives	Proportion affected of child's relatives
33	History of one or more seizures in 31 and 32 combined	Proportion affected of child's relatives, 0 = no, 1 = yes
34	Father with history of one or more seizures	0 = no, 1 = yes
35	History of one or more seizures in paternal second degree relatives	Proportion affected of child's relatives

36	History of one or more seizures in paternal third degree relatives	Proportion affected of child's relatives
37	History of one or more seizures in 34 and 35 combined	Proportion affected of child's relatives; 0 = no, 1 = yes
38	History of one or more seizures in 34 to 36 combined	Proportion affected of child's relatives; 0 = no, 1 = yes
39	History of one or more seizures in maternal or paternal second degree relatives	Proportion affected of child's relatives
40	History of one or more seizures in maternal or paternal third degree relatives	Proportion affected of child's relatives
41	History of one or more seizures in 34 and 39 combined	Proportion affected of child's relatives; 0 = no, 1 = yes
42	History of one or more seizures in 34, 39 and 40 combined	Proportion affected of child's relatives; 0 = no, 1 = yes

Reproductive History

43	Pregnancy order	Numbers 1-6 for women with reproductive history of 0 to 5 pregnancies prior to the pregnancy under study
44	Birth order	Numbers 1-5 for women with reproductive history of 0-4 births prior to the birth under study
45	Previous fetal loss	Spontaneous abortion or perinatal death preceding pregnancy under study, 0 = no, 1 = yes
46	Previous therapeutic abortion	Therapeutic abortion preceding pregnancy under study, 0 = no, 1 = yes

47	Trimester of first obstetric antenatal visit	1= first trimester, 2= second trimester, 3 = third trimester
48	Number of obstetrical antenatal visits	0 = 7+ visits, 1= < 7 visits
49	Gestation by last menstrual period (LMP)	Length of pregnancy in weeks determined exclusively from LMP
50	Gestation by LMP or ultrasound	Length of pregnancy in weeks determined by LMP or early ultrasound (<24 weeks) when available
51	Maternal weight gain	Maximum weight gain in kg during pregnancy

Maternal Illnesses and Pregnancy Complications

52	Maternal endocrine disorder	Chronic hypothyroidism or gestational diabetes, 0 = no, 1 = yes
53	Maternal infection treated in first trimester	respiratory, cardiac, intestinal, urogenital infection treated by antibacterial or antifungal agents, 0 = no, 1 = yes
54	Maternal infection treated anytime during pregnancy	respiratory, cardiac, intestinal, urogenital infection treated by antibacterial or antifungal agents, 0 = no, 1 = yes
55	Fever first trimester	0 = no, 1 = yes
56	Fever anytime during pregnancy	0 = no, 1 = yes
57	Maternal anemia	Haemoglobin < 10 5 g. per 100 ml at anytime during pregnancy , 0 = no, 1 = yes
58	Bleeding in first trimester	0 = no, 1 = yes
59	Bleeding in second trimester	0 = no, 1 = yes
60	Bleeding in third trimester	0 = no, 1 = yes

61	Toxemia or chronic hypertension	Toxemia: (1) high blood pressure (BP) ≥ 140/90 or systolic BP increase above 30 mm Hg over usual BP or diastolic BP increase above 15 mm Hg over usual BP, on at least 2 occasions, with (2) proteinuria ≥ 1+, and/or (3) significant edema. Chronic hypertension. history of BP ≥ 140/90 preceding pregnancy. 0 = no, 1 = yes
62	Nausea +/- vomiting treated by antinauseants	0 = no, 1 = yes
63	Premature contractions	Treated in the majority of cases, 0 = no, 1 = yes
64	Abnormal quantity of amniotic fluid	0 = no, 1= polyhydramnios, 2 = oligohydramnios
65	Blood group sensitization	0 = no, 1 = yes

Physical Agents

66	Abdominal or pelvic x-rays in first trimester	0 = no, 1 = yes
67	Abdominal or pelvic x-rays anytime during pregnancy	0 = no, 1 = yes

Drugs Other Than Anticonvulsants

68	Sex hormones first trimester	Birth control and other indications 0 = no, 1 = yes
69	Sex hormones anytime during pregnancy	Birth control and other indications 0 = no, 1 = yes
70	Other hormones first trimester	Thyroid, corticosteroid 0 = no, 1 = yes
71	Other hormones anytime during pregnancy	Thyroid, corticosteroid 0 = no, 1 = yes
72	Alcohol consumption anytime during pregnancy	0 = no, 1 = yes

73	Cigarette smoking anytime during pregnancy	0 = none, 1 = < 1/2 package/ day, 2 = 1/2 package - 1 package/ day, 3 = > 1 package/ day

Maternal Epilepsy

74	Type of epilepsy	1 = primary generalized, 2 = secondary generalized with or without focalization, 3 = partial complex, 4 = partial simple, 5 = partial, focus undetermined, 6 = unclassified; 7 = epilepsy unlikely
75	Age at onset of first seizure	Age in years
76	Age at onset of habitual seizures	Age in years
77	Duration of epilepsy Years from onset of first seizure to LMP	Number 6 above minus number 75 above
78	Duration of epilepsy: Years from onset of habitual seizures to LMP	Number 6 above minus number 76 above
79	Frequency of major seizures first trimester	0 = none; 1= 1-2 seizures/ 3 months; 2 = 3-5 seizures/ 3 months; 3 = ≥6 seizures/ 3 months
80	Frequency of major seizures during entire pregnancy	0 = none, 1= 1-2 seizures / 9 months, 2 = 3-5 seizures/ 9 months, 3 = 6-9 seizures / 9 months, 4 = > 1 / month
81	Frequency of minor seizures first trimester	0 = none; 1= < 1/month, 2 = 1-3/month; 3 =1-6/week, 4 = ≥ 1/day
82	Frequency of minor seizures during entire pregnancy	0 = none, 1= < 1/month, 2 = 1-3/month, 3 = 1-6/week, 4=≥ 1/day

Maternal Anticonvulsant Therapy

83	Age at commencement of anticonvulsant therapy	Age in years
84	Duration of anticonvulsant therapy	Number 6 above minus number 83 above

85	Number of anticonvulsant drugs in first trimester	0 to 3 drugs
86	Number of anticonvulsant drugs anytime during pregnancy	0 to 3 drugs

Comparisons of specific anticonvulsant regimens:

87 Type of monotherapy in first trimester:

Phenytoin
vs
Phenobarbital and/or Primidone
vs
Carbamazepine
vs
Valproic acid
vs
More than one drug taken consecutively in monotherapy

88 Type of monotherapy anytime during pregnancy.

Phenytoin
vs
Phenobarbital and/or Primidone
vs
Carbamazepine
vs
Valproic acid
vs
More than one drug taken consecutively in monotherapy

89 Type of polytherapy in first trimester.

Phenytoin + phenobarbital
vs
Primidone + other anticonvulsants
vs
Valproic acid + other anticonvulsants
vs
Carbamazepine + other anticonvulsants (excluding primidone or valproic acid)
vs
Other combinations of anticonvulsants

APPENDIX C

90 Type of polytherapy anytime during pregnancy·

Phenytoin + phenobarbital
VS
Primidone + other anticonvulsants
VS
Valproic acid + other anticonvulsants
VS
Carbamazepine + other anticonvulsants
(excluding primidone or valproic acid)
VS
Other combinations of anticonvulsants

91 Monotherapy vs polytherapy in first trimester.

Phenytoin alone
VS
Phenytoin + other anticonvulsants

92 Monotherapy vs polytherapy in first trimester:

Phenobarbital alone
VS
Phenobarbital + other anticonvulsants

93 Monotherapy vs polytherapy in first trimester:

Carbamazepine alone
VS
Carbamazepine + other anticonvulsants

94 Monotherapy vs polytherapy in first trimester·

Valproic acid alone
VS
Valproic acid + other anticonvulsants

95 Monotherapy vs polytherapy anytime during pregnancy.

Phenytoin alone
VS
Phenytoin + other anticonvulsants

APPENDIX C

96 Monotherapy vs polytherapy anytime during pregnancy·

Phenobarbital alone
VS
Phenobarbital + other anticonvulsants

97 Monotherapy vs polytherapy anytime during pregnancy·

Carbamazepine alone
VS
Carbamazepine + other anticonvulsants

98 Monotherapy vs polytherapy anytime during pregnancy·

Valproic acid alone
VS
Valproic acid + other anticonvulsants

99 Phenytoin, phenobarbital, primidone in monotherapy or polytherapy in first trimester

Phenytoin alone
VS
Phenobarbital alone
VS
Phenytoin + primidone
VS
Phenytoin + phenobarbital
VS
Phenytoin + phenobarbital + primidone

100 Phenytoin, phenobarbital, primidone in monotherapy or polytherapy anytime during pregnancy:

Phenytoin alone
VS
Phenobarbital alone
VS
Phenytoin + primidone
VS
Phenytoin + phenobarbital
VS
Phenytoin + phenobarbital + primidone

APPENDIX C

101 Carbamazepine, phenytoin in monotherapy or polytherapy in first trimester

Carbamazepine alone
vs
Phenytoin alone
vs
Phenytoin + carbamazepine

102 Carbamazepine, phenytoin in monotherapy or polytherapy anytime during pregnancy

Carbamazepine alone
vs
Phenytoin alone
vs
Phenytoin + carbamazepine

103	Mean daily dose of phenytoin first trimester	Daily dose in mg averaged over the trimester
104	Mean daily dose of phenytoin second trimester	Daily dose in mg averaged over the trimester
105	Mean daily dose of phenytoin third trimester	Daily dose in mg averaged over the trimester
106	Mean daily dose of phenytoin entire pregnancy	Daily dose in mg averaged over the pregnancy
107	Mean daily dose of phenobarbital first trimester	Daily dose in mg averaged over the trimester
108	Mean daily dose of phenobarbital second trimester	Daily dose in mg averaged over the trimester
109	Mean daily dose of phenobarbital third trimester	Daily dose in mg averaged over the trimester
110	Mean daily dose of phenobarbital entire pregnancy	Daily dose in mg averaged over the pregnancy
111	Mean daily dose of primidone first trimester	Daily dose in mg averaged over the trimester

112	Mean daily dose of primidone second trimester	Daily dose in mg averaged over the trimester
113	Mean daily dose of primidone third trimester	Daily dose in mg averaged over the trimester
114	Mean daily dose of primidone entire pregnancy	Daily dose in mg averaged over the pregnancy
115	Mean daily dose of carbamazepine first trimester	Daily dose in mg averaged over the trimester
116	Mean daily dose of carbamazepine second trimester	Daily dose in mg averaged over the trimester
117	Mean daily dose of carbamazepine third trimester	Daily dose in mg averaged over the trimester
118	Mean daily dose of carbamazepine entire pregnancy	Daily dose in mg averaged over the pregnancy
119	Mean daily dose of ethosuximide first trimester	Daily dose in mg averaged over the trimester
120	Mean daily dose of ethosuximide second trimester	Daily dose in mg averaged over the trimester
121	Mean daily dose of ethosuximide third trimester	Daily dose in mg averaged over the trimester
122	Mean daily dose of ethosuximide entire pregnancy	Daily dose in mg averaged over the pregnancy
123	Mean daily dose of valproic acid first trimester	Daily dose in mg averaged over the trimester
124	Mean daily dose of valproic acid second trimester	Daily dose in mg averaged over the trimester
125	Mean daily dose of valproic acid third trimester	Daily dose in mg averaged over the trimester

126	Mean daily dose of valproic acid entire pregnancy	Daily dose in mg averaged over the pregnancy
127	Dose of phenytoin at LMP	Dose in mg/kg
128	Mean dose of phenytoin in first trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
129	Mean dose of phenytoin in second trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
130	Mean dose of phenytoin in third trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
131	Mean dose of phenytoin for entire pregnancy at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the pregnancy
132	Dose of phenobarbital at LMP	Dose in mg/kg
133	Mean dose of phenobarbital in first trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
134	Mean dose of phenobarbital in second trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
135	Mean dose of phenobarbital in third trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester

136	Mean dose of phenobarbital for entire pregnancy at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the pregnancy
137	Dose of primidone at LMP	Dose in mg/kg
138	Mean dose of primidone in first trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
139	Mean dose of primidone in second trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
140	Mean dose of primidone in third trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
141	Mean dose of primidone for entire pregnancy at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the pregnancy
142	Dose of carbamazepine at LMP	Dose in mg/kg
143	Mean dose of carbamazepine in first trimester at time of monitoring of plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
144	Mean dose of carbamazepine in second trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
145	Mean dose of carbamazepine in third trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester

146	Mean dose of carbamazepine for entire pregnancy at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the pregnancy
147	Dose of ethosuximide at LMP	Dose in mg/kg
148	Mean dose of ethosuximide in first trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
149	Mean dose of ethosuximide in second trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
150	Mean dose of ethosuximide in third trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
151	Mean dose of ethosuximide for entire pregnancy at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the pregnancy
152	Dose of valproic acid at LMP	Dose in mg/kg
153	Mean dose of valproic acid in first trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
154	Mean dose of valproic acid in second trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester
155	Mean dose of valproic acid in third trimester at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the trimester

156	Mean dose of valproic acid for entire pregnancy at time of monitoring plasma anticonvulsant levels	Dose in mg/kg averaged over the pregnancy
157	Mean plasma level of phenytoin first trimester	Plasma levels (µg/ml) averaged over the trimester
158	Mean plasma level of phenytoin second trimester	Plasma levels (µg/ml) averaged over the trimester
159	Mean plasma level of phenytoin third trimester	Plasma levels (µg/ml) averaged over the trimester
160	Mean plasma level of phenytoin entire pregnancy	Average of the means of plasma levels (µg/ml) in three trimesters
161	Mean plasma level of phenobarbital first trimester	Plasma levels (µg/ml) averaged over the trimester
162	Mean plasma level of phenobarbital second trimester	Plasma levels (µg/ml) averaged over the trimester
163	Mean plasma level of phenobarbital third trimester	Plasma levels (µg/ml) averaged over the trimester
164	Mean plasma level of phenobarbital entire pregnancy	Average of the means of plasma levels (µg/ml) in three trimesters
165	Mean plasma level of primidone first trimester	Plasma levels (µg/ml) averaged over the trimester
166	Mean plasma level of primidone second trimester	Plasma levels (µg/ml) averaged over the trimester

167	Mean plasma level of primidone third trimester	Plasma levels (µg/ml) averaged over the trimester
168	Mean plasma level of primidone entire pregnancy	Average of the means of plasma levels (µg/ml) in three trimesters
169	Mean plasma level of carbamazepine first trimester	Plasma levels (µg/ml) averaged over the trimester
170	Mean plasma level of carbamazepine second trimester	Plasma levels (µg/ml) averaged over the trimester
171	Mean plasma level of carbamazepine third trimester	Plasma levels (µg/ml) averaged over the trimester
172	Mean plasma level of carbamazepine entire pregnancy	Average of the means of plasma levels (µg/ml) in three trimesters
173	Mean plasma level of ethosuximide first trimester	Plasma levels (µg/ml) averaged over the trimester
174	Mean plasma level of ethosuximide second trimester	Plasma levels (µg/ml) averaged over the trimester
175	Mean plasma level of ethosuximide third trimester	Plasma levels (µg/ml) averaged over the trimester
176	Mean plasma level of ethosuximide entire pregnancy	Average of the means of plasma levels (µg/ml) in three trimesters
177	Mean plasma level of valproic acid first trimester	Plasma levels (µg/ml) averaged over the trimester

178	Mean plasma level of valproic acid second trimester	Plasma levels (µg/ml) averaged over the trimester
179	Mean plasma level of valproic acid third trimester	Plasma levels (µg/ml) averaged over the trimester
180	Mean plasma level of valproic acid entire pregnancy	Average of the means of plasma levels (µg/ml) in three trimesters
181	Detoxification defects of arene oxide metabolites of phenytoin	In vitro response of lymphocytes (cell death) to challenge with arene oxide metabolites of phenytoin generated in the presence of phenytoin and a microsomal enzyme system, as compared with that for the microsomal system alone. 0 = no significant increase in cell death (negative response); 1 = significant increase in cell death (positive response)

Folate

182	Folic acid supplements in first trimester	0 = no folic acid supplements; 1 = folic acid supplements, 2 = vitamins of unknown content
183	Folic acid supplements anytime during pregnancy	0 = no folic acid supplements; 1 = folic acid supplements; 2 = vitamins of unknown content
184	Mean serum folate level first trimester	Serum levels (ng/ml) averaged over the trimester
185	Mean serum folate level second trimester	Serum levels (ng/ml) averaged over the trimester
186	Mean serum folate level third trimester	Serum levels (ng/ml) averaged over the trimester
187	Mean serum folate level entire pregnancy	Average of the means of serum levels (ng/ml) in three trimesters
188	Mean red cell folate level first trimester	Red cell folate levels (ng/ml) averaged over the trimester

189	Mean red cell folate level second trimester	Red cell folate levels (ng/ml) averaged over the trimester
190	Mean red cell folate level third trimester	Red cell folate levels (ng/ml) averaged over the trimester
191	Mean red cell folate level entire pregnancy	Average of the means of red cell folate levels (ng/ml) in three trimesters
192	At least one serum folate level < 4 ng/ml in first trimester	Serum folate ≥4 ng/ml = 0; Serum folate <4 ng/ml = 1
193	At least one serum folate level < 4 ng/ml in second trimester	Serum folate ≥4 ng/ml = 0; Serum folate <4 ng/ml = 1
194	At least one serum folate level < 4 ng/ml in third trimester	Serum folate ≥4 ng/ml = 0, Serum folate <4 ng/ml = 1
195	At least one serum folate level < 4 ng/ml anytime during pregnancy	Serum folate ≥4 ng/ml = 0; Serum folate <4 ng/ml = 1
196	At least one red cell folate level < 175 ng/ml in first trimester	Red cell folate ≥175 ng/ml = 0, Red cell folate <175 ng/ml = 1
197	At least one red cell folate level < 175 ng/ml in second trimester	Red cell folate ≥175 ng/ml = 0; Red cell folate <175 ng/ml = 1
198	At least one red cell folate level < 175 ng/ml in third trimester	Red cell folate ≥175 ng/ml = 0, Red cell folate <175 ng/ml = 1
199	At least one red cell folate level < 175 ng/ml anytime during pregnancy	Red cell folate ≥175 ng/ml = 0, Red cell folate <175 ng/ml = 1
200	Mean serum folate and red cell folate levels (ng/ml) first trimester	Serum folate level ≥ 4 and red cell folate level ≥ 385 = 0 , serum folate level <4 and red cell folate level < median level of 385 =1, serum folate level < 4 or red cell folate level < 385 = 2

APPENDIX D

Mean or Median values of Continuous Risk factors in All Pregnancies (N=116[a])

RISK FACTOR	NO. PREGNANCIES	MEAN or MEDIAN	STANDARD DEVIATION or RANGE
Personal Characteristics			
Child's age at last exam (length of follow-up)(yr)	109	1.2	day 5-10.8
Maternal age (yr)	116	26.1	4.8
Paternal age (yr)	114	27.9	19.4-50.9
Mother's height(cm)	107	158.8	147.0-172.7
Father's height(cm)	93	172.7	157.5-188.0
Mid-parent height(cm)	93	167.3	4.9
Mother's prepregnancy weight (kg)	114	55.6	35.4-76.7
Mother's ponderal index (gm/m^2)	106	22.0	3.0
Mother's head circumference (cm)	73	54.5	51.5-59.7
Father's head circumference (cm)	45	57.6	1.8
Mid-parent head circumference (cm)	45	56.3	1.2

[a] Excluding 2 therapeutic abortions and 1 ectopic pregnancy with unknown embryopathology

APPENDIX D

RISK FACTOR	NO. PREGNANCIES	MEAN or MEDIAN	STANDARD DEVIATION or RANGE
Pregnancy Characteristics			
Length of gestation by LMP (wk)	116	39.4	6.4-43.3
Length of gestation by US or LMP (wk)	116	39.1	6.4-43.3
Mother's weight gain during pregnancy (kg)	108	14.3	4.4
Maternal Epilepsy			
Age at onset of first seizure (yr)	115	12.3	6.9
Age at onset of habitual seizures (yr)	115	13.3	6.7
Duration epilepsy (yrs from 1st seizure to LMP)	115	13.7	6.8
Duration epilepsy (yrs from onset of habitual seizures to LMP)	115	12.7	6.6
Maternal AED Therapy			
Age at commencement of AED (yr)	116	15.1	7.0
Duration AED therapy (yr)	116	9.5	0.2-32.0

APPENDIX D

Family History of Major Congenital Anomalies and of Seizures

FAMILY HISTORY OF MAJOR CONGENITAL ANOMALIES & SEIZURES (RELATIONSHIP OF AFFECTED RELATIVE TO CHILD IN PROSPECTIVE STUDY)	NO. CHILDREN	MEDIAN PROPORTION OF RELATIVES AFFECTED FOR ALL CHILDREN (RANGE)
Maternal 2nd degree relatives with major anomalies	107	0 (0-0.40)
Maternal 3rd degree relatives with major anomalies	102	0 (0-0.50)
Maternal 1st & 2nd degree relatives with major anomalies	108	0 (0-0.43)
Maternal 1st,2nd & 3rd degree relatives with major anomalies	102	0 (0-0.36)
Paternal 2nd degree relatives with major anomalies	109	0 (0-0.50)
Paternal 3rd degree relatives with major anomalies	99	0 (0-0.15)
Paternal 1st & 2nd degree relatives with major anomalies	109	0 (0-0.60)

APPENDIX D

Family History of Major Congenital Anomalies and of Seizures

FAMILY HISTORY OF MAJOR CONGENITAL ANOMALIES & SEIZURES (RELATIONSHIP OF AFFECTED RELATIVE TO CHILD IN PROSPECTIVE STUDY)	NO. CHILDREN	MEDIAN PROPORTION OF RELATIVES AFFECTED FOR ALL CHILDREN (RANGE)
Paternal 1st,2nd & 3rd degree relatives with major anomalies	99	0 (0-0.50)
Maternal & paternal 2nd degree relatives with major anomalies	104	0 (0-0.29)
Maternal & paternal 3rd degree relatives with major anomalies	91	0 (0-0.26)
Maternal & paternal 1st & 2nd degree relatives with major anomalies	104	0 (0-0.33)
Maternal & paternal 1st, 2nd & 3rd degree relatives with major anomalies	91	0 (0-0.21)
Maternal 2nd degree relatives with seizures	111	0 (0-0.50)

APPENDIX D

Family History of Major Congenital Anomalies and of Seizures

FAMILY HISTORY OF MAJOR CONGENITAL ANOMALIES & SEIZURES (RELATIONSHIP OF AFFECTED RELATIVE TO CHILD IN PROSPECTIVE STUDY)	NO. CHILDREN	MEDIAN PROPORTION OF RELATIVES AFFECTED FOR ALL CHILDREN (RANGE)
Maternal 3rd degree relatives with seizures	102	0 (0-0.33)
Maternal 2nd & 3rd degree relatives with seizures	102	0 (0-0.20)
Paternal 2nd degree relatives with seizures	109	0 (0-0.50)
Paternal 3rd degree relatives with seizures	99	0 (0-0.13)
Paternal 1st & 2nd degree relatives with seizures	108	0 (0-0.33)
Paternal 1st, 2nd & 3rd degree relatives with seizures	98	0 (0-0.13)
Maternal & paternal 2nd degree relatives with seizures	105	0 (0-0.27)

APPENDIX D

Family History of Major Congenital Anomalies and of Seizures

FAMILY HISTORY OF MAJOR CONGENITAL ANOMALIES & SEIZURES (RELATIONSHIP OF AFFECTED RELATIVE TO CHILD IN PROSPECTIVE STUDY)	NO. CHILDREN	MEDIAN PROPORTION OF RELATIVES AFFECTED FOR ALL CHILDREN (RANGE)
Maternal & paternal 3rd degree relatives with seizures	91	0 (0-0.14)
Maternal & paternal 1st & 2nd degree relatives with seizures	104	0 (0-0.25)
Maternal & paternal 1st, 2nd & 3rd degree relatives with seizures	91	0 (0-0.13)

APPENDIX E

Unordered Categorical and Ordinal Risk factors in All Pregnancies (N=116[a])

Social Data

FATHERS OCCUPATION

FA'S OCCUP	PREGNANCIES[b] FREQ	%	PREGNANCIES CUM FREQ	CUM %	FATHERS[c] FREQ	%	FATHERS CUM FREQ	CUM %
Unknown	4				4			
1	19	17.0	19	17.0	14	15.2	14	15.2
2	23	20.5	42	37.5	18	19.6	32	34.8
3	10	8.9	52	46.4	10	10.9	42	45.7
4	21	18.8	73	65.2	19	20.7	61	66.3
5	39	34.8	112	100.0	31	33.7	92	100.0

MOTHER'S EDUCATION

MO'S EDUC	PREGNANCIES FREQ	%	PREGNANCIES CUM FREQ	CUM %	MOTHERS[c] FREQ	%	MOTHERS CUM FREQ	CUM %
Unknown	4				3			
1	3	2.7	3	2.7	3	3.3	3	3.3
2	15	13.4	18	16.1	10	11.1	13	14.4
3	18	16.1	36	32.1	16	17.8	29	32.2
4	39	34.8	75	67.0	30	33.3	59	65.6
5	22	19.6	97	86.6	18	20.0	77	85.6
6	15	13.4	112	100.0	13	14.4	90	100.0

[a] Excluding 2 therapeutic abortions and 1 ectopic pregnancy with unknown embryopathology

[b] Proportion of pregnancies [c] Proportion of mothers or fathers

APPENDIX E

FATHER'S EDUCATION

FA'S EDUC	PREGNANCIES FREQ	%	PREGNANCIES CUM FREQ	CUM %	FATHERS FREQ	%	FATHERS CUM FREQ	CUM %
Unknown	11				10			
1	9	8.6	9	8.6	7	8.1	7	8.1
2	21	20.0	30	28.6	16	18.6	23	26.7
3	22	21.0	52	49.5	17	19.8	40	46.5
4	18	17.1	70	66.7	17	19.8	57	66.3
5	16	15.2	86	81.9	14	16.3	71	82.6
6	19	18.1	105	100.0	15	17.4	86	100.0

Personal Characteristics

SEX OF CHILD

SEX	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
0	53	48.2	53	48.2
1	57	51.8	110	100.0

Family History

MAJOR CONGENITAL MALFORMATIONS IN MOTHER

MA MALF MOTHER	PREGNANCIES FREQ	%	PREGNANCIES CUM FREQ	CUM %	MOTHERS FREQ	%	MOTHERS CUM FREQ	CUM %
0	108	93.1	108	93.1	87	93.5	87	93.5
1	8	6.9	116	100.0	6	6.5	93	100.0

APPENDIX E

MAJOR CONGENITAL MALFORMATIONS IN MATERNAL 1ST OR 2ND DEGREE RELATIVES

MAJ MALF RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	8			
0	76	70.4	76	70.4
1	32	29.6	108	100.0

MAJOR CONGENITAL MALFORMATIONS IN MATERNAL 1ST,2ND OR 3RD DEGREE RELATIVES

MAJ MALF RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	9			
0	63	58.9	63	58.9
1	44	41.1	107	100.0

MAJOR CONGENITAL MALFORMATIONS IN FATHER

MAJ MALF FATHER	PREGNANCIES FREQ	%	PREGNANCIES CUM FREQ	CUM %	FATHERS FREQ	%	FATHERS CUM FREQ	CUM %
Unknown	6				6			
0	106	96.4	106	96.4	87	96.7	87	96.7
1	4	3.6	110	100.0	3	3.3	90	100.0

APPENDIX E

MAJOR CONGENITAL MALFORMATIONS IN PATERNAL 1ST OR 2ND DEGREE RELATIVES

MAJ MALF RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	7			
0	99	90.8	99	90.8
1	10	9.2	109	100.0

MAJOR CONGENITAL MALFORMATIONS IN PATERNAL 1ST, 2ND OR 3RD DEGREE RELATIVES

MAJ MALF RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	12			
0	85	81.7	85	81.7
1	19	18.3	104	100.0

MAJOR CONGENITAL MALFORMATIONS IN MOTHER OR FATHER

MAJ MALF RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	6			
0	98	89.1	98	89.1
1	12	10.9	110	100.0

MAJOR CONGENITAL MALFORMATIONS IN MATERNAL OR PATERNAL 1ST OR 2ND DEGREE RELATIVES

MAJ MALF RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	12			
0	64	61.5	64	61.5
1	40	38.5	104	100.0

APPENDIX E

MAJOR CONGENITAL MALFORMATIONS IN MATERNAL OR PATERNAL 1ST, 2ND, OR 3RD DEGREE RELATIVES

MAJ MALF RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	17			
0	50	50.5	50	50.5
1	49	49.5	99	100.0

SEIZURES IN MATERNAL 2ND OR 3RD DEGREE RELATIVES

SEIZ RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	7			
0	74	67.9	74	67.9
1	35	32.1	109	100.0

SEIZURES IN FATHER

SEIZ FATHER	PREGNANCIES FREQ	PREGNANCIES %	PREGNANCIES CUM FREQ	PREGNANCIES CUM %	FATHERS FREQ	FATHERS %	FATHERS CUM FREQ	FATHERS CUM %
Unknown	7				7			
0	106	97.2	106	97.2	86	96.6	86	96.6
1	3	2.8	109	100.0	3	3.4	89	100.0

SEIZURES IN PATERNAL 1ST OR 2ND DEGREE RELATIVES

SEIZ RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	8			
0	99	91.7	99	91.7
1	9	8.3	108	100.0

APPENDIX E

SEIZURES IN PATERNAL 1ST, 2ND, OR 3RD DEGREE RELATIVES

SEIZ RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	12			
0	91	87.5	91	87.5
1	13	12.5	104	100.0

SEIZURES IN MATERNAL OR PATERNAL 1ST OR 2ND DEGREE RELATIVES

SEIZ RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	12			
0	72	69.2	72	69.2
1	32	30.8	82	100.0

SEIZURES IN MATERNAL OR PATERNAL 1ST,2ND OR 3RD DEGREE RELATIVES

SEIZ RELATIVE	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	16			
0	57	57.0	57	57.0
1	43	43.0	43	100.0

Reproductive History

PREGNANCY ORDER

PREGN ORDER	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
1	55	47.4	55	47.4
2	27	23.3	82	70.7
3	24	20.7	106	91.4
4	7	6.0	113	97.4
5	2	1.7	115	99.1
6	1	0.9	116	100.0

APPENDIX E

BIRTH ORDER

BIRTH ORDER	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
1	68	61.8	68	61.8
2	28	25.5	96	87.3
3	12	10.9	108	98.2
5	2	1.8	110	100.0

PREVIOUS FETAL LOSS

FETAL LOSS	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
0	29	56.9	29	56.9
1	22	43.1	51	100.0

PREVIOUS THERAPEUTIC ABORTION

THERAP ABOR	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
0	38	64.4	38	64.4
1	21	35.6	59	100.0

Pregnancy Characteristics

TRIMESTER OF FIRST OBSTETRIC VISIT

TRIM VISIT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	4			
1	91	81.3	91	81.3
2	20	17.9	111	99.1
3	1	0.9	112	100.0

APPENDIX E

NUMBER OF OBSTETRIC VISITS

NO. VISIT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	7			
0	99	97.1	99	97.1
1	3	2.9	102	100.0

Maternal Illnesses and Pregnancy Complications

ENDOCRINE DISORDER

ENDOCR DISORD	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
0	110	94.8	110	94.8
1	6	5.2	116	100.0

INFECTION TREATED TRIM 1

INF TR TRIM1	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	6			
0	103	93.6	103	93.6
1	7	6.4	110	100.0

INFECTION TREATED ANYTIME

INF TR ANYT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	5			
0	92	82.9	92	82.9
1	19	17.1	111	100.0

APPENDIX E

FEVER TRIM I

FEVER TRIM I	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	6			
0	99	90.0	99	90.0
1	11	10.0	11	100.0

FEVER ANYTIME

FEVER ANYT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	9			
0	83	77.6	83	77.6
1	24	22.4	107	100.0

ANEMIA

ANEMIA	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	12			
0	99	95.2	99	95.2
1	5	4.8	104	100.0

BLEEDING TRIM I

BLEED TRIM I	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
0	93	84.5	93	84.5
1	17	15.5	110	100.0

BLEEDING TRIM II

BLEED TRIM II	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
0	106	96.4	106	96.4
1	4	3.6	110	100.0

APPENDIX E

BLEEDING TRIM III

BLEED TRIM III	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
0	106	96.4	106	96.4
1	4	3.6	110	100.0

CHRONIC HYPERTENSION OR TOXEMIA

HYPERT,TOX	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
0	104	89.7	104	89.7
1	12*	10.3	116	100.0

*(10 with toxemia; 2 with chronic hypertension)

NAUSEA+/- VOMITING, TREATED

NAUSEA TR	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	2			
0	92	80.7	92	80.7
1	22	19.3	114	100.0

PREMATURE CONTRACTIONS, TREATED

PREM CONTR TR	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
0	103	93.6	103	93.6
1	7	6.4	110	100.0

OLIGOHYDRAMNIOS,POLYHYDRAMNIOS

OLIGOH,POLYH	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	1			
0	99	90.8	99	90.8
1	6	5.5	105	96.3
2	4	3.7	109	100.0

BLOOD GROUP SENSITIZATION

BLOOD GP SENS	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	6			
0	106	96.4	106	96.4
1	4	3.6	110	100.0

Physical Agents

ABDOMINAL OR PELVIC X-RAY EXPOSURE TRIM 1

X-RAYS TRIM 1	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	4			
0	108	96.4	108	96.4
1	4	3.6	112	100.0

ABDOMINAL OR PELVIC X-RAY EXPOSURE ANYTIME

X-RAYS ANYT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	4			
0	98	87.5	98	87.5
1	14	12.5	112	100.0

Drugs other than AED

SEX HORMONES TRIM 1

SEX HORM TRIM 1	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	3			
0	107	94.7	107	94.7
1	6	5.3	113	100.0

APPENDIX E

SEX HORMONES ANYTIME

SEX HORM ANYT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	3			
0	107	94.7	107	94.7
1	6	5.3	113	100.0

OTHER HORMONES TRIM 1

OTH HORM TRIM 1	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	4			
0	107	95.5	107	95.5
1	5	4.5	112	100.0

OTHER HORMONES ANYTIME

OTH HORM ANYT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	4			
0	105	93.8	105	93.8
1	7	6.3	112	100.0

ALCOHOL

ALCOHOL	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	3			
0	79	69.9	79	69.9
1	34	30.1	113	100.0

APPENDIX E

CIGARETTE SMOKING

CIG SMOKING	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	1			
0	63	54.8	63	54.8
1	10	8.7	73	63.5
2	34	29.6	107	93.0
3	8	7.0	115	100.0

Maternal Epilepsy

FREQUENCY OF MAJOR SEIZURES TRIM 1

MA SEIZ TRIM 1	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	3			
0	87	77.0	87	77.0
1	14	12.4	101	89.4
2	9	8.0	110	97.3
3	3	2.7	113	100.0

FREQUENCY OF MAJOR SEIZURES ANYTIME

MA SEIZ ANYT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	2			
0	69	60.5	69	60.5
1	21	18.4	90	78.9
2	8	7.0	98	86.0
3	7	6.1	105	92.1
4	9	7.9	114	100.0

FREQUENCY OF MINOR SEIZURES TRIM I

MI SEIZ TRIM I	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	20			
0	58	60.4	58	60.4
1	11	11.5	69	71.9
2	9	9.4	78	81.3
3	10	10.4	88	91.7
4	8	8.3	96	100.0

FREQUENCY OF MINOR SEIZURES ANYTIME

MI SEIZ ANYT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	21			
0	52	54.7	52	54.7
1	13	13.7	65	68.4
2	10	10.5	75	78.9
3	11	11.6	86	90.5
4	9	9.5	95	100.0

Folate

FOLATE SUPPLEMENTS TRIM I

FOLATE TRIM I	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	22			
0	43	45.7	43	45.7
1	50	53.2	93	98.9
2	1	1.1	94	100.0

APPENDIX E

FOLATE SUPPLEMENTS ANYTIME

FOLATE ANYT	FREQUENCY	PERCENT	CUMULATIVE FREQUENCY	CUMULATIVE PERCENT
Unknown	7			
0	17	15.6	17	15.6
1	74	67.9	91	83.5
2	18	16.5	109	100.0

APPENDIX F: Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,†0.001<p≤0.005,††0.0001<p≤0.001

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2. **N=58, R^2=0.4456**

SEXOFFSP	***OCCUPE***	**HCM**;	**SZF**;	**SZ12DE6P**;	**SZ12DE6MP**;	**SZ123DE6MP**;	***SMOKING***
-0.730* **	**0.193***						**-0.347*** **
(0.250)	**(0.087)**						**(0.125)**

SEXHORM;	**TYPEEP**;	***MAJSZI***;	FMAJSZA;	**FMINSZI**;	FMINSZA ;	***MAEDI***	NAEDA;
		-0.807*				**-0.425***	
		(0.347)				**(0.180)**	

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

2A. **N=60, R^2=0.4596**

SEXOFFSP	***OCCUPE***	**HCM**;	**SZF**;	**SZ12DE6P**;	**SZ12DE6MP**;	**SZ123DE6MP**;	***SMOKING***
-0.567*	**0.215** **						**-0.331*** **
(0.240)	**(0.083)**						**(0.121)**

SEXHORM;	**TYPEEP**;	**MAJSZI**;	FMAJSZA;	***MINSZI***;	FMINSZA ;	***MAEDI***	NAEDA;
				-0.674* **		**-0.387***	
				(0.248)		**(0.179)**	

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

APPENDIX F: Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,'0.001< p≤0.005,''0.0001<p≤0.00

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3. **N=56, R^2=0.5324**

SEXOFFSP	***OCCUPF***	**HCM**; **SZF**; **SZ12DE6P**; **SZ12DE6MP**; **SZ123DE6MP**;	***SMOKING***
-0.570*	**0.265'**		**-0.413'**
(0.236)	**(0.083)**		**(0.121)**

SEXHORM; **TYPEEP**; MAJSZI;	***FMAJSZA***	FMINSZI;	***FMINSZA***	NAEDI;	**NAEDA**;
	-0.404'		**-0.241****		
	(0.125)		**(0.094)**		

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

3A. **N=66, R^2=0.4993**

SEXOFFSP	OCCUPF;	***HCM***; **SZF**; **SZ12DE6P**; **SZ12DE6MP**; **SZ123DE6MP**;	***SMOKING***
-0.773'	**0.233'**		**-0.342*****
(0.227)	**(0.076)**		**(0.118)**

SEXHORM; **TYPEEP**; MAJSZI;	***FMAJSZA***	FMINSZI; **MINSZA**;	NAEDI;	***NAEDA***
	-0.411''			**-0.367***
	(0.112)			**(0.161)**

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

APPENDIX F: Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print), *0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,'0.001< p≤0.005,''0.0001<p≤0.001

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5. **N=35, R^2=0.4549**

SEXOFFSP; ***OCCUPF***; **HCM**; **SZF**; **SZ12DEGP**; **SZ12DEGMP**; **SZ123DEGMP**; **SMOKING**;
-1.008' **0.268****
(0.312) **(0.101)**

SEXHORM; **TYPEEP**; **MAJSZI**; FMAJSZA; **FMINSZI**; FMINSZA; ***NAEDI***; NAEDA;
-0.609 ***
(0.221)

MPPHTPBPRMI; MPPHTPBPRMA; **PHTmg/kgLMP**; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

5A. **N=37, R^2=0.4565**

SEXOFFSP; ***OCCUPF***; **HCM**; **SZF**; **SZ12DEGP**; **SZ12DEGMP**; **SZ123DEGMP**; **SMOKING**;
-0.672* **0.291'**
(0.310) **(0.096)**

SEXHORM; **TYPEEP**; **MAJSZI**; FMAJSZA; ***MINSZI***; FMINSZA; **NAEDI**; NAEDA;
-0.944***
(0.331)

MPPHTPBPRMI; MPPHTPBPRMA; **PHTmg/kgLMP**; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

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7. N=28, R^2=0.4869

SEXOFFSP; ***OCCUPF***; **HCM**; SZF; SZ12DEGP; **SZ12DEGMP**; SZ123DEGMP; ***SMOKING***;
0.264**
(0.098)
-0.368*
(0.168)

SEXHORM; ***TYPEEP***; **MAJSZI**; FMAJSZA; **FMINSZI**; FMINSZA; **NAEDI**; NAEDA;
-0.225*
(0.105)

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

7A. N=27, R^2=0.4606

SEXOFFSP; **OCCUPF**; **HCM**; SZF; SZ12DEGP; SZ12DEGMP; ***SZ123DEGMP***; **SMOKING**;
-1.080†
(0.321)

SEXHORM; ***TYPEEP***; **MAJSZI**; FMAJSZA; **FMINSZI**; FMINSZA; **NAEDI**; NAEDA;
-0.301***
(0.104)

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

APPENDIX F: Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,†0.001< p≤0.005,††0.0001<p≤0.001

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9. **N=35, R^2=0.6753**

SEXOFFSP **OCCUPF; HCM; SZF; SZ12DE6P; SZ12DE6MP; SZ123DE6MP;** ***SMOKING***;
-0.831†
(0.252)
-0.416†
(0.126)

SEXHORM; TYPEEP; MAJSZI; ***FMAJSZA***; FMINSZI; **FMINSZA;** NAEDI; **NAEDA;**
-0.512†
(0.153)

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; ***PHTPLALL***; RCFIII
-0.184††
(0.047)

9A. **N=44, R^2=0.6274**

SEXOFFSP **OCCUPF; HCM; SZF; SZ12DE6P; SZ12DE6MP;** ***SZ123DE6MP*** **SMOKING;**
-1.020††
(0.240)
-0.878††
(0.245)

SEXHORM; TYPEEP; MAJSZI; ***FMAJSZA***; FMINSZI; **MINSZA;** NAEDI; **NAEDA;**
-0.577††
(0.125)

MPPHTPBPRMI; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; **PHTPLALL;** RCFIII
-0.205*
(0.100)

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11. **N=26, R^2=0.7372**

SEXOFFSP; ***OCCUPF***; **HCM** ; SZF; SZ12DEGP; SZ12DEGMP; **SZ123DEGMP**; ***SMOKING***;
0.280'
(0.080)
-0.328*
(0.153)

SEXHORM; ***TYPEEP***; MAJSZI; **FMAJSZA** ; FMINSZI; **FMINSZA**; NAEDI; ***NAEDA***;
-0.225**
(0.084)
-1.742**
(0.660)

MPPHTPBPRMI; ***MPPHTPBPRMA***; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;
-0.869*
(0.410)

PHTmg/kgALL; PHTPLALL; RCFIII

11A. **N=32, R^2=0.7033**

SEXOFFSP; **OCCUPF**; ***HCM***; **SZF**; **SZ12DEGP**; **SZ12DEGMP**; ***SZ123DEGMP***; **SMOKING**;
-0.675**
(0.262)
0.240'
(0. 078)
-1.079''
(0.252)

SEXHORM; ***TYPEEP***; MAJSZI; ***FMAJSZA***; FMINSZI; **MINSZA**; NAEDI; **NAEDA**;
-0.224**
(0.090)
-0.714''
(0.163)

MPPHTPBPRMI; **MPPHTPBPRMA**; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; PHTPLII;

PHTmg/kgALL; PHTPLALL; RCFIII

APPENDIX F: Multiple Regression Analysis of Risk Factors in Relation to Head Circumference at Birth. Risk factors entered into proposed model are in bold print. Regression coefficients and standard errors are given for significant risk factors (bold italic print),*0.02<p≤0.05,**0.01<p≤0.02,***0.005<p≤0.01,†0.001< p≤0.005,††0.0001<p≤0.001

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12. **N=24, R^2=0.7948**

SEXOFFSP; **OCCUPF**; **HCM**; SZF; SZ12DEGP; **SZ12DEGMP**; SZ123DEGMP; **SMOKING**;
-0.956'
(0.253)

SEXHORM; ***TYPEEP***; MAJSZI; **FMAJSZA**; FMINSZI; **FMINSZA**; NAEDI; ***NAEDA***;
-0.195*
(0.083)
-0.575'
(0.177)

MPPHTPBPRM1; MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; **PHTPLII**;

PHTmg/kgALL; ***PHTPLALL***; RCFIII
-0.186''
(0.042)

12A. **N=28, R^2=0.6603**

SEXOFFSP; **OCCUPF**; **HCM**; SZF; SZ12DEGP; SZ12DEGMP; ***SZ123DEGMP***; **SMOKING**;
-0.873***
(0.304)
-1.274''
(0.285)

SEXHORM; **TYPEEP**; MAJSZI; ***FMAJSZA***; FMINSZI; **MINSZA**; NAEDI; **NAEDA**;
-0.854''
(0.061)

MPPHTPBPRM1, MPPHTPBPRMA; PHTmg/kgLMP; PHTmg/kgI; PHTPLI; PHTmgII; **PHTPLII**;

PHTmg/kgALL, **PHTPLALL**; RCFIII