PERINATAL SULFUR AMINO ACID TOXICITY

by

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ABSTRACT

Accumulation of methionine and its metabolites in the mother and fetus following methionine administration and the resulting effects upon fetal development were studied in the sow, ewe, and rat.

The placenta exerted limited control over the rate of methionine transport to the fetus, however, ultimate control rested with the maternal plasma methionine concentration. When maternal plasma methionine concentrations were elevated for prolonged intervals, accumulation of high concentrations of methionine in fetal blood occurred. Fetal capability to reduce elevated blood methionine concentrations was limited relative to maternal capacity, as a result of limited fetal to maternal transport, low fetal tissue uptake, a low excretion rate into amniotic fluid, and limited fetal methionine metabolism.

Pregnant rats fed 3.2% dietary methionine displayed decreased fetal weights in utero. This growth defect was reversed in the post natal period. No permanent effects of gestational dietary methionine level were observed over 3 generations.

RÉSUMÉ

L'accumulation de la méthionine et ses métabolites chez la mère et le foetus suivant l'administration de la méthionine et les effets qui s'ensuivent sur le développement du foetus ont été étudiés sur la truie, la brebis et le rat.

Le placenta exerce un contrôle limité sur la vitesse du transport de méthionine au foetus. Toutefois, le contrôle ultime se trouve dans la concentration de méthionine dans le plasma maternel. Quand on élève pendant des intervalles prolongés les concentrations de méthionine dans le plasma maternel, il en résulte une accumulation de haute concentration de méthionine dans le sang du foetus. La capacité du foetus de réduire des concentrations élevées de méthionine dans le sang se trouvait limitée en rapport avec la capacité maternelle, comme conséquence du transport limité du foetus à la mère, une basse absorption par le tissu du foetus, une basse excrétion dans le liquide amniotique et un métabolisme limité de méthionine par le foetus.

Des rats gravides nourries un régime de 3.2% de méthionine démontraient un poids diminué du foetus in utero. Ce défaut de croissance se trouvait renversé pendant la période post natale. Dans trois générations, on n'observa aucun effet permanent causé par le niveau de méthionine dans le régime pendant la gestation.

Suggested Short Title:

Perinatal Sulfur Amino Acid Toxicity

Knipfel

CLAIMS TO ORIGINALITY

As far as the author is aware, studies with the sow represent the first attempts to cannulate chronically the venous system of the pig fetus in utero, and the only reports of plasma amino acid concentrations in the sow and her fetus in an unanesthetized in utero condition.

The rate and extent of placental transport of methionine has until now been considered to be under control of the placenta. These studies have shown that while the rate of placental transport of methionine appears to be a placental function, the extent of transport is under control of the maternal plasma methionine concentration.

To the author's knowledge, no previous studies have shown the transport of methionine and its metabolites from maternal to fetal blood and from fetal blood to amniotic fluid in terms of turnover rate.

Changes in maternal and fetal plasma and amniotic fluid amino acid concentrations in response to methionine loading have not been previously reported.

Until these investigations were undertaken, there was a lack of information regarding the expression of prenatal methionine toxicity in the post natal period.

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EXPLANATION OF ABBREVIATED DESIGNATIONS OF AMINO ACIDS

Abbreviations for amino acids used in the thesis have been listed below:

Asp	-	Aspartic Acid
Thr	-	Threonine
Ser	-	Serine
Glu	-	Glutamic Acid
Gly	-	Glycine
Ala	-	Alanine
Val	-	Valine
Cys[1]	-	Cysteine (1/2 cystine)
Met	-	Methionine
Ileu	-	Isoleucine
Leu	-	Leucine
Tyr	-	Tyrosine
Phe	-	Phenylalanine
Lys	-	Lysine
His	-	Histidine
Arg	-	Arginine
Orn	-	Ornithine
Cys Ac	-	Cysteic Acid
Cystat	-	Cystathionine
Tau	-	Taurine
Met sulfox	-	Methionine Sulfoxide
Cit	-	Citrulline
Glu NH_2	-	Glutamine
Asp NH_2	-	Asparagine
AAB	-	α-aminobutyric Acid

[1] Cystine is formed by oxidation of the sulfhydryl groups of two cysteine molecules to give a disulfide linkage, thus the molecular weight of cystine is twice that of cysteine less 2 hydrogen atoms.

INTRODUCTION

Inborn errors of amino acid metabolism in humans have been characterized by the presence of excessive concentrations of the amino acid and/or its metabolites in body fluids. Mental retardation is often associated with this excessive concentration of amino acid (Daniel and Waisman, 1968). Many such inherited disorders have been shown to be the result of a deficiency of an enzyme involved in the catabolism of the amino acid, thus creating a "block" in the catabolic pathway and accumulation of metabolites formed in steps prior to the block. The mechanisms by which the accumulation of metabolites leads to clinical manifestation of the disease remain unknown.

The administration of large amounts of single amino acids has been observed to result in the appearance of an assortment of toxic effects. The degree of toxicity appears to be related to the relative elevation in the plasma concentration of that amino acid above its normal level (Daniel and Waisman, 1968). The mechanisms of amino acid toxicity remain obscure.

Although the origin of the inherited disorders of amino acid metabolism is genetic, while that of amino acid toxicity is prescribed, there is similarity between the two situations in the accumulation of an excessive plasma concentration of the amino acid. Such accumulation suggests that more of the

amino acid was presented to the body than it was capable of metabolizing, whether the limiting factor was a low concentration of enzyme(s) relative to normal as in the case of inborn errors, or a high concentration of amino acid relative to normal as in toxicity studies. The mechanism(s) by which the detrimental effect(s) are produced may be similar or identical and differences in clinical expression of the effect(s) may be due to the duration and timing of the exposure to the high plasma amino acid concentration.

In general, enzymes involved in the catabolism of amino acids exist only at low levels in the developing fetus increasing after birth. The ability of the fetus to metabolize a high blood level of an amino acid may be analagous to the case of an inborn error of metabolism.

Fetal blood amino acid concentrations have been repeatedly shown (Curet, 1970) to be higher than the corresponding maternal concentration. If this phenomenon holds regardless of maternal amino acid concentration, then a high level of circulating amino acid in the mother would be reflected to an exaggerated degree in the fetal blood. If the maternal level was elevated for an extended period the fetus might show symptoms similar to those of inborn errors of metabolism even though the causative agent was not genetic. Evidence has accumulated which would suggest the correctness of such a hypothesis for phenylalanine (Kerr et al., 1968). Whether these abnormalities are permanent or could be corrected by

therapy in the postnatal period remains open to question. Similarly there is a lack of information regarding the effects of elevation of maternal blood levels of amino acids during pregnancy on fetal development over several generations.

Methionine has been repeatedly shown to be the most toxic of the naturally occurring amino acids (Sauberlich, 1961; Daniel and Waisman, 1968) and has been implicated in several inborn errors of amino acid metabolism. The possibility exists that excessive intakes of methionine may occur as a result of incorrect supplementation of foods. High levels of methionine are presently recommended for use therapeutically as a lipotropic agent in humans (National Formulary, 1970). In view of the toxic nature of methionine, such usage in pregnant women could have adverse effects.

An examination of the effects of maternal methionine administration on maternal and fetal accumulation of methionine would be of interest in attempting to illucidate possible mechanisms of methionine toxicity. The metabolic activity of the fetus toward methionine has not been examined in detail. As well, the relationships of maternal blood methionine concentration to fetal development and post partum performance would be of utmost importance in preparing recommendations from both clinical and regulatory standpoints.

LITERATURE REVIEW

Effects of Maternal Dietary Protein Adequacy upon Fetal Development and Post Partum Performance

Early in this century, it was demonstrated that the long term effects of temporary malnutrition depended upon the stage of life during which the malnutrition is imposed (Chow et al., 1968). Systematic studies of the effects of maternal nutritional plane on fetal development began about 1935. Hale (1937 cited by Hurley, 1968) reported that Vitamin A deficiency in pregnant sows resulted in congenital malformations. Since that time considerable effort has been expended to illucidate the effects of maternal diet on fetal development and postnatal performance (Giroud, 1968). It has been generally agreed that the nutritional status of the dam during pregnancy may have profound effects upon both pre- and post-natal development of the offspring. The quantity and/or quality (adequacy) of maternal dietary protein plays a central role in this development (Miller, 1970).

Inadequate dietary protein has long been known to lead to reproductive disturbances. Nelson(1959) reported decreased birth weight amongst rats whose mothers were fed diets deficient in protein during pregnancy. The extent of the decrease appeared to be proportional to the severity of the

maternal deficiency. In addition, the number of fetal resorptions increased and the percentage of successful litters decreased with increasing severity of protein deficiency. These observations were thought to be the result of a lack of ovarian hormones. Others have demonstrated maintenance of pregnancy, in rats subjected to protein deficiency, by hormone therapy (Callard and Leatham, 1970; Leatham et al., 1968).

Chow and Lee (1964) reported that feed restriction of female rats during gestation and/or lactation resulted in retarded growth of the progeny which was not corrected during post natal life by provision of an adequate dietary intake. Gestational or lactational protein restriction alone were later shown (Chow et al., 1967) to result in growth retardation (Table 1.1). These workers concluded that the dominant factor causing growth retardation was the protein value of the diet, either in terms of quality or quantity of protein. A number of other abnormalities were also attributed to the maternal dietary protein adequacy (Table 1.1). In most instances, an additional effect of restriction during lactation was observed over that of gestation alone.

Chow et al. (1967) failed to disclose any effects due to lactational stress alone other than growth retardation and suggested that gestational diet potentiated the lactational effect and that although some of the effects of gestational

TABLE 1.1 Effects of Maternal Dietary Protein Restriction on Growth and Metabolism of Progeny[1]

Effect on Progeny[2]	Maternal Dietary Regimen		
	Gestation and Lactation		Gestation
	50% Restriction	Ad lib poor quality protein	50% Restriction
growth retardation	****	****	*
nitrogen wastage	****		
food wastage	****	****	***
glucose intolerance	***	***	
proteinuria	****		**
amino aciduria	****		
retarded neurological development	****		**
neurological abnormalities	*****		**

[1] From Chow et al (1967)

[2] Relative to controls.

deficiency were permanent, the severity of many effects could be modified by adequate nutrition during lactation.

The congenitally malnourished female rat produced stunted offspring even though she was not subjected to protein restrictions at any time from weaning until the conclusion of her own pregnancy (Chow et al., 1967). Zeman (1968) observed morphological and histochemical changes in the kidney of young rats whose mothers were fed low protein diets during pregnancy. Glomerular filtration rate and urinary excretion were reduced (Hall and Zeman, 1968). Functional changes in the kidneys of congenitally malnourished rats were confirmed by Lee and Chow (1968) and Brown and Guthrie (1968), indicating that an important result of maternal protein inadequacy was altered kidney function which could imply an alteration in transport characteristics of the kidney.

Other tissues have been observed (Guthrie and Brown, 1968; Zeman, 1970; Kenney, 1969) to be affected by maternal dietary protein adequacy. The extent of recovery of organ function in congenitally malnourished offspring depended upon the organ studied (Roeder and Chow, 1972).

Brain development has been studied exhaustively in relation to maternal gestational protein adequacy. General agreement has been reached that altered brain structure and function may result from protein deficiency during pregnancy in the rat (Simonson and Chow, 1970; Zamenhoff et al., 1971;

Simonson et al., 1969; Frankova and Barnes, 1968a,b; Guthrie and Brown, 1968); the pig (Barnes et al., 1970); and humans (Dobbing, 1970; Winnick, 1969; Winnick and Rosso, 1969; Winnick, 1971). The severity of this effect was summarized by Winnick (1969) and Dobbing (1970). These authors suggested the possibility of impaired brain function amongst a large segment of the world's population perhaps as a result of protein malnutrition during gestation.

The studies of Chow et al. (1967) using poor quality (deficient in one or more essential amino acids) protein indicated that specific amino acid deficiencies as well as suboptimal protein intake may affect fetal development (Table 1.1). Daniel and Olson (1968) and Gwatkin (1969) have demonstrated strict requirements for several of the essential amino acids during the early stages of embryogenesis.

Infusion of chick embryos with a methionine deficient medium was associated with reduced survival and growth of the embryo (Grau, 1968). Roeder and Chow (1970) fed pregnant rats a methionine deficient diet and observed growth retardation in both the fetus and neonate. A similar observation was made by Newberne et al., (1970) whose data indicated that the essentiality of methionine was in part due to its function as a lipotropic agent. Injection of ethionine, a methionine antagonist, into pregnant rats (Chow and Agustin, 1965) induced premature birth, suggesting that methionine was

essential for the maintenance of pregnancy.

Phenylalanine deprivation in the pregnant and lactating rat resulted in decreased weight gains of their progeny as well as a reduction in development of liver phenylalanine hydroxylase activity (Bessman et al., 1969). Several other investigators (Pineda, 1968; Kerr et al., 1969) have suggested that treatment of phenylketonuria by feeding a diet low in phenylalanine to pregnant women resulted in symptoms of phenylalanine deficiency and altered developmental patterns in babies. Kerr et al., (1969) demonstrated a similar effect in the Rhesus monkey.

From the preceding discussion, it appears that the effects of maternal dietary protein adequacy during pregnancy may be a reflection of the supply of amino acids reaching the fetus and the ability of the fetus to assimilate this supply into its metabolic activities. In this regard, the role of the placenta in mediating the supply of amino acids transported from maternal to fetal blood must be examined.

Amino Acid Transport Across the Placenta

Mechanisms for transport of amino acids across membranes

Considerable effort has been made in the field of biological transport; the abundant information on this subject has been adequately reviewed (Czaky, 1965; Benson and Rampone, 1966).

Van Slyke and Meyer (1913) were the first to demonstrate that free amino acid concentrations were much higher in tissues than in blood, and that elevations in blood amino acid levels following infusion of a protein hydrolyzate were reflected to an exaggerated degree in tissues. Since that time these findings have been confirmed by many workers and reviewed by Christensen (1964).

The transport of amino acids into and out of cells has been studied extensively and the general consensus has been that amino acids are transported across cellular membranes by carrier-mediated transport systems (Czaky, 1965) which may be of several types:

1. Facilitated diffusion has as a driving force the concentration difference on either side of the membrane.

2. Exchange diffusion appears to be a specialized case of facilitated diffusion wherein the substances are translocated without energy expenditure. In some instances an apparent net transport against a concentration gradient

may occur.

3. Active transport is an energy requiring process which may be either uphill or downhill with respect to the concentration gradient.

There appears to be little evidence that different carriers are required for these processes. Most experimental evidence is consistent with the concept of a specific, receptor site for any given molecule (Czaky, 1965) with the affinity for the concentrative mechanism(s) depending upon the configuration of the carrier-substrate complex. The nature of the carriers involved in amino acid transport has not been fully established (Young and Freedman, 1971), although a protein has been postulated as the carrier.

The kinetics of amino acid transport have been suggested to resemble those of enzyme reactions although with less stringent structural requirements (Christensen, 1964) and have been reviewed by Christensen (1969).

Most of the information regarding amino acid transport has been derived from studies measuring uptake and release by isolated cells and tissues. The transport of amino acids across membrane systems such as the intestine, kidney, or placenta has received considerably less attention with a large portion of the data coming from clearance studies in the kidney or transepithelial flux measurements in the intestine (Young and Freedman, 1971).

Amino acid group classification by transport characteristics

A number of groupings of amino acids with common transport characteristics have been proposed, with additional subgroupings within the major groups. Young and Freedman (1971) classified the amino acids into four transport groups as follows:

1. Neutral system - including aliphatic, branched chain, heterocyclic, aromatic and amide containing amino acids, methionine and cystine. Evidence for the existence of a common system for such amino acids has come from genetic studies (Cusworth and Dent, 1960), comparisons of competition for transport (Bergeron and Morel, 1969) and the effects of infusion of single neutral amino acids on clearance of other neutral amino acids (Webber et al., 1961; Kamin and Handler, 1951). Oxender and Christensen (1963) demonstrated within the neutral amino acid transport group the existence of two separate transport systems which were designated the "L" or leucine-preferring system and the "A" or alanine-preferring system. These two systems transported nearly all the neutral amino acids to varying degrees. The L-system has been proposed (Christensen, 1969) to be Na^+ independent, with a widely variable maximum velocity (Vmax) for different amino acids and a strong tendency to exchange amino acids on both sides of the membrane so that net operation of the system

may be difficult to demonstrate. Some selectivity for transport of specific amino acids was shown. The A-system was nonselective with respect to transport of neutral amino acids, showed a relatively constant Vmax for most neutral amino acids except branched chain amino acids and was Na^+ dependent. The transport appeared to be only weakly reversible thus resulting in steep uphill gradients. Most of the neutral amino acids are transported to some extent by both these systems (Christensen, 1969); each neutral amino acid showed inhibition of the uptake of other neutral amino acids. Further breakdowns of the L and A systems have been proposed (Christensen, 1969). The existence of these various systems has been questioned (Guroff et al., 1964), however, the proposal of Oxender and Christensen (1963) is consistent with other data (Jacquez, 1967).

2. Basic system - including lysine, arginine, ornithine and possibly cystine. While lysine, arginine and ornithine undeniably share a common transport system, the relationship of cystine to this system remains in question (Whelan and Scriver, 1968; Schneider et al., 1968).

3. Acidic system - including aspartic and glutamic acids.

4. Imino-glycine system - including proline, hydroxyproline and glycine. This system has been suggested to be an oversimplification in that while the overall system is operative, there are certainly multiple mediations within

this group (Young and Freedman, 1971).

Groupings of amino acids such as that proposed by Young and Freedman (1971) have been established merely in an attempt to classify common transport characteristics; whether this particular classification should be expanded or revised remains open to question. A number of alternate proposals have been put forth (Benson and Rampone, 1966; Munck, 1966; Baker and George, 1971; Lerner and Burrill, 1971).

Intergroup interactions

Numerous examples of group interactions have been reported. Mohyuddin and Scriver (1968) demonstrated alanine inhibition of proline transport. Munck and Schultz (1969) observed stimulation of lysine transport by methionine and leucine but not by other neutral amino acids in the rabbit ileum. Other workers have demonstrated interactions between the neutral and basic transport systems (Thomas et al., 1971; Christensen, 1968), as well as between the neutral and acidic systems (Webber, 1962). Two possible theories could account for these interactions (Young and Freedman, 1971). The first would have a limited number of transport systems which would have wide and overlapping substrate specificities. The second would postulate the existence of two basic types of transport systems, one with high capacity and low specificity, somewhat similar to that of the first theory, while the other

system would have low capacity and high specificity. The second theory would, on the basis of experimental evidence to date, appear to be consistent with available data (Young and Freedman, 1971) although the second theory appears to be an extension of the first.

From the preceding discussion, it becomes obvious that transport systems for amino acids are not well understood and that considerable investigation is required to elucidate the actual transport processes.

Proposed role of methionine as a mediator of neutral amino acid transport

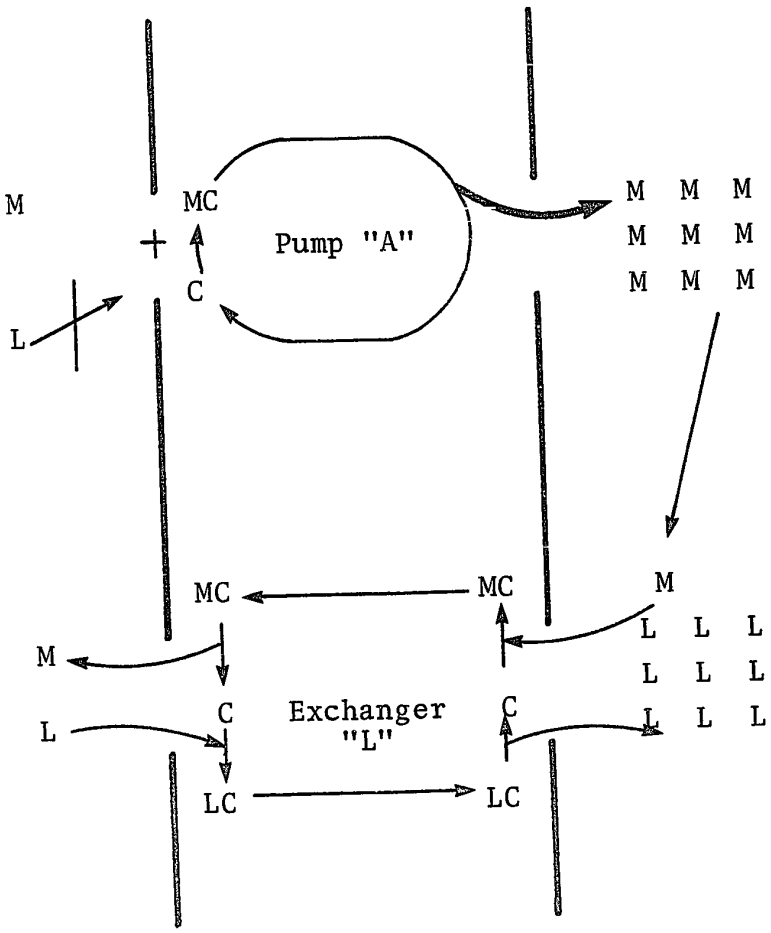
The A system for transport of neutral amino acids as outlined by Christensen (1964) may result in steep gradients for amino acids across membranes being attained. The affinity of this system appears to be greatest for short chain amino acids such as alanine. Branching of the C-chain almost completely eliminated transport by the A system. Valine, isoleucine and leucine would therefore be transported by the L system, which appears to have little ability to create a steep concentration gradient. Methionine has been shown (Oxender and Christensen, 1963) to have high affinity for both systems. The result of this dual affinity is proposed (Christensen, 1964; Oxender and Christensen, 1963) to be a strong concentration of methionine in cells by the A system.

Since the L system has high affinity for methionine also, the exchange of methionine through the L system serves to drive the uphill transport of the branched chain amino acids. A schematic representation is shown in Fig.1. Such behavior would make methionine extremely significant in the transport of other amino acids which appear to be transported only by exchange. The application of this theory to suggested mechanisms of methionine toxicity is discussed in later sections.

Placental transport of amino acids

It has been suggested that concentrations of free amino acids in umbilical cord blood are a reflection of placental transport, placental structural integrity, maternal diet, maternal free amino acid levels, and fetal metabolic processes (Kerr, 1968).

Christensen and Streicher (1948) observed fetal (F)/ maternal (M) ratios (F/M ratios) for blood amino acid concentrations which were greater than 1 for guinea pigs and rabbits. Others have observed similar ratios in humans, sheep and other species (reviewed by Curet, 1970). Dancis et al. (1958) produced saturation of the placental transport system for histidine by infusing histidine into the mother. Preferential placental transfer of L-histidine over the D-isomer was demonstrated by Page et al. (1957). The exact nature of placental transport mechanisms has not



Methionine (M) is strongly concentrated by pump "A", which does not transport Leucine (L). Methionine also has strong affinity for the exchanger "L", therefore is exchanged for Leucine which would then be concentrated uphill to a greater extent than would occur by their weak affinity for pump "A". From Christensen (1964).

Fig. 1. Proposed Role of Methionine as a Mediator of Amino Acid Transport

been fully illucidated due to the complexity of the placenta as a membrane system. However, these data suggest that amino acids are actively transported across the placenta against a concentration gradient, a phenomenon which would result in F/M ratios greater than one.

Considerable variation occurs in morphological structure of the placenta from species to species (Nalbandov, 1959) and one might expect placental transport characteristics to vary with the different types of placentas. Curet (1970), in reviewing the transport of amino acids across the placenta pointed out that the F/M ratios of different species varied to some extent; perhaps this variability may be in part due to differing placentation types which would alter the efficiency of transport. Regardless of the placental structure, all species studied to date have shown evidence of active transport of amino acids from the maternal to fetal circulations.

Blood plasma amino acid concentrations in mother and fetus

During pregnancy maternal concentrations of free amino acids have in many cases been observed to decrease (Bjornesjo, 1968; Christensen and Streicher, 1948; Kerr, 1968). This effect has been attributed to removal of amino acids from the maternal circulation by the placenta (Christensen and Streicher, 1948; Bjornesjo, 1968), increased cellular uptake of amino

acids by the mother (Bjornesjo, 1968), and hyperaminoaciduria during pregnancy (Jones et al., 1969). Regardless of the factors resulting in lowered maternal blood amino acid concentrations during pregnancy, the F/M ratios for amino acids would depend on both maternal and fetal influences and may show considerable variation during pregnancy. Kerr (1968) noted a progressive decrease in the F/M ratio for all amino acids with advancing pregnancy in the Rhesus monkey, while Lichenstein (1931) and Ghadmi and Pecora (1964) observed highest levels of amino acid nitrogen in the most immature human fetuses.

Several authors have studied the effects of altered placental function on amino acid concentrations in fetal blood. Clemetson and Churchman (1954) observed a fall in F/M ratio for total alpha amino nitrogen concentration rather than decreased fetal level in toxemic pregnancies during mild toxemia. Curet (1970) suggested that with more severe toxemia, uterine blood flow was reduced and the resulting impairment of placental function reduced the amino acid supply to the fetus. Foley et al. (1967) demonstrated in Guernsey cattle with placental insufficiency that the F/M ratios for valine, methionine, isoleucine, leucine, and arginine were reduced to less than or close to 1, due to reduced fetal blood levels of these amino acids. These data suggest that the maternal system has some regulatory

capability for supply of amino acids reaching the fetus but that the placenta is the major regulator.

Studies (Antonov, 1947; Smith, 1947) with post war European children indicated that severe and prolonged undernutrition of pregnant mothers was required before significant effects on the fetus were seen, further confirming that the placenta may regulate the supply of amino acids reaching the fetus regardless of maternal levels, within physiological limits. In most other species, however, the weight of the fetus or litter may be markedly affected by protein restriction, which would affect the supply of amino acids reaching the fetus. Slater and Mellor (1972) produced a decrease in plasma concentrations of threonine, valine, leucine and phenylalanine with a concurrent increase in non-essential amino acids in pregnant ewes subjected to a low protein diet. Similar changes occurred in fetal lamb plasma. F/M ratios for most amino acids were somewhat higher or similar to those of ewes fed adequate protein during gestation, suggesting that the placenta regulated the supply of amino acids reaching the fetus to eliminate some, but not all the effects of maternal nutrition.

Hopkins et al. (1971a) observed relatively high plasma amino acid concentrations in fetal lamb blood compared with that from the ewe with threonine and serine particularly elevated in the fetal plasma. These results are in

accord with those of numerous other researchers cited by Curet (1970), regarding higher blood levels in the fetal blood than in the mother for different species. In further studies, Young (1971) and Hopkins et al. (1971b) injected mixtures of amino acids belonging to the "A" and "L" preferring neutral amino acid groups (Oxender and Christensen, 1963). Transport of "L" preferring amino acids was rapid and resulted in increased fetal blood levels following injection of the amino acid mixture into the maternal jugular vein. Fetal levels of amino acids belonging to the "A" preferring neutral group did not increase following maternal injection. These results were explained on the basis of relatively free exchange of the "L" preferring amino acids between the placenta and fetal blood, while the "A" preferring amino acids (with little exchange capacity) remained in the placental tissue once taken up from maternal blood by the placenta. These researchers proposed that on the basis of their results the placenta secreted amino acids from the maternal to fetal circulations at a constant rate which was little influenced by the maternal blood level.

Amino acid concentrations in the placenta

Free amino acid changes in the blood of the mother and fetus may yield considerable useful information regarding

net placental transport of amino acids. However, the placental tissue is very active metabolically and concentrations of free amino acids within placental tissue are considerably higher than those observed in the circulations on either side of the placenta (Dancis et al., 1968; Pearse and Sornsen, 1969). In order to establish actual mechanisms of amino acid transport from maternal to fetal circulations, a knowledge of the magnitude of the free amino acid pool of the placenta and the relationships of this pool to the maternal and fetal blood amino acid concentrations is necessary.

Dancis et al. (1968) studied the passage of an unmetabolizable amino acid, ^{14}C-alpha aminoisobutryic acid (^{14}C-AIB) across the placenta of the guinea pig. Placental tissue consistently contained higher levels of radioactivity than did either maternal or fetal blood (Table 1.2). In addition, the fetal placenta (trophoblast) appeared to concentrate ^{14}C to a considerably higher level than did the maternal placenta (decidua). Lorincz and Kuttner (1969) reported decreased maternal serum/uterine tissue ratios for amino acids during pregnancy in humans and rats which were a result of both decreased maternal free amino acid levels and increased uterine tissue free amino acid content. Concentrations of free amino acids in actively growing tissues have been shown to increase (Noall et al., 1957;

TABLE 1.2 Relative concentrations of ^{14}C-α amino isobutyric acid in maternal and fetal tissue and fluids following infusion into pregnant guinea pigs[1]

Expt. Number	Infusion (min.)	Fetus Number[2]	Ratio $\frac{F}{M}$[3][4]	$\frac{D}{M}$[5]	$\frac{T}{M}$[6]	$\frac{T}{F}$	$\frac{ML}{M}$[7]	$\frac{FL}{T}$[8]
1	115	1	0.6	1.3	2.4	4.2	34.0	—
		2	0.7	1.1	2.2	3.4		
2	105	1	1.4	1.1	2.8	2.0	7.0	0.9
		2	1.4	1.6	2.8	2.0		0.9
3	225	1	2.9	3.9	13.0	4.7	51.0	1.1
		2	2.4	2.9	13.0	5.5		1.1
4	230	1	3.2	1.9	16.0	8.4	28.0	1.4
		2	2.5	2.2	17.0	6.8		1.0
5	240	1	4.8	17.0	3.0	0.7	36.0	1.3
		2	3.4	12.0	9.0	2.8		1.4
6	240	1	2.0	1.6	3.4	1.7	39.0	1.6
		2	1.9	1.7	3.3	1.7		1.2

[1] From Dancis et al (1968)

[2] Two fetuses were analyzed in each experiment

[3] Fetal Plasma - F

[4] Maternal Plasma - M

[5] Maternal Placenta - D

[6] Fetal Placenta - T

[7] Maternal Liver - ML

[8] Fetal Liver - FL

Christensen et al., 1958; Christensen and Streicher, 1948) leading to elevated tissue/blood free amino acid ratios. Reproductive tissues during pregnancy undergo profound changes in size and function (Lorincz and Kuttner, 1969). It is possible that the increase in maternal placental ^{14}C-AIB content observed by Dancis et al. (1968) was a reflection of uptake by a metabolically active tissue and not a reflection of placental transport per se. This supposition implies therefore that the transport of amino acids to the fetus is a function of the fetal placenta. Comparison of the data of Lorincz and Kuttner (1969) with that of Page (1969) also disclosed considerably higher tissue/blood ratios of amino acids for placental tissue than for the uterine tissue in humans.

Pearse and Sornsen (1969) compared the free amino acid concentrations of normal human placentas with those from placentas derived from humans affected with a variety of pathological conditions. Low free amino acid levels in infarcted placental tissue (Table 1.3) were associated with placental insufficiency indicating that the placenta was unable to concentrate amino acids from the maternal circulation to normal levels. Pre-eclampsia has also been associated with placental insufficiency (Curet, 1970), however, the levels of free amino acids in a pre-eclamptic situation (Table 1.3) were actually higher than those of

TABLE 1.3 Free amino acid concentrations in normal and abnormal placental tissue of the human [1]

Amino Acid	Term Placenta	Infarcted Placenta	Preeclamptic Placenta	Hydatiform Mole
	(μM/100 g wet tissue)			
Asp	105	63	163	19
Thr	54	33	82	35
Ser	57	26	137	27
Glu NH_2 + Asp NH_2	69	66	86	59
Pro	34	21	T [2]	28
Glu	223	70	272	83
Gly	134	60	247	63
Ala	97	60	114	73
Val	30	5	T	40
Cys	23	11	70	40
Met	6	T	8	3
Ileu	17	T	29	14
Leu	38	T	70	28
Tyr	17	10	27	12
Phe	19	9	31	15
Lys	62	31	88	52
His	17	10	23	11
Arg	28	12	56	6
Total	1030	487	1503	608

[1] From Pearse and Sornson (1969)

[2] Trace present

normal term placenta with the exception of proline and valine. The fact that these two apparently conflicting situations both result in placental insufficiency, suggests that several mechanisms may be involved in placental insufficiency. In the case of infarction, placental insufficiency may be due to impairment of active transport of amino acids from the maternal circulation to the placenta resulting in a lowered gradient from placenta to fetus, thus less transport. Fetal growth has been observed to correlate well with placenta size (Dawes, 1968), suggesting that a larger area for nutrient transport may result in increased nutrient passage. Following infarction the surface area of placenta available for transport of amino acids to the fetus would be decreased, thus the placental insufficiency might also result from decreased amounts of active tissue (Dawes, 1968). In the case of pre-eclampsia the transport of amino acids from the maternal blood into the placental tissue does not appear to be lowered (Table 1.3) thus the transfer of amino acids from the placenta to the fetal circulation may be impaired. If the observations of Christensen and Streicher (1948) and others that actively growing tissue maintained a higher level of free amino acids than did less active tissue are correct, perhaps the pre-eclamptic placenta was regenerating and thus using amino acids for its own requirements.

Lorincz and Kuttner (1969) reported that protein

restriction of rats during pregnancy, resulted in a fall in the uterine tissue/maternal serum ratio for most amino acids as a result of reduced uterine tissue uptake. Whether this effect occurred for placental tissue was not determined. If such were the case, the effects of maternal dietary protein deficiency in reducing birth weight could be established as due to decreased amino acid supply to the fetus.

Placental transport mechanisms

Curet (1970) found that the amounts of amino acids leaving the uterine circulation had little relationship to the amount of amino acids appearing in the umbilical blood at any given time. Under physiological conditions the amount of alpha amino acid nitrogen crossing the placenta appeared to be independent of the maternal level. As previously shown (Table 1.2), free amino acid concentrations in the placenta were considerable higher than for either maternal or fetal plasma. These observations suggested that amino acids taken up by the placenta may not be immediately transferred to the fetal circulation but stored in the placental tissue. Based on these data, Curet (1970) proposed a scheme for the placental transport of amino acids which is shown in Fig. 2.

Stage 1 was proposed to represent a dynamic equilib-

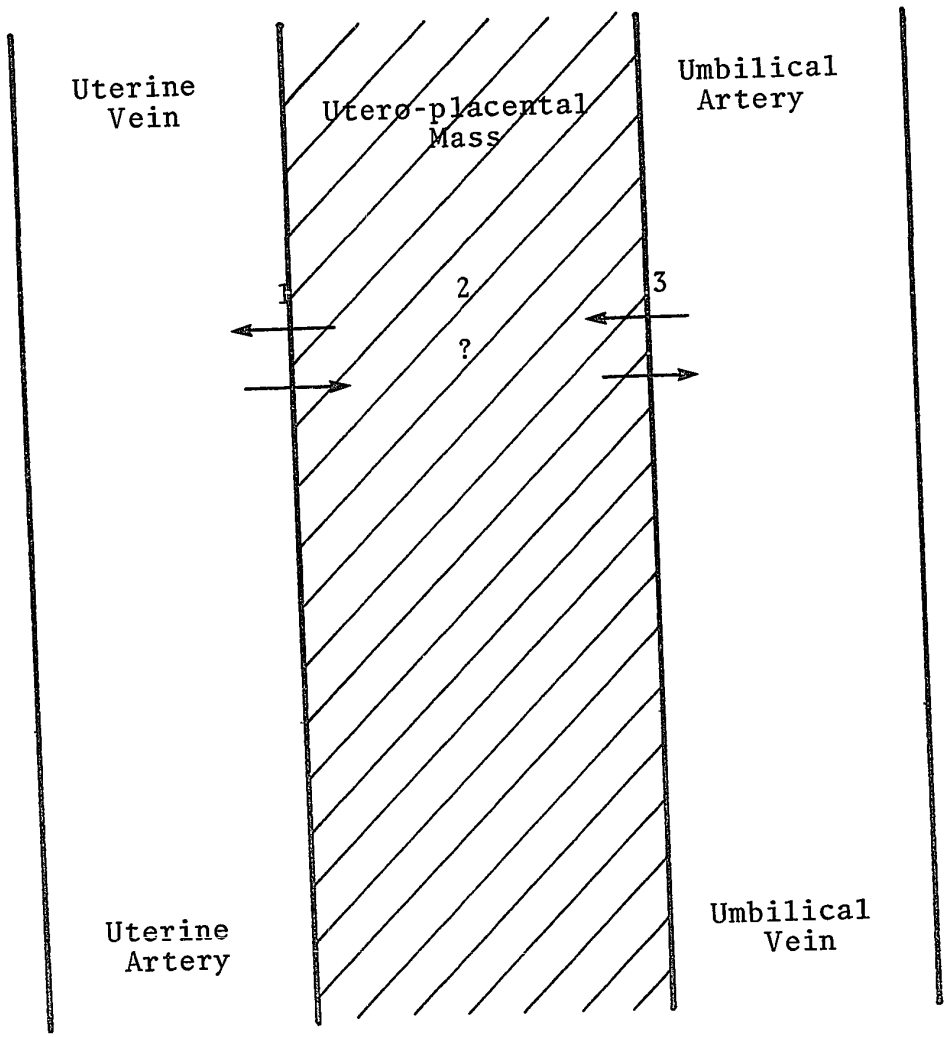


Fig. 2. Schematic Representation of Stages Involved in Placental Transport of Amino Acids. From Curet (1970).

rium between uterine blood and the placental tissue. Active transport mechanism(s) were thought to be confined to this stage. Stage 2 constitutes the storage and/or metabolism of amino acids within the placenta. Stage 3 represents a diffusion process. It must be realized that this proposal is an extremely simplified system, however, the overall net effect of such a system is consistent with much of the data from studies on blood and tissue amino acid concentrations in mother, placenta, and fetus, providing a number of assumptions are made. The concept of stage one, involving active transport processes is likely correct as shown for uptake processes in other tissues (Czaky, 1965). There is little reason to suspect that the placenta reacts differently, especially considering the high intracellular amino acid concentrations of placental tissue relative to maternal blood (Table 1.2). Stage 2 may also be a correct hypothesis, based on the high free amino acid concentrations of placental tissue relative to levels found in maternal and fetal circulations (Table 1.2). The free amino acid pool of the placenta potentially represents a significant reservoir of amino acids. The concept of placental-umbilical blood transfer of amino acids by simple diffusion implies unidirectional flow of amino acids from placenta to the fetal circulation down a concentration gradient. However, Sturman et al. (1971) reported that methionine or cystine injected into the fetal

circulation appeared in the maternal circulation, although this transfer was very slow. In order for an amino acid to return to the maternal circulation, an active process of transport from fetal blood to placental tissue would be required. The very slow transfer of cystine and methionine from fetus to mother indicated that the active transport process from fetal circulation to placental tissue may have been extremely limited quantitatively, however, some capacity must have been present. Lines and Waisman (1971) observed a slow clearance of phenylalanine from fetal to maternal rat plasma. Pearse and Sornson's (1969) observation of higher free amino acids in pre-eclamptic placenta suggests that a mechanism other than simple diffusion mediates transport from placenta to fetal blood. An increase in the free amino acid content of the placenta would be expected to increase the free amino acid supply to the fetus, rather than the reverse, if simple diffusion was involved. Results of transport studies with other tissues (Czaky, 1965) indicated that the "downhill" transport process was an active rather than a passive one.

Amino acid concentrations in amniotic fluid

The amino acid composition of amniotic fluid has been suggested (Saifer et al., 1970) as a diagnostic tool in

the determination of possible disorders of amino acid metabolism and their treatment during pregnancy. Some interest has also been raised (Curet, 1970) in the possibility of transamniotic alimentation of the fetus in cases of placental insufficiency. Kerr and Kennan (1969) found lower levels of free amino acids in amniotic fluid than in fetal blood in the Rhesus monkey. The amniotic fluid pattern of amino acids did not bear any particular resemblance to either maternal or fetal blood amino acid levels. These workers suggested that a tendency for amniotic fluid amino acid concentrations to decrease during advancement of pregnancy may have resulted from absorption of the amino acids from the fetal gut subsequent to swallowing of the amniotic fluid. Free amino acid levels in human amniotic fluid increased considerably from the ninth to sixteenth week of gestation then decreased steadily until term (Emery et al., 1970). In addition, most amino acids were present at higher concentrations in amniotic fluid than in maternal or fetal plasma during early pregnancy, but this trend decreased steadily until term when only taurine and ethanolamine were higher in amniotic fluid than in maternal or fetal blood. Levy and Montag (1969) indicated a generally lower level of free amino acids in amniotic fluid compared with levels in maternal or fetal serum at term in humans with taurine

present in the amniotic fluid at considerably higher levels than in maternal serum. On the other hand, Cockburn et al. (1970) observed somewhat higher free amino acid concentrations in amniotic fluid than in maternal plasma although the differences were slight.

Controversy exists regarding the origin of amniotic fluid. During early gestation amniotic fluid has been considered to be a dialyzate of maternal plasma and/or a secretion of the amniotic epithelium, while later in pregnancy the fetus contributes most of the amniotic fluid via micturition (Cockburn et al., 1970; Kerr and Kennan, 1969). Levy and Montag (1969) on the basis of comparisons of amino acid concentrations in maternal and fetal blood, fetal urine and amniotic fluid suggested that the other fluids all contributed to amniotic fluid.

Saifer et al. (1970) suggested that amino acids are rapidly cleared from the amniotic fluid by the maternal circulation; the mechanism by which this would occur is unclear since there is limited direct connection between the maternal circulation and that of the amniotic sac. Kerr and Kennan (1969) and Curet (1970) suggested that much of the decrease in amniotic fluid amino acids occurred as a result of fetal swallowing and reabsorption of the amino acids during passage through the digestive tract. If this

were the case, the proposal of Saifer et al. (1970) becomes untenable. Amniotic fluid constituents have been shown (Tyson et al., 1969) to have a slow turnover rate, which would further decrease the liklihood of Saifer et al's. (1970) hypothesis.

The nutritional significance of amniotic fluid amino acids to the fetus remains unclear but represents a pool of amino acids which may have a role in maintaining homeostasis of fetal amino acid levels. The fetus appears to be dependent upon placental transport of amino acids from the maternal circulation to supply its amino acid requirements. A pool of free amino acids exists in amniotic fluid, from which the fetus may derive some of its needs, but direct transport from maternal to fetal blood would appear to be of much greater importance nutritionally than the amniotic fluid pool.

In cases of amino acid excesses the placenta apparently maintains the F/M ratio in the face of increased maternal amino acid concentrations (Kerr et al., 1968) which could act to the detriment of the fetus in cases of toxicity. As indicated by Sturman et al. (1971), transfer of methionine from the fetal circulation to the maternal blood was limited. Thus an accumulation of excessively high concentrations of methionine in fetal blood might be expected to persist even though the maternal level was returned to normal following

administration of a large load of methionine to the mother.

Methionine has been shown to be extremely active metabolically and several metabolites are readily formed by methionine degradation. The extent to which these metabolites may cross the placenta and produce toxic effects in the fetus has not been investigated. There is, however, a considerable body of evidence regarding metabolism and toxicity of methionine and its degradative products in the postnatal subject which may also be relevant to the fetus.

Metabolism and Toxicity of Sulfur Amino Acids

Metabolism

Degradation of methionine and its metabolites

The sulfur amino acids have been shown by many workers to be extremely active metabolically, participating in a large number of metabolic pathways in the intact animal.

The major metabolic functions of methionine have been summarized by Finkelstein and Mudd (1967) as follows:

1. Utilization for protein synthesis;
2. Conversion to S-adenosylmethionine, the dominant biological methyl group donor;
3. Conversion via the transsulfuration pathway to cystathionine, cysteine and other metabolites.

The pathways for the latter two functions involve a common path initially, i.e. the formation of S-adenosyl methionine, S-adenosylhomocysteine and homocysteine (Fig.3). Homocysteine may be metabolized by two metabolic routes, the first being conversion, via remethylation, to methionine. The alternative route for homocysteine metabolism is an irreversible one in which homocysteine, in the presence of serine, is converted initially to cystathionine followed by cleavage to homoserine and cysteine. The degradation of methionine to cysteine effectively results in transfer of the S-atom of methionine to the C-chain of serine (transsul-

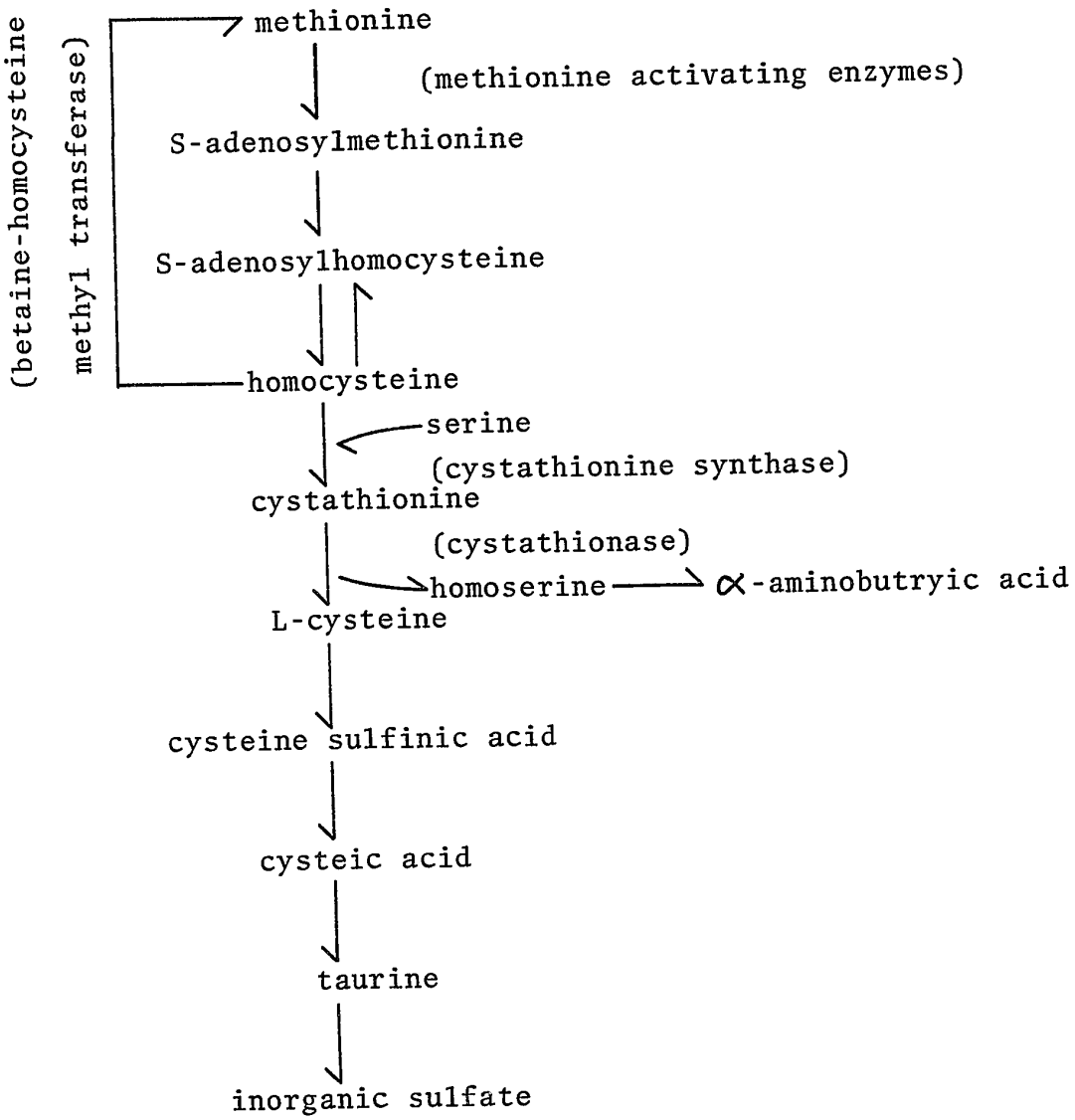


Fig. 3. A Simplified Scheme of Methionine Degradation

furation), yielding cysteine. This transsulfuration pathway has been established as the dominant scheme for conversion of methionine to inorganic sulfate (Laster et al., 1965), although a number of other pathways may be operative as well (Meister, 1965). A comprehensive review of metabolic pathways for methionine and its metabolites has been prepared by Meister (1965).

Cysteine is further metabolized to its major excretory products, inorganic sulfur (80%) and taurine (Finkelstein and Mudd, 1967; Laster et al., 1965). Only the predominant pathway of cysteine metabolism is shown in Fig. 3.

Activity of methionine metabolism

Finkelstein and Mudd (1967) suggested that a regulatory system exists for the transsulfuration pathway, based on the relative activities of cystathionine synthase and betaine homocysteine methyltransferase. These workers observed that when diets low in methionine and supplemented with cysteine were fed to rats, a decrease in hepatic cystathionine synthase activity occurred, with the decrease beginning within 24 hours and reaching minimum activity in 3 days. However, betaine homocysteine methyltransferase levels were unaffected. These results were taken to indicate that a larger proportion of homocysteine would be remethylated to methionine, thus sparing methionine for other metabolic

activities. A decrease in the activity of the methionine activating enzyme also occurred with cysteine supplementation of low methionine diets, which would effectively reduce the amount of methionine entering the transsulfuration cycle. The mechanism(s) by which cysteine exerts these effects on the enzymes involved in methionine degradation is as yet unknown. However, repression of the enzymes by cysteine and derepression by methionine were suggested to be areas for investigation.

These results were thought (Finkelstein and Mudd, 1967) to be of clinical importance in that low methionine, high cystine diets are routinely utilized in treatment of homocystinuria to reduce the accumulation of methionine metabolites and prevent cysteine deficiency. The investigations of Finkelstein and Mudd indicated that a further decrease in cystathionine synthase activity would occur as a result of such therapy and that if cystathionine had some biological function other than its role in transsulfuration then cystathionine deficiency might occur. Therapy aimed at this type of pathological disorder would appear to be of more benefit if stimulation of cystathionine synthase activity could be achieved.

Toxicity

Effects of ingestion of disproportional amounts of amino acids

Adverse effects as a result of ingestion of disproportionate amounts of amino acids are uncommon in healthy subjects and are usually experimentally produced (Harper, 1970). However, in certain disease states such as liver damage, protein malnutrition, or genetic disorders of amino acid metabolism, tolerance to amino acid loading may be reduced and result in the appearance of adverse effects.

The severity of the adverse effect varies both with the nature and degree of amino acid disproportionality and also with the nutritional adequacy of the diet (Sauberlich, 1961).

Classification of adverse effects of disproportionate amounts of amino acids

A number of classifications have developed for the effects of disproportionate amounts of amino acids. The most widely accepted classification has been summarized by Harper (1970) as follows:

1. Amino Acid Toxicity has been used to describe the adverse effects resulting from the ingestion of large amounts of individual amino acids, which do not appear to have common features. There likely is no common basis for the

adverse effects of individual amino acids; rather the effects are due to both chemical structure and metabolic inter-relationships of such amino acids.

2. Amino Acid Antagonism as a concept arose from the antimetabolite theory of structural analogues. It can be applied to those changes in amino acid pattern of a diet which cause a growth depression which is largely or completely alleviated by the addition of a small amount of a structurally similar amino acid (or amino acids) not originally limiting in the diet.

3. Amino Acid Imbalance has been defined as a change in the proportions of amino acids in a diet which results in a depression in food intake or growth rate that can be completely prevented by a supplement of the indispensable amino acid present in the least amount in the diet in relation to the amount required for optimal performance.

Toxicity of dietary excesses of amino acids

Excessive intakes of most indispensable and several dispensable amino acids depress growth (Sauberlich, 1961; Daniel and Waisman, 1968), and the general statement has been made that massive doses of any amino acid would likely be toxic to an organism (Harper, 1970). The quantity required to cause toxicity symptoms varies greatly amoung

individual amino acids (Milne, 1968), indicating that the basis of the effect is probably dependent upon the structure and metabolism of the particular amino acid.

Those amino acids which are metabolically active in several pathways appear to be the most toxic, the toxicity decreasing with decreasing metabolic activity and ease of oxidation (Harper, 1970). In keeping with the metabolic activity-toxicity theory, methionine and its sulfur-containing metabolites which are rapidly metabolized, are among the most toxic of the amino acids when given in excessive amounts to animals (Milne, 1968; Harper, 1970; Sauberlich, 1961).

Effects of excessive intakes of S-containing amino acids

Numerous studies have shown that methionine, homocystine, and cystine when fed or injected in large amounts give rise to toxic effects which are characteristic for each of these compounds. As seen from Table 1.4, methionine caused the most severe growth depression, followed by homocystine and cystine. For a more intensive treatment of the subject, the reader is referred to the exhaustive review of Harper (1970). The specific toxic effects due to these compounds and their metabolites have been summarized below.

Cystine - The toxic effects of ingestion of excess cystine have been known for many years, however, investigations

TABLE 1.4 Relative growth depressing effects of Methionine, Homocysteine and Cysteine fed to growing rats[1]

Diet	Weight change in 7 days (g)
10% Casein + 0.3% L-Methionine	45
10% Casein + 0.3% L-Methionine + 2.4% DL-homocysteine (free base)	13
10% Casein + 0.3% L-Methionine + 2.4% L-cysteine (free base)	13
10% Casein + 0.3% L-Methionine + 1.2% L-cysteine (free base)	35
10% Casein + 0.3% L-Methionine + 0.6% L-cysteine (free base)	45
10% Casein + 3.0% L-Methionine	0

[1] Taken from Harper (1970)

into the mechanism(s) of the toxicity have been limited (Harper, 1970). Tissue damage caused by excess cystine appears to be confined to the liver and kidney (Harper, 1970; Milne, 1968) with the incidence and severity of the damage increasing with dietary cystine level (Harper, 1970).

Growth rates of rats fed dietary cystine levels close to 2% of diet decreased (Harper, 1970), while at lower levels growth rate was not significantly affected (Benevenga et al., 1968; Cohen et al., 1958). At dietary cystine levels higher than 2% growth depression and mortality were common (Benevenga et al., 1968; Sauberlich, 1961).

Earle and Victor (1942) found that increased dietary protein levels were of no benefit in reducing the adverse effects of excess dietary cystine, however, Sauberlich (1961) observed that when lactalbumin was fed in place of casein to rats given excess cystine in the diet, growth was nearly normal and no deaths occurred. The apparent discrepancy in these studies was suggested to be a consequence of the balance of amino acids in the overall diet (Sauberlich, 1961).

Earle et al. (1942), in attempting to determine whether cystine or its metabolites were responsible for the toxic effects, found that cysteic acid did not cause tissue damage or growth depression in rats until dietary levels of 12 to 15% were fed. No pathological effects were observed when

taurine was fed at dietary levels up to 10%. These workers suggested that the lesions caused by excess dietary cystine were not a result of the disulfide linkage of cystine, oxidation of the sulfur, the formation and excretion of sulfate in large quantities, or the presence of an amino group separated by a 2 carbon chain from the sulfur molecule. Thus, the toxic effects of cystine may be a result of some effect other than the specific toxicity of cystine itself, although this question remains to be resolved.

Homocystine - Addition of 0.54% homocystine to a 15% casein diet deficient in pyridoxine resulted in reduction of survival time of rats to about 50% of that of rats not fed homocystine (Debey et al., 1952). Supplementation of the pyridoxine deficient diets with choline or betaine partially alleviated the growth depressing activity of 0.54% homocystine. This observation was later confirmed by Cohen et al., (1958). Several studies (Benevenga and Harper, 1967; Benevenga, 1969) have shown that homocystine toxicity in otherwise adequate diets could be at least partially prevented by supplements of serine or glycine. Although homocystine toxicity is similar in several respects to that of methionine (Harper, 1970), growth depression caused by excess homocysteine appears to be less than that caused by excess methionine (Table 1.4). The role of homocysteine in

expression of methionine toxicity is further discussed on page 51.

Methionine - In studies of the relative toxicities of amino acids, methionine has consistently been found to be among the most toxic (Milne, 1968; Daniel and Waisman, 1968). Several studies (Wretlind and Rose, 1950; Sauberlich, 1961) have shown L-methionine to be more toxic than D-methionine. The growth depressing effects of methionine have been observed with dietary levels greater than 1% of diet (Harper, 1970). Four percent of methionine in the diet of pregnant rats lowered fetal weight (Leatham et al., 1968; Viau et al., 1969).

Direct toxic effects of methionine have been reported. The use of cold temperature to increase food intake in rats fed high dietary levels of methionine, resulted in increased methionine toxicity (Beaton, 1967). Tissue damage due to methionine excess did not appear to be permanent, and adult animals appeared to be less sensitive to methionine toxicity than weanling or growing rats (Harper, 1970).

Large amounts of methionine administered to a subject result in a number of metabolic alterations. In rats, pronounced elevations in the plasma methionine concentration occurred following excess methionine intake whether the method of administration was dietary (Sauberlich, 1961; Sanchez and Swendseid, 1969) or by injection (Harper, 1970;

Daniel and Waisman, 1969). Concentrations of methionine and its metabolites were elevated in liver and muscle tissue of mature rats (Sanchez and Swendseid, 1969) and in brain and liver tissue of weanling rats (Daniel and Wasiman, 1969) following the increase in blood methionine level. Several other amino acids were also affected by the high methionine concentration. Plasma glycine (Sanchez and Swendseid, 1969; Harper, 1970) and serine (Harper, 1970) have been observed to decrease following elevation of methionine concentrations in plasma. This was not surprising in view of the role of serine in methionine degradation (Fig. 3) and the ready formation of serine from glycine (Meister, 1965).

The activity of several enzymes involved in methionine metabolism appears to be substrate dependent although some controversy exists in this regard. Sanchez and Swendseid (1969) reported no changes in methionine adenosyltransferase and cystathionase activities following force-feeding of 4% methionine to rats. Finkelstein and Mudd (1967) observed enhanced activities of methionine adenosyltransferase, cystathionine synthetase, and cystathionase when animals fed low dietary methionine levels were injected with methionine. The addition of cystine to a low methionine diet was found (Finkelstein and Mudd, 1967) to reduce hepatic methionine adenosyltransferase activity. No effect was observed when a

diet adequate in methionine was fed. The addition of methionine to a protein-free diet markedly increased the activity of methionine adenosyltransferase (Pan and Tarver, 1967), however, the same addition to a diet containing adequate protein was without effect.

Methionine ingestion appears to stimulate glucocorticoid production (Finkelstein, 1967). Injection of glucocorticoids have also been observed to cause increases in the activity of methionine adenosyltransferase and cystathionine synthetase (Chase et al., 1968; Volpe and Laster, 1972). Munro (1964) implied that the consumption of excess methionine may indirectly stimulate the activity of enzymes involved in methionine metabolism through stimulation of corticosterone production. Whether the addition of methionine to diets adequate in protein would have the same effect remains to be elucidated.

Feeding large amounts of methionine caused an elevation of S-adenosylmethionine concentration and a reduction in adenosine triphosphate (ATP) concentration in rat liver (Choitz and Kurrie, 1968). Depletion of hepatic ATP was suggested to be the mechanism through which methionine toxicity was mediated (Hardwick et al., 1970), however, the validity of this postulation has not been established.

In vitro studies (Kutzbach and Stockstad, 1967) have

shown that concentrations of S-adenosylmethionine comparable to those found in vivo in rats following methionine loading inhibit the activity of methylene tetrahydrofolate reductase to less than 50% of control values, thereby reducing the supply of N^5-methyltetrahydrofolate available for remethylation of homocystine. However, the hepatic activity of betaine-homocystine transmethylase has been observed to increase (Ericson and Harper, 1956) in rats fed excess methionine, which may allow methionine formation from homocystine, and at least partially compensate for the reduced methylene tetrahydrofolate reductase activity.

In the rat, diets containing 4% methionine caused a marked increase in hepatic threonine-serine dehydratase activity (Sanchez and Swendseid, 1969), while a low methionine diet supplemented with cystine decreased serine dehydratase activity substantially (Finkelstein and Mudd, 1967). Addition of 2.5% methionine to a low protein (2% casein) diet reduced the activity of 3-phosphoglycerate dehydrogenase, the initial enzyme in a pathway for serine biosynthesis, 4 fold from activities observed in control animals fed the unsupplemented diet (Fallon et al., 1968). The mechanism(s) by which high levels of methionine affects the enzymes involved in serine degradation and biosynthesis is unknown. Administration of cortisol increased the activity of serine

dehydratase 10 fold. At the same time a 10 fold reduction in 3-phosphoglycerate dehydrogenase activity was noted (Fallon and Byrne, 1967). Harper (1970) suggested that the effects of methionine excess on the activity of enzymes involved in serine metabolism were mediated through increased glucocorticoid production. This proposal was based on the similarity of changes in the activity of serine metabolizing enzymes caused by the hormone and the amino acid. These observations on alterations in enzyme activities following methionine loading suggest that the supply of serine for synthesis of cystathionine may be reduced both by increased destruction of serine and decreased biosynthesis; such an effect would result in an accumulation of methionine. Dietary supplements of serine and/or glycine appear to reduce plasma methionine levels in animals fed high methionine diets (Harper, 1970). The effect of glycine and serine on alleviation of some of the toxic effects of methionine may be partially a result of increased serine availability allowing more rapid catabolism of methionine (Benevenga and Harper, 1967).

Suggested mechanisms of methionine toxicity

Metabolite accumulation as a basis for methionine toxicity

Numerous attempts have been made to elucidate the

cause of methionine toxicity. Cohen et al. (1958) observed no toxic effects from loading rats with homoserine, a product of the cystathionase reaction (Fig. 3). Products of homoserine metabolism did not show toxic properties which would implicate them as the active principles in methionine toxicity.

Any toxic effects due to an excess of methyl groups from methionine would likely not be a direct effect of the methyl group per se, since the ability of several methyl acceptors to reduce methionine toxicity varies widely (Cohen et al., 1958; Sauberlich, 1961). Also, the toxicity of methyl donors varies widely (Harper, 1970).

Methionine toxicity cannot be attributed to accumulation of high levels of cysteine since glycine and serine alleviate methionine and homocysteine toxicity but not that of cysteine (Benevenga, 1969).

Homocysteine does not appear to be the sole toxic principle in methionine toxicity since the growth depressing effects of methionine are greater than those of homocysteine on an equimolar basis (Benevenga and Harper, 1967). In addition, alleviation of homocysteine toxicity by glycine or serine is much more rapid than is relief of methionine toxicity by these two compounds (Harper, 1970). Also, the histopathological lesions of methionine and homocysteine

toxicity are somewhat different (Klavins and Johansen, 1965). Methionine loading results in high blood and tissue concentrations of methionine but not homocysteine (Daniel and Waisman, 1969).

From the preceeding discussion it is obvious that the relative importance of various products of methionine degradation, in promoting the symptoms of methionine toxicity, have not been fully assessed. Biochemical mechanisms by which the effect may be brought about remain subject to controversy also.

Transport as a basis for methionine toxicity

The structural requirements of transport systems with respect to amino acids appear to be less rigid than those of enzymes. Thus excesses of one amino acid may have a greater effect on the transport of other amino acids than would occur in the more specific enzyme systems (Christensen, 1964). In view of the proposed central role of methionine in amino acid transport (page 16), the relatively high toxicity of methionine relative to other amino acids (Sauberlich, 1961; Milne, 1968; Daniel and Waisman, 1968) may be to some extent the result of an alteration in the transport of amino acids caused by the presence of excess methionine. Daniel and Waisman (1969) observed alterations in the free

amino acid patterns in liver and brain tissue of rats in the face of high plasma levels of methionine. Acute effects following injection of methionine were more pronounced than were chronic effects caused by the presence of high levels of methionine in the diet. Daniel and Waisman (1969) noted an increase in methionine and its metabolites, lysine and ornithine in liver tissue following intraperitoneal injection of methionine. Hepatic levels of aspartic acid, threonine, serine, glutamic acid, glutamine, glycine, alanine and γ aminobutryic acid were decreased. Similar changes were noted in brain tissue. A generalized aminoaciduria was observed by these workers following excessive methionine intake. Sanchez and Swendseid (1969) reported decreases in the content of most amino acids other than methionine and its metabolites in liver and muscle tissue of rats after feeding 4% methionine. The effect of methionine in reducing tissue levels of other amino acids was suggested to be an indirect effect resulting from stimulation of enzymes involved in the metabolism of these amino acids rather than an alteration in transport. Sanchez and Swendseid (1969) did not report urinary excretion patterns following ingestion of excess methionine, therefore the possibility of reduction in plasma and tissue free amino acid levels due to excretion remained open. In a later study Sanchez et al. (1972)

reported a decrease in the plasma and liver concentrations of a number of amino acids following methionine loading in rats. While increases in activity of several enzymes involved in the metabolism of those amino acids which decreased were observed, decreases were also noted which were not correlated with enzyme activity and which may have been a result of transport interactions. Block et al. (1969) commented that following oral loading of human subjects with methionine, cystine, cysteine, or taurine, plasma levels of most other amino acids were either maintained or decreased below fasting levels. Spaide et al. (1971), studying schizophrenic patients, observed that increases in the plasma concentration of most amino acids occurred following chronic oral methionine loading over an 18 to 20 day period.

Excess methionine appeared to affect amino acid concentrations in plasma and tissues differently in the above investigations. The effects of excess methionine on the concentration of other free amino acids in plasma and tissues appears open to question although effects undoubtedly do occur. Block et al. (1971) suggested that the effects were dose dependent, thus the apparent controversy may have been a consequence of differing experimental conditions.

Although free amino acid concentrations in plasma and tissues may allow some insight into the overall processes of amino acid transport, it is important to realize that

amino acids are extensively removed from the free pool in tissues both by degradation and incorporation into protein. The observed free amino acid patterns therefore represent the net result of both transport and metabolic processes. Evidence accumulated to date has not clarified the relative importance of transport and metabolic alteration in the pathogenesis of methionine toxicity.

Development of Amino Acid Metabolism in the Fetus

Protein and amino acid metabolism achieve their highest level of activity during fetal development. Protein deposition in the fetus occurs at a rapidly increasing rate during the latter portion of gestation, while the growth of the placenta is most rapid during earlier stages of pregnancy (Waisman and Kerr, 1965). This observation implies the necessity for placental maturation prior to fetal development. With development of individual organs in the fetus the emphasis on protein synthesis gradually changes from structural to functional (enzymatic) synthesis, and during this period new metabolic pathways emerge creating new requirements for amino acids (Waisman and Kerr, 1965).

In general, enzymes involved in the metabolism of energy yielding substrates and in essential metabolic processes have been observed to appear early in gestation (Sereni and Principi, 1965; Waisman and Kerr, 1965). Enzymes concerned with processes which can be performed by the placenta or mother usually appear later in gestation or postnatally (Waisman and Kerr, 1965). Many enzymes concerned with amino acid catabolism fall into the latter category, and do not achieve adult levels of activity until after birth. This raises questions regarding the

ability of the fetus to react to changes in the supply of amino acids reaching it, and the amino acid requirements of the fetus.

Development of tyrosine metabolizing enzymes in the fetus has been observed in rats (Sereni and Principi, 1965; Msuya and Schepartz, 1969; Wicks, 1968) to occur in the immediately postnatal period, with very low activity in the fetus prior to birth. Induction of enzyme activity was suggested (Sereni and Principi, 1965; Msuya and Schepartz, 1969; Reynolds and Potter, 1971) to be a result of hormonal stimulation. Wicks (1969) induced tyrosine transaminase activity in the fetal rat liver by the administration of glucocorticoids, insulin, or glucagon to hypophysectomized fetuses. In the intact fetus the induction was steroid resistant, suggesting repression of enzyme activity. The repression was suggested to be caused by inhibition of enzyme synthesis by growth hormone. Wicks also suggested an <u>in utero</u> repression of other enzymes.

Tryptophan pyrolase did not show any appreciable activity during fetal life (Sereni and Principi, 1965), however, the increased postnatal activity was delayed for a considerable period of time, indicating a different mechanism for control of activity than that of tyrosine transaminase. Glutamine synthetase (Knox et al., 1971),

threonine dehydratase (Yeung and Yeung, 1972) and serine dehydratase (Reynolds and Potter, 1971) as well as a number of other enzymes (Sereni and Principi, 1965) have shown low activities in fetal tissues.

Fetal Sulfur Amino Acid Metabolism

Chase et al. (1968) reported a sharp increase in the activity of the methionine activating enzyme in rat liver within 48 hours following birth. In brain tissue the activity did not change significantly from the 18th day of gestation until adult life. Similarly, Volpe and Laster (1970a) could detect no change in the activity of the methionine activating enzyme in the brain of the Rhesus monkey from early fetal stage to the 2 to 3 year old animal, while liver activity of the same enzyme increased approximately four fold following birth. Gaull et al. (1972) observed a similar pattern of activity development for the methionine activating enzyme in the human. These results were explained by these investigators on the basis that the brain had a high requirement for S-adenosylmethionine to serve as a methyl donor.

No detectable cystathionine synthase activity was found in rat brain until late in gestation (Volpe and Laster, 1972), with most activity developing postnatally

in response to increased requirements of the brain for metabolites of the transsulfuration pathway. The development of cystathionine synthase activity in fetal rat liver was also very limited until after parturition (Volpe and Laster, 1972). In the Rhesus monkey (Volpe and Laster, 1970b) the increase in hepatic cystathionine synthase activity was less dramatic than that observed in the neonatal rat while the activity of brain cystathionine synthase did not change significantly from fetal to adult life. The reasons for these species differences in the development of cystathionine synthase activity are unknown. However, it has been repeatedly observed (Tallan, Stein and Moore, 1958; Brenton et al., 1965) that the primate brain contained considerably higher levels of cystathionine than did the brain of non primates.

Cystathionase was reported to be absent in human fetal tissues (Gaull et al., 1972; Sturman et al., 1970). An increase in the cystathionase activity in various organs of the rat post partum (Finkelstein, 1967) was not striking, however, the apparent differences between the rat and human could not be construed as a true species variation since the samples were not taken at similar developmental stages.

Mechanism(s) for control of the enzymes involved in transsulfuration during fetal life remain unknown. Volpe

and Laster (1972) reported that adrenal corticoids were not the major determinants of increases in neonatal enzyme activity. Finkelstein (1967) in studies of hormonal and dietary effects on transsulfuration enzymes, observed that the activities of methionine activating enzyme, cystathionine synthase and cystathionase varied independently of each other and that the response of any of the enzymes varied depending upon the tissue studied in the neonatal rat. In this study hormonal or dietary therapy had little effect on the induction of cystathionine synthase.

On the basis of the previous discussion, the fetus seems to have a limited capability for production of transsulfuration products such as cystathionine and cysteine and metabolites of cysteine such as taurine. This observation implies that the fetus is largely dependent on a supply of these products reaching it via the placenta. Placental tissue has been shown (Gaull et al., 1972; Sturman et al., 1970) to have negligible transsulfuration activity, thus placental transport of the products of methionine catabolism from the maternal circulation appeared to be of considerable importance in supplying the requirements of the fetus for such metabolites.

Excess Amounts of Amino Acids as Related to Fetal Development

During the past decade a considerable number of inborn errors of metabolism have been discovered, many of which

are the result of defects in amino acid metabolism. These diseases have been suggested to be a result of enzyme and/or transport defects (Holtzman, 1970), and although the basic defect may be known, the processes leading to clinical manifestation of the diseases are largely unknown (Howell, 1970).

In phenylketonuria, phenylalanine and several of its metabolites accumulate in blood and tissues due to a lack of the enzyme phenylalanine hydroxylase (Kerr et al., 1969). It has been suggested that the accumulation of these metabolites results in altered brain metabolism and function (McKean et al., 1968; Lowden and LaRamee, 1969). Current therapy for treatment of this disease is dietary, the object being to maintain blood levels of phenylalanine within the normal range. Several studies with humans (Hsia, 1971; Allen and Brown, 1968; Frankenburg et al., 1968) have indicated that high levels of phenylalanine in maternal blood during pregnancy could be damaging to development of the fetal brain. Intrauterine growth retardation was also observed (Frankenburg et al., 1968; Fisch et al., 1968; Stevenson and Huntley, 1967) as a result of maternal phenylketonuria.

Homocystinuria, an inherited disorder of amino acid metabolism is characterized by a lack of cystathionine synthase, resulting in an accumulation of methionine and

homocystine in blood and urine. The symptoms have been attributed to homocysteine excess and deficiencies of folic acid and homocystine metabolites (Holtzman, 1970). Several therapeutic treatments have been attempted with varying degrees of success and the proposal has been made that while the disorder may be diagnosed in utero, little is known regarding the mechanism(s) of toxicity (Holtzman, 1970) or its management.

A number of other inborn errors of amino acid metabolism have also been demonstrated (Berry, 1969). The outstanding feature of these metabolic defects is the accumulation of high levels of certain amino acids and/or their metabolites in blood and tissues. The toxic mechanisms by which high levels of these metabolites exert their effect are largely unknown, however, interference with transport and/or metabolism may be involved.

Kerr et al. (1968) produced the symptoms of phenylketonuria in infant Rhesus monkeys whose mothers had been subjected to large doses of phenylalanine during pregnancy.

Longenecker et al. (1970) produced the symptoms of phenylketonuria in neonatal rats by loading them orally with phenylalanine. The fetus displays little or no phenylalanine hydroxylase activity (Freedland et al., 1962) and thus, from an enzyme defect standpoint, appears to be similar to the classical postnatal phenylketonuric. Considering the

concentrative ability of the placenta, the fetus may be exposed to even higher levels of phenylalanine than present in the maternal circulation, thus a loading of the maternal system may produce grave effects in the fetus, as previously described (Kerr et al., 1968; Longenecker et al., 1970).

There is little information regarding the effects of high levels of methionine in the maternal circulation on fetal development. However, one might expect that a situation similar to that occurring with phenylalanine might occur. The severity of such a situation may be greater for methionine, in view of its apparent role in transport and its relatively high toxicity.

Viau et al. (1969) observed a decrease in the fetal and placental weights of rats fed 4% methionine in an 18% casein diet. Protein and DNA contents of the placenta were also reduced. Feeding of a low protein diet (6% casein) plus 4% methionine resulted in complete failure to maintain pregnancy. The administration of steroids to the animals fed the low protein plus methionine diet allowed pregnancy to be maintained. No reduction in litter size was observed, but the fetuses, placentas, and the uterus were reduced in weight. The fetal liver and brain protein content were unaffected but the placental protein concentration increased along with a decrease in placental DNA.

A number of workers (Sturman et al., 1971; Kerr et al., 1968) have reported elevated fetal blood methionine concentrations relative to the circulating level in the mother in untreated animals. If such a situation persisted in the face of a high circulating methionine level in the maternal blood, the fetal level would reflect to an exaggerated degree the elevated maternal level. Kerr et al. (1968) suggested that normal placental function, which maintains free amino acids at higher concentrations in the fetal blood than in maternal blood, does not "turn off" when normal fetal levels are reached, but rather maintains the F/M ratio. A similar proposal was made by Curet (1970). In addition, the capacity of the fetal system to clear an amino acid present at high levels in the blood would likely be low in view of very low catabolic activity (Sereni and Principi, 1965; Waisman and Kerr, 1965) and the relatively slow transport of amino acids from fetus to mother (Sturman et al., 1971). This combination of factors suggest that if the maternal blood level of an amino acid was elevated, the fetal blood level would increase to a greater extent than did that of the mother, and that this elevated level may be maintained for a prolonged period of time in the fetus. In view of the suggested inability of the fetus to metabolize amino acids, one is tempted to surmise that the toxic effects

of an amino acid to the fetus are a result of interference in the transport mechanism and/or with other fetal metabolic processes as a result of the presence of a high circulating level of that amino acid. It must be kept in mind, however, that with a high maternal amino acid level an increase in the extent of maternal catabolism of the amino acid may occur and that the resulting metabolites may cross the placenta to produce toxic effects also. Wong et al. (1972) produced a disaggregation of polyribosomes in the cerebral cortex of the fetal rat following intravenous injection of either phenylalanine or tryptophan into the mother. This disaggregation was associated with an increased cerebral cortical concentration of the amino acid in the fetus. These data suggest inhibition of protein synthesis, caused by increased brain amino acid levels, as a possible explanation for toxicity in the fetus. Despite considerable effort, the mechanisms of amino acid toxicity in the fetus remain largely unknown. The work of Wong et al. (1972) implied that the mechanism might be similar for several amino acids, however, this problem remains to be solved.

Potential for Excess Methionine Intake by the Pregnant Female

The toxicity of excess methionine has been well documented in previous sections. In spite of this knowledge superoptimal intakes of methionine may occur under a variety of nutritional or therapeutic regimens.

Methionine has been recommended as a lipotropic agent for humans to combat the hepatic disease syndrome referred to as fatty infiltration of the liver. Amounts of methionine ranging from 3 to 20 g daily have been recommended (National Formulary, 1970) for this therapeutic use. Severe symptoms of methionine toxicity have been observed (Milne, 1968) following administration of 24 g of methionine intravenously in man. Single oral loads of 5 g (Block et al., 1969) did not appear to cause toxic symptoms in man although plasma patterns of methionine were elevated considerably. Spaide et al. (1971) noted very high plasma methionine levels in schizophrenic subjects following oral loading with 40 g methionine; several toxic symptoms occurred in this group as a result of such loading. Prescription of methionine as a lipotropic agent for a pregnant woman might be expected to result in an elevation of the plasma methionine level and undoubtedly elevate the fetal blood level also, perhaps to the detriment of fetal development.

Intraperitoneal injections of methionine or cystine into

sheep in amounts up to 100 g have been suggested (Downes et al., 1970) to dramatically increase wool production. Loads of this magnitude in the pregnant ewe could result in elevations in maternal and fetal blood levels with concomitant adverse effects in the fetus and/or ewe.

Supplementation of sulfur amino acid deficient foodstuffs with methionine to meet the recommended requirements of children resulted in the appearance of adverse effects attributed to the methionine supplement (Bressani et al., 1958). The consequences of error in determining correct supplementation levels may be widespread.

Prolonged exposure of the mother to methionine intakes in excess of requirement levels might result in an accumulation of methionine in the fetus as a result of a somewhat elevated maternal blood level. Little is known of the effects of exposure to such levels of methionine by the pregnant female.

Symptoms of methionine toxicity in the postnatally exposed animal do not appear to be permanent (Harper, 1970). Results of studies on inborn errors of methionine metabolism and the knowledge that excess levels of amino acids during pregnancy may affect fetal development imply that the toxic effects of methionine on the fetus may be more pronounced and less reversible than those observed for postnatal animals. Investigation of these possibilities was the subject of the studies reported herein.

EXPERIMENTAL

The following investigations were undertaken to assess the effects of excessive intakes of methionine by the pregnant female on the development of the fetus and its post partum performance. Studies were conducted using the sow, ewe and rat as experimental animals.

Most studies of transport of amino acids across the placenta have involved collection of samples at termination of pregnancy, whether the termination was natural or by design. Under such conditions the placental transport of amino acids may not be indicative of the normal in utero condition. Therefore attempts were made to surgically insert indwelling cannulas into the maternal and fetal venous circulations of the sow and ewe. Such a preparation would allow repeated blood samplings from the unanesthetized dam and her in utero fetus during studies of placental transport of methionine in these two species.

The rat was used as an experimental animal in assessing the effects of maternal consumption of a series of differing dietary methionine levels during pregnancy on the pre and postnatal growth and metabolism of the offspring.

Studies with each species of experimental animal have been reported separately.

Experimental procedures which were routinely used in these studies were compiled in Appendix A.

Studies with Sows

The sow was chosen as an experimental model for placental transport studies for several reasons:

1. As a monogastric omnivore, its metabolism and amino acid requirements may approximate those of the human more closely than would those of some other species.

2. An extensive body of knowledge has been accumulated with regard to the use of swine in biomedical research (Bustad and McClellan, 1966) and standard biochemical and physiological parameters are well established.

3. Cannulas may be conveniently exteriorized since the sow has limited mobility in reaching various areas of her body.

4. Size of the sow and her fetuses may be such that venous cannulation of both can be achieved without undue surgical difficulties.

A total of 12 surgical preparations were undertaken. The initial 9 attempts utilized mini-sows (Hormel strain) purchased from the Department of Animal Science, MacDonald College[1] or their offspring bred at the Health Protection Branch[2] facilities from the originally purchased stock. The

[1] Ste. Anne deBellevue, Quebec

[2] Department of National Health and Welfare, Tunney's Pasture Ottawa, Ontario

remaining 3 animals were commercial crossbred sows purchased from one dealer. The mini-sows were all bred by the same boar. No records other than date of service were available for the commercial sows. One acute preparation was carried out with a mini-sow at MacDonald College. The chronically prepared sows were housed individually in 1.2 x 2.4m pens bedded with wood shavings. A commercial sow ration (Ralston Purina of Canada, Woodstock, Ont.) was fed ad libitum with water freely available. The ration contained 14% CP (0.10% Met., 0.25% Cys.).

Placental transport of ^{35}S following intraarterial injection of ^{35}S-methionine (1.6 mg/kg) to the acutely prepared sow

Objectives:

1. To establish surgical techniques for use in subsequent chronic preparations.

2. To study the transport of ^{35}S across the sow placenta following injection of ^{35}S-methionine at a level which would not markedly elevate the maternal plasma methionine concentration.

3. To study uptake of ^{35}S by the maternal and fetal liver following intraarterial injection of ^{35}S-methionine into the sow.

Procedures:

Anesthesia was induced with thiopental (Pentothal Sodium,

Abbott Laboratories, Montreal, Quebec) and maintained with halothane (Fluothane, Ayerst Laboratories, Montreal, Quebec) for the duration of the experiment. A laparotomy incision was made and the maternal uterine vein cannulated. Five hundred μCi ^{35}S-L-methionine (1.6 mg L-methionine/kg body weight) was injected into the uterine artery. Samples of maternal and fetal blood and fetal liver were obtained at the post injection times indicated in Table 2.1. A sample of the maternal liver was obtained upon termination of the experiment. At each sampling time, one fetus was removed from the uterus following ligation of the umbilical vessels and weighed. A blood sample was taken from each fetus by heart puncture and the fetal liver removed, weighed and homogenized prior to sampling. Maternal blood samples were obtained via the uterine vein cannula. The uterine and abdominal incisions were kept closed with towel clips except during sample collection. The total radioactivity attributable to ^{35}S (DPM/ml whole blood or /g wet tissue) was determined for maternal blood, fetal blood, maternal liver, and fetal liver using methods for sample digestion and radioactivity determination outlined in Appendix A. The sow was euthanized by thiopental overload at the conclusion of the experiment.

Results:

Highest levels of ^{35}S in both maternal and fetal blood

occurred at the first post injection sampling time (Table 2.1). However, maximum ^{35}S activities may have occurred at an earlier time. The maternal blood ^{35}S level remained higher than that of fetal blood throughout the test period (Table 2.1), indicating that only a small proportion of the injected dose had crossed the placenta to the fetus. Comparisons of blood volumes of the sow and the fetuses were not made. Based on equal blood volumes as a percentage of body weight, however, the sow would have had approximately 15 times as much blood as the total number of fetuses combined. While the fetuses might have been expected to have a higher percentage of body weight as blood, the maternal circulation would still contain considerably more blood than the fetuses. Thus the proportion of injected methionine which crossed the placenta from the maternal circulation was probably even smaller than would be apparent from examining activities on a per unit basis.

The fetal liver exhibited some tendency to accumulate ^{35}S (Table 2.1). However, this tendency was very much less than that of the maternal liver at the end of the test period. The extremely high uptake of ^{35}S by maternal liver suggests that limited transport of the injected dose of ^{35}S across the placenta may have been a consequence of dilution of the label by maternal blood and maternal tissue uptake. The decrease observed in fetal blood ^{35}S level at later times

TABLE 2.1 Distribution of ^{35}S Between Mini-Sow and Fetus Following Intraarterial Injection of ^{35}S-L-Methionine to the Acutely Prepared Sow

Time after Injection (min.)	Sow		Fetus	
	Blood	Liver	Blood	Liver
	(DPM/ml whole blood or g wet tissue)			
15	879800	-[1]	29895	1720
30	9590	-	3255	331
45	7000	-	880	444
60	6700	-	490	591
75	5700	-	370	410
90	5340	-	100	708
110	4515	58890	280	904

[1] No samples of sow liver taken at these times.

may have been a result of fetal liver uptake, however, the decrease observed between 15 and 30 minutes post injection could not be accounted for by liver uptake (Table 2.1). Part of this decrease may have been a result of more uniform distribution of ^{35}S in the blood but the route of removal of ^{35}S from fetal blood by extra hepatic tissues and/or other fetal fluids remains unknown.

This experiment indicated that the fetal pig at 100 days of gestation was sufficiently large to allow chronic venous cannulation without undue difficulty. The preparation was of value in that some familiarity with anatomical features of the subject was obtained which could be applied to later studies.

Because the experiment was performed under anesthesia and the method of fetal sampling involved considerable uterine stress, there was some doubt whether the results were representative of the in utero situation in the unanesthetized animal. In subsequent studies, therefore, indwelling cannulas were implanted in veins of the sow and her fetus to allow experiments to be undertaken in the unanesthetized intact maternal-fetal preparation.

During the preparation of blood samples for liquid scintillation counting to determine ^{35}S activity, some samples were not completely decolorized. To eliminate this effect in later experiments, samples taken were centrifuged (Appendix A) to remove erythrocytes from the sample and

all subsequent determinations were made on the basis of plasma.

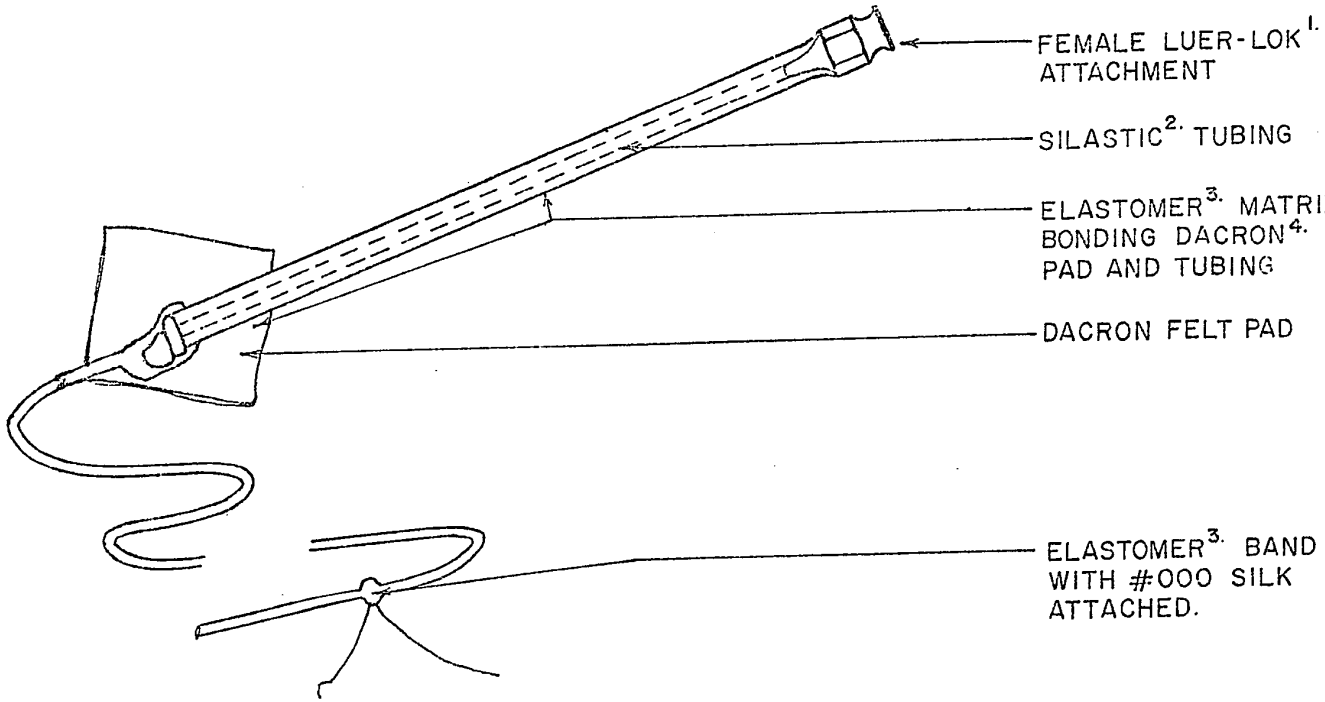
Surgical preparation of sows for chronic studies of placental ^{35}S transport in utero

The techniques used were adapted to the sow from those described for the ewe by Willes et al. (1970).

All sows were fasted overnight (approximately 16 hours) prior to surgery. Immediately prior to surgery the sow was clipped from the abdominal midline to the dorsal midline on the left side, leaving an area over the flank of about 60 cm width which was free of hair. Atropine sulfate (1.8mg/animal) was administered intramuscularly, at least 30 minutes prior to induction of anesthesia, to reduce salivation. The sow was thoroughly scrubbed with a hexachlorophrene containing soap (pHisohex, Winthrop Laboratories, Aurora, Ontario) and disinfected with a 5% Dettol solution (Reckitt and Coleman Canada Ltd., Montreal, Quebec). Anesthesia was induced by intravenous administration of Thiopental to effect and maintained with halothane following endotrachial intubation. The sow was placed in left lateral recumbancy on the operating table and the entire flank area was disinfected with tincture of Zephiran chloride (Winthrop Laboratories, Aurora, Ontario). Aseptic procedures were strictly observed

throughout the surgery.

A 15-20 cm diagonal flank incision was made through the skin and the underlying tissues separated by blunt dissection to the peritoneum. The peritoneum was incised within a purse string suture of no. 0 chromic gut. The uterus was manipulated such that one rear leg of a fetus was positioned beneath the peritoneal incision. A 3 to 5 cm incision was made through the uterine wall and amniotic membranes, exposing the fetal rear leg. The saphenous vein was exposed and cannulated with silastic rubber tubing (Silastic Medical Grade Tubing, Dow-Corning Corporation, Midland, Mich.) of suitable diameter (0.76 mm ID) for the vein. Design of the cannulas are shown in Fig. 4. Sufficient cannula tubing was inserted so that the tip of the cannula lay in the area of the posterior vena cava of the fetus. The cannula was secured to the vein and the skin of the fetal leg with no. 00 silk sutures. The fetal leg incision was closed with interrupted silk sutures of no. 00 silk. The amniotic sac cannula (1.02 mm ID tubing) was looped around the fetal leg and secured with several no. 00 silk sutures. The leg was repositioned within the amniotic sac and the membranes sutured with no. 00 chromic gut (continuous suture line). The uterine incision was closed with inverted horizontal mattress sutures of no. 00 chromic gut. The peritoneum was



1 Leave approximately 5 cm exterior to skin incision
2 Inner cannula tube of suitable diameter for vein with outer tube (0.32 x 0.64 mm) to provide mechanical strength
3 Used for bonding components together
4 Dacron pad rapidly becomes secured by tissue infiltration to provide a secure anchor for the cannula and a mechanical barrier to infection

Fig. 4. Construction of Cannulas. From Knipfel et al. (1973)

closed with simple interrupted no. 1 chromic gut sutures. Approximately 15 cm of cannula tubing was left within the uterus and an additional 20 cm within the abdominal cavity. The cannulas were passed subcutaneously to the lumbar region of the sow and exteriorized. All skin incisions were closed with vertical mattress sutures using no. 0 silk.

The saphenous vein of the sow was cannulated in a manner identical to that described for the fetus.

A variety of exteriorization techniques were employed. Sows prior to number 7 (Table 2.2) had cannulas exteriorized by the method of Willes et al. (1970). Sow 7 had cannulas exteriorized through a stab wound in the skin. The cannulas were placed in a plastic bag which in turn was inside a leather bag sutured to the skin of the sow. The plastic bag was kept filled with Zephiran tincture allowing the cannulas to be submerged when not in use. In later studies exteriorization was accomplished as described by Withey et al. (1973).

Cannulas were fitted with sterile 3-way stopcocks (Travenol Laboratories, Inc., Morton Grove, Ill.). The sow was injected with 300,000 I.U. Benzathine Penicillin G and 300,000 I.U. Procaine Penicillin G (Derapen, Ayerst Laboratories, Montreal, Quebec) intramuscularly.

Cannulas were flushed daily with sterile physiological

saline solution containing 10 units/ml of Heparin. Prior to flushing, administration of test solutions, or withdrawl of samples, the stopcocks were thoroughly flushed with Zephiran tincture.

Sows were separately housed after surgery. Experiments were not conducted on the chronic preparations until the day following surgery. The sow was allowed free access to feed and water immediately following surgery up until the performance of each experiment. During the course of the experiment, the animal was confined in a 0.9 x 1.5 m cage, to facilitate collection of blood samples. The post surgical performance of all preparations is indicated in Table 2.2.

Placental transport of ^{35}S following intravenous injection of ^{35}S-methionine (1.6 mg/kg) to cannulated sow-fetal preparations

Objectives:

1. To study the extent of placental transport of ^{35}S following intravenous injection of ^{35}S-methionine to the unanesthetized sow.

2. To study the rate of appearance of ^{35}S in amniotic fluid and the rate of disappearance of ^{35}S from fetal and maternal plasma following intravenous injection of ^{35}S-methionine.

3. To compare maternal and fetal blood plasma free amino acid concentrations.

TABLE 2.2 Post Surgical Performance of Cannulated Sow-Fetal Preparations

Sow Number	Sow Type	Post Surgical Duration of Pregnancy	Gestational Age (days) at Surgery	Comments
1.	Mini	24 hours	110	Aborted? Fetuses appeared normal.
2.	Mini	48 hours	102	Aborted.
3.	Mini	24 hours	106	Aborted.
4.	Mini	16 days	95	Fetuses too small to cannulate.
5.	Mini	acute study	100	Conducted at MacDonald College.
6.	Mini	24 hours	104	Aborted.
7.	Mini	6 days	102	Sow tore out cannulas on day 5. Aborted 24 hours later, likely due to infection after apparatus destruction.
8.	Mini	-	105	Sow died during surgery-pneumonia.
9.	Mini	72 hours	108	Aborted.
10.	Cross bred commercial	7 days	100	Gave progesterone - sow died from toxemia. Fetuses dead.
11.	Cross bred commercial	16 hours	102	Aborted? Infection of fetus through cannulas? Remainder of litter born alive.
12.	Cross bred commercial	48 hours	104	Aborted.

Procedures:

Twenty-four hours after surgery, each of three sows was injected with 1.6 mg/kg L-methionine containing 0.1 mCi ^{35}S-methionine via the maternal saphenous vein cannula. Two of the animals were mini-sows, the other a commercial cross-bred sow. Blood samples were collected from mother and fetus at the intervals indicated in Fig. 5. Amniotic fluid samples were collected at the same intervals from one preparation. Total ^{35}S in maternal and fetal plasma and amniotic fluid were determined as described in Appendix A. Amino acid analyses were undertaken using standard procedures (Appendix A). No radioactivity appeared in amniotic fluid during the test period, therefore no further analyses of amniotic fluid were undertaken.

Mean values for amino acid concentration and ^{35}S activity in maternal and fetal blood were calculated with standard errors (Steele and Torrie, 1962); ^{35}S data were plotted, and the ratio of fetal concentration to maternal concentration for the free amino acids calculated.

In an additional experiment to determine rate of clearance of ^{35}S from fetal blood, 3.7 mg/kg L-methionine (20 μCi ^{35}S-methionine) was injected into the fetal circulation via the fetal saphenous vein cannula in sow number 7. Blood samples were obtained at the intervals indicated in Table 2.3. Total ^{35}S activity was determined for each sample by usual tech-

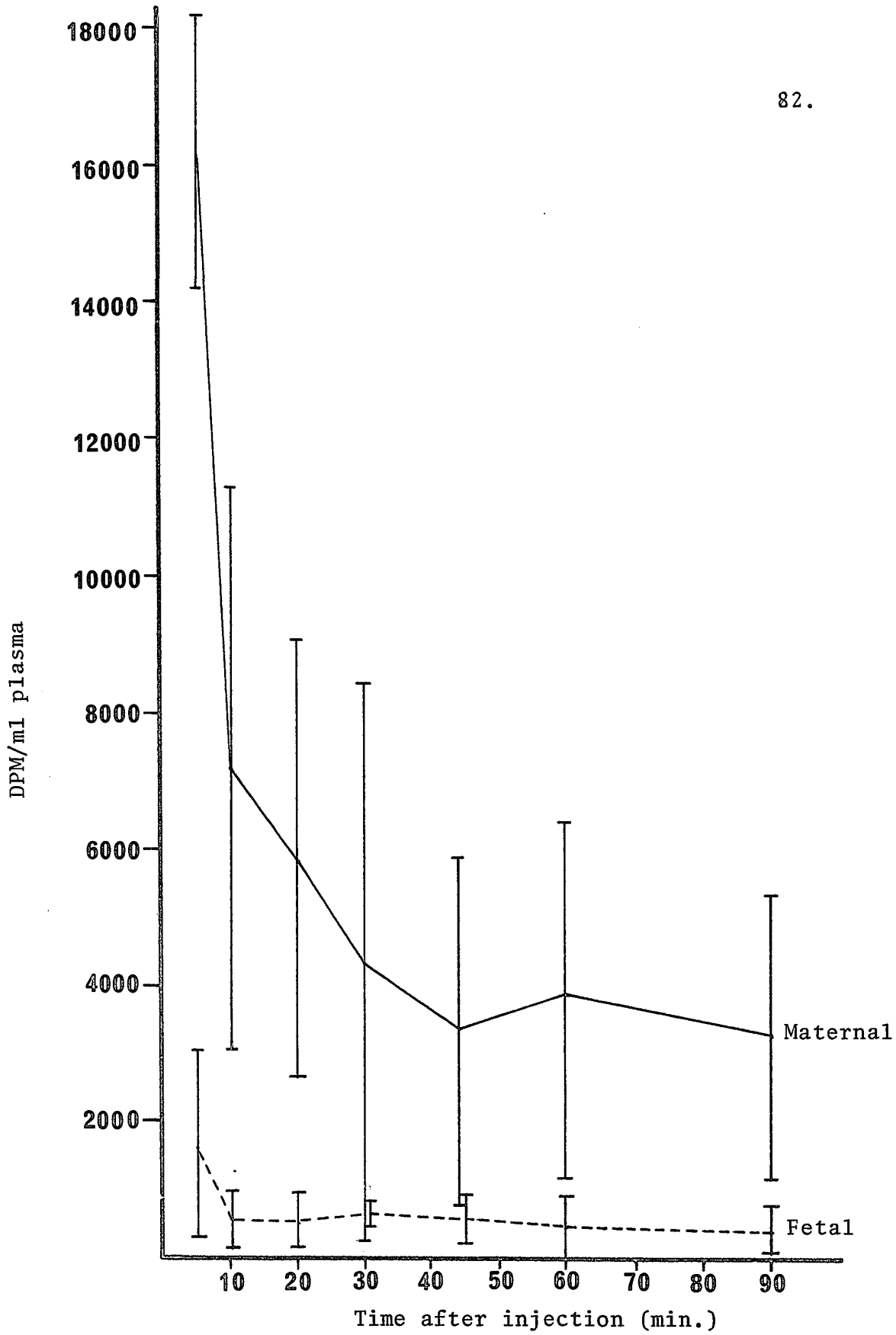


Fig. 5. Maternal and Fetal Plasma ^{35}S Activity Following Intravenous Injection of ^{35}S-Methionine (1.6mg/kg) to Sow

TABLE 2.3 ^{35}S Activity in Fetal Blood Plasma Following Intravenous Injection of ^{35}S-Methionine (3.7 mg/kg) to Fetus

Time after Injection (min.)	^{35}S Activity (DPM/ml blood)
5	180,742
10	36,971
15	50,208
20	28,048
30	38,115
45	39,359
60	37,717

niques (Appendix A). Maternal blood samples were unobtainable due to cannula blockage.

Results:

In spite of the size differences between the mini-sows and the commercial sow, the ^{35}S and plasma amino acid concentrations did not differ markedly. Thus the data were pooled to facilitate presentation. As observed in the previous study, the levels of ^{35}S attained in the fetal blood were considerably lower than those attained in the sow (Fig. 5). In addition, the decrease in fetal blood ^{35}S level with time after intravenous injection appeared to be less than that observed for the sow (Fig. 5) from 10 minutes post injection to the end of the experiment. This observation may have indicated a reduced ability of the fetus to clear the accumulated ^{35}S from its blood by tissue uptake or excretion relative to that of the sow. When the fetus was injected intravenously with ^{35}S-methionine (Table 2.3) the highest ^{35}S level was observed at 5 minutes post injection, likely as a result of incomplete dilution by the fetal blood and/or incomplete flushing of the cannula following administration of the dose. The fetal plasma ^{35}S level did not change markedly from that at 10 minutes post injection during the remainder of the study indicating slow clearance from fetal plasma. The suggestion of a slower clearance of ^{35}S from

fetal blood following maternal injection would appear to have been confirmed by fetal injection results.

Plasma amino acid levels in the sow exhibited only minor changes following intravenous injection of methionine (Table 2.4). A tendency for the concentration of most amino acids to decrease somewhat with time may have occurred but this effect was not marked. The plasma methionine level in the sow increased from the pre-injection level to 5 minutes post injection (Table 2.4) but had returned to pre-injection levels by later sampling times.

There was not a noticable tendency toward changes in fetal plasma amino acid levels with time (Table 2.5). The fetal plasma methionine level did not change noticably with time. This gave a further indication of limited transfer of methionine across the placenta to the fetus as a result of a small transient increase in maternal blood methionine level.

F/M ratios for plasma amino acid levels indicated that not all amino acids were present in higher concentrations in fetal blood than in maternal blood. A characteristic ratio appeared to exist for each amino acid (Table 2.6). The concentrations of several of the essential amino acids (valine, isoleucine, leucine, phenylalanine) were consistently lower in fetal than in maternal plasma. Only threonine and lysine concentrations remained higher in fetal than maternal plasma. Concentrations of non-essential amino acids

TABLE 2.4 Free amino acid concentrations in sow plasma following intravenous injection of ^{35}S-methionine (1.6 mg/kg) into sow

Amino Acid	Time After Injection (min.) 0	5	10
	(concentrations in µM/100 ml)		
Asp	1.1	T[1]	1.6
Thr	14.4 ± 1.6(3)[2]	12.3 ± 0.3(2)	13.2 ± 2.5(3)
Ser	–[3]	–	–
Glu	11.0 ± 1.1(3)	11.7 ± 2.1(2)	9.9 ± 1.8(3)
Pro	31.5 ± 9.9(2)	34.4 ± 25.2(2)	30.4 ± 22.9(2)
Gly	42.3 ± 24.3(3)	34.1 ± 22.1(2)	38.4 ± 18.1(3)
Ala	42.7 ± 12.0(3)	49.8 ± 16.6(2)	36.4 ± 11.2(3)
Val	31.3 ± 14.5(3)	36.5 ± 16.0(2)	28.4 ± 14.3(3)
Cys	5.5	T	4.8
Met	4.4 ± 0.4(3)	11.0 ± 4.4(2)	5.3 ± 2.2(3)
Ileu	14.4 ± 4.1(3)	13.6 ± 6.9(2)	13.0 ± 6.2(3)
Leu	23.1 ± 8.8(3)	22.3 ± 11.2(2)	21.5 ± 11.8(3)
Tyr	6.8 ± 1.3(3)	4.3 ± 0.3(2)	6.5 ± 1.6(2)
Phe	7.9 ± 0.7(3)	5.5 ± 1.3(2)	8.0 ± 1.2(2)
Lys	20.3	*[4]	24.4
His	3.3	*	6.6
Arg	9.2	*	9.4
Orn	10.3	*	7.9
Cit	4.7	*	3.8

[1] Trace present

[2] Mean ± standard error. Number in brackets indicates number of observations.

[3] Unable to quantitate due to poor resolution on amino acid analyzer.

[4] Insufficient sample for analysis.

. . . continued

TABLE 2.4 Free amino acid concentrations in sow plasma following intravenous injection of ^{35}S-methionine (1.6 mg/kg) into sow (continued)

Amino Acid	Time After Injection (min.) 20	30	45
	(concentrations in μM/100 ml)		
Asp	1.4	1.5	1.6
Thr	14.0 ± 2.3(3)	12.8 ± 1.5(3)	13.1 ± 1.5(3)
Ser	—	—	—
Glu	9.9 ± 2.6(3)	8.8 ± 0.9(3)	10.2 ± 2.4(3)
Pro	27.8 ± 20.2(2)	20.7 ± 11.6(2)	24.9 ± 17.0(2)
Gly	37.9 ± 18.0(3)	33.9 ± 19.8(3)	36.0 ± 20.6(3)
Ala	36.0 ± 11.0(3)	31.9 ± 7.8(3)	34.2 ± 13.1(3)
Val	32.0 ± 16.2(3)	24.0 ± 6.5(3)	30.0 ± 13.0(3)
Cys	4.8	4.5	5.4
Met	4.9 ± 2.3(3)	3.0 ± 0.8(2)	4.0 ± 1.2(3)
Ileu	12.8 ± 6.5(3)	10.9 ± 2.0(3)	12.6 ± 4.1(3)
Leu	21.0 ± 12.1(3)	16.5 ± 3.6(3)	18.7 ± 9.2(3)
Tyr	7.0 ± 1.1(3)	5.7 ± 0.8(3)	6.2 ± 0.2(3)
Phe	9.3 ± 4.1(2)	6.6 ± 0.6(2)	7.7 ± 0.8(3)
Lys	29.0	22.1	19.9
His	5.1	2.5	T
Arg	8.6	T	7.2
Orn	9.7	7.1	7.1
Cit	3.5	3.5	4.0

[1] Trace present

[2] Mean ± standard error. Number in brackets indicates number of observations.

[3] Unable to quantitate due to poor resolution on amino acid analyzer.

[4] Insufficient sample for analysis.

. . . continued

TABLE 2.4 Free amino acid concentrations in sow plasma following intravenous injection of ^{35}S-methionine (1.6 mg/kg) into sow (continued)

Amino Acid	Time After Injection (min.) 60	90
	(concentrations in µM/100 ml)	
Asp	1.5	2.4
Thr	13.1 ± 1.7(3)	12.6 ± 0.5(3)
Ser	—	—
Glu	10.9 ± 4.0(3)	8.1 ± 1.7(3)
Pro	23.3 ± 12.7(2)	17.8 ± 12.1(2)
Gly	31.4 ± 17.8(3)	29.2 ± 16.5(3)
Ala	36.3 ± 21.9(3)	28.2 ± 12.8(3)
Val	27.9 ± 14.1(3)	26.1 ± 9.1(3)
Cys	2.3	4.2
Met	3.4 ± 0.5(3)	3.0 ± 1.3(3)
Ileu	11.3 ± 3.1(3)	11.8 ± 4.4(3)
Leu	18.6 ± 8.0(3)	22.5 ± 14.0(3)
Tyr	6.5 ± 1.1(3)	6.0 ± 0.3(2)
Phe	8.9 ± 2.2(3)	8.0 ± 0.3(2)
Lys	16.4	13.3
His	T	6.5
Arg	8.0	6.4
Orn	8.9	6.2
Cit	5.9	3.2

[1] Trace present

[2] Mean ± standard error. Number in brackets indicates number of observations.

[3] Unable to quantitate due to poor resolution on amino acid analyzer.

[4] Insufficient sample for analysis.

TABLE 2.5 Free amino acid concentrations in fetal plasma following intravenous injection of ^{35}S-methionine (1.6 mg/kg) into sow

Amino Acid	Time After Injection (min.) 0	5	10
	(concentrations in μM/100 ml)		
Thr	44.3 ± 20.2(3)[1]	27.4	37.0 ± 2.8(2)
Ser	–[2]	–	–
Glu	59.7 ± 42.2(3)	47.7	66.5
Pro	29.8 ± 8.6(3)	19.6	28.4
Gly	78.2 ± 21.5(3)	53.2	85.8 ± 7.7(2)
Ala	55.0 ± 17.8(3)	41.2	52.3 ± 4.6(2)
Val	22.0 ± 12.9(3)	12.5	22.5 ± 11.3(2)
Cys	T[3]	T	T
Met	3.9 ± 0.3(3)	4.2	5.4
Ileu	5.1 ± 2.5(3)	3.1	3.6
Leu	9.1 ± 5.1(3)	4.9	10.3 ± 5.8(2)
Tyr	6.3 ± 3.6(3)	3.6	7.2 ± 0.4(2)
Phe	3.4 ± 1.1(3)	2.6	4.5 ± 1.1(2)
Lys	30.9	*[4]	25.4
His	3.8	*	T
Arg	20.5	*	T
Orn	18.5	*	9.7
Cit	T	*	T

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Unable to quantitate due to poor resolution on amino acid analyzer.

[3] Trace present

[4] Insufficient sample for analysis.

. . . continued

TABLE 2.5 Free amino acid concentrations in fetal plasma following intravenous injection of ^{35}S-methionine (1.6 mg/kg) into sow (continued)

Amino Acid	Time After Injection (min.) 20	30	45
	(concentrations in μM/100 ml)		
Thr	47.2 ± 17.4(2)	39.0 ± 6.2(2)	38.5 ± 14.0(2)
Ser	—	—	—
Glu	45.6 ± 20.9(2)	38.0 ± 30.5(2)	39.8 ± 0.3(2)
Pro	27.7 ± 0.9(2)	26.2 ± 5.9(2)	29.0 ± 16.5(2)
Gly	77.0 ± 17.7(2)	72.7 ± 18.6(2)	68.5 ± 26.7(2)
Ala	65.8 ± 23.6(2)	47.8 ± 6.5(2)	55.0 ± 25.5(2)
Val	22.4 ± 12.2(2)	17.6 ± 6.9(2)	19.1 ± 10.7(2)
Cys	T	T	T
Met	3.8 ± 1.1(2)	3.6 ± 2.5(2)	4.0 ± 0.9(2)
Ileu	5.7 ± 3.5(2)	4.9 ± 1.5(2)	5.9 ± 3.6(2)
Leu	12.3 ± 8.8(2)	9.1 ± 4.0(2)	10.8 ± 8.4(2)
Tyr	9.4 ± 6.4(2)	8.5 ± 3.0(2)	8.4 ± 5.2(2)
Phe	3.9 ± 1.3(2)	3.7 ± 0.6(2)	5.5 ± 3.2(2)
Lys	31.7	28.4	32.2
His	T	T	5.7
Arg	17.8	19.6	20.9
Orn	13.5	13.4	21.8
Cit	T	T	T

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Unable to quantitate due to poor resolution on amino acid analyzer.

[3] Trace present

[4] Insufficient sample for analysis.

. . . continued

TABLE 2.5 Free amino acid concentrations in fetal plasma following intravenous injection of ^{35}S-methionine (1.6 mg/kg) into sow (continued)

Amino Acid	Time After Injection (min.) 60	90
	(concentrations in μM/100 ml)	
Thr	40.6 ± 8.7(2)	44.0 ± 20.3(2)
Ser	—	—
Glu	47.5 ± 10.5(2)	39.6 ± 5.2(2)
Pro	24.8 ± 11.3(2)	24.4 ± 5.8(2)
Gly	83.0 ± 5.4(2)	67.6 ± 25.8(2)
Ala	54.6 ± 18.9(2)	52.3 ± 19.3(2)
Val	29.8 ± 3.7(2)	10.6
Cys		T
Met	4.0 ± 0.0(2)	3.5
Ileu	5.7 ± 2.8(2)	2.9
Leu	6.2 ± 0.1(2)	10.5 ± 7.4(2)
Tyr	7.8 ± 3.8(2)	7.8 ± 3.4
Phe	4.5 ± 1.8(2)	3.9 ± 1.7
Lys	29.7	42.1
His	8.0	7.5
Arg	19.5	15.3
Orn	19.3	15.7
Cit	T	T

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Unable to quantitate due to poor resolution on amino acid analyzer.

[3] Trace present

[4] Insufficient sample for analysis.

TABLE 2.6 Mean F/M ratios for plasma amino acid concentrations following intravenous injection of ^{35}S-methionine (1.6 mg/kg) into sow

Amino Acid	Time After Injection (min.)							
	0	5	10	20	30	45	60	90
Thr	3.1	2.2	2.8	3.4	3.0	2.9	3.1	3.5
Glu	5.4	4.1	6.7	4.6	4.3	3.9	4.4	4.9
Pro	1.0	0.6	0.9	1.0	1.3	1.2	1.1	1.4
Gly	1.8	1.6	2.2	2.0	2.1	1.9	2.6	2.3
Ala	1.3	0.8	1.4	1.8	1.5	1.6	1.5	1.9
Val	0.7	0.3	0.8	0.7	0.7	0.6	1.1	0.4
Met	0.9	0.4	1.0	0.8	1.2	1.0	1.2	1.2
Ileu	0.4	0.2	0.3	0.4	0.4	0.5	0.5	0.2
Leu	0.4	0.2	0.5	0.6	0.6	0.6	0.3	0.5
Tyr	0.9	0.8	1.1	1.3	1.5	1.4	1.2	1.3
Phe	0.4	0.5	0.6	0.4	0.6	0.7	0.5	0.5
Lys	1.5	–[1]	1.0	1.1	1.3	1.6	1.8	3.2
His	0.8	–	–	–	–	–	–	1.2
Arg	2.2	–	–	2.1	–	2.9	2.4	2.4
Orn	1.8	–	1.2	1.4	1.9	3.1	2.2	2.5

[1] Unable to calculate

were generally higher in fetal than maternal plasma (Table 2.6).

No radioactivity was detected in amniotic fluid during the experimental period, thus no further analyses of this fluid were carried out in future experiments. This observation indicated that the fetus did not dispose of its blood ^{35}S by excretion into amniotic fluid over the experimental period studied and that decreases in fetal blood methionine concentration during the period must have involved mechanism(s) other than excretion into the amniotic fluid.

Placental transport of ^{35}S following intragastric introduction of ^{35}S-methionine (5.5 mg/kg) into the sow

Objectives:

1. To attempt to increase the duration and extent of elevation of maternal blood ^{35}S level by introduction of the dose into the stomach of the sow.

2. To assess the extent of ^{35}S transport to the fetus following maternal intragastric introduction.

Procedures:

A gastric fistula similar to that used in studies with the ewe (Fig. 6) was implanted in addition to the cannulas. Two hundred μCi of ^{35}S-methionine (5.5 mg/kg) were introduced into the sow via the fistula. Blood samples were obtained

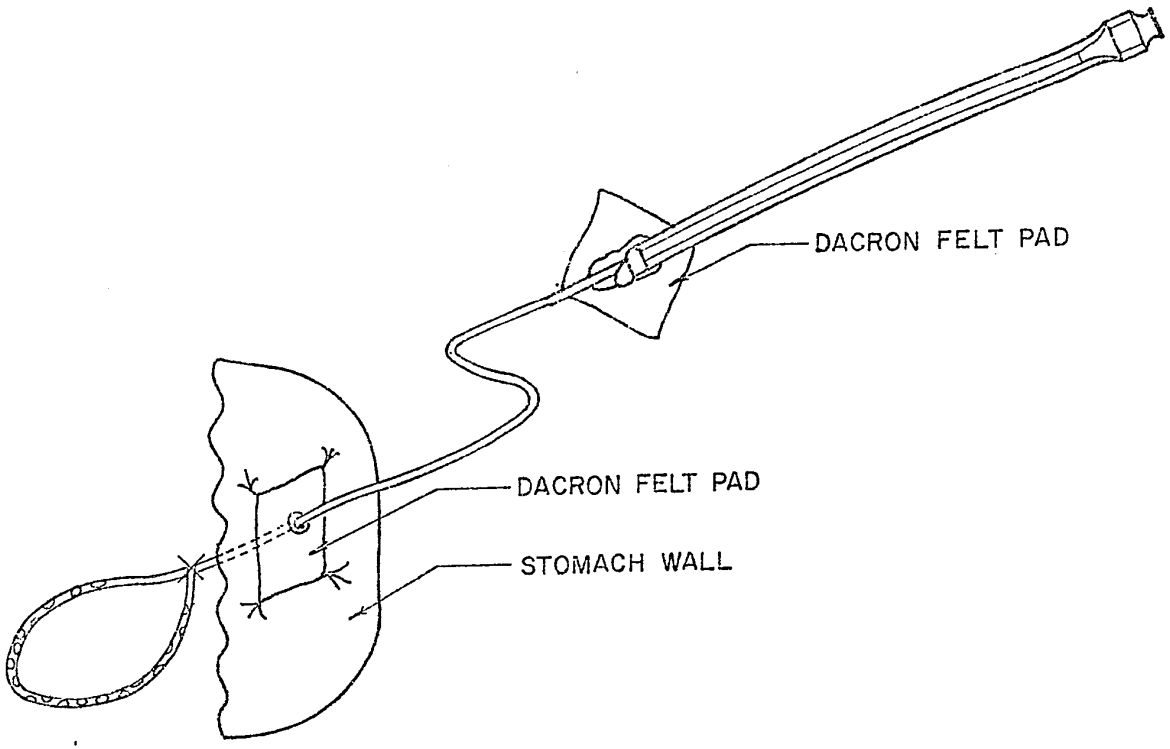


Fig. 6. Construction and Insertion of Gastric or Abomasal Fistula. From Knipfel et al. (1973).

at intervals indicated in Table 2.7. Total ^{35}S activity was determined by standard methods (Appendix A).

Results:

Only a small amount of ^{35}S appeared in maternal blood after intragastric introduction of ^{35}S-methionine (Table 2.7). The highest ^{35}S activity was detected 60 minutes post injection in maternal plasma, indicating a sustained period of absorption of ^{35}S from the gastrointestinal tract. The fetal blood ^{35}S level was considerably lower than that observed in the sow (Table 2.7) at all times studied and showed considerable fluctuation between sampling times. This variation may have been partially due to the low activity in the samples resulting in less accurate estimates of radioactivity. The dose of labelled methionine given was obviously of insufficient magnitude to result in pronounced elevations of maternal and fetal blood ^{35}S levels. This may have been to a large extent the result of maternal liver uptake. In order to increase the blood ^{35}S level following an intragastric dose of methionine, a sufficiently large methionine load, to overcome the ability of the liver and gastrointestinal tract to take up the label, must be given.

TABLE 2.7 Distribution of ^{35}S Between the Commercial Sow and its Fetus Following Intragastric Introduction of ^{35}S-Methionine (5.5 mg/kg) into Sow

Time after Injection (min.)	Sow	Fetus
	(Activity in DPM/ml blood)	
0	0	0
5	2392	387
10	1582	517
20	1268	157
30	1182	387
45	1682	147
60	7412	166
90	1852	326
120	2582	326

Discussion of Swine Studies

Following intravenous injection of ^{35}S-methionine into the sow, only a small proportion of the injected ^{35}S was transferred to the fetal circulation. A similar situation occurred when the dose was given intraarterially in the acute preparation. Intragastric introduction resulted in even less of the administered dose being transported to the fetus. The plasma methionine level in the fetus did not exhibit any tendency to increase following maternal intravenous injection. These findings were perhaps a consequence of dose dilution by maternal tissue uptake and by maternal blood. The maternal liver was observed to contain an extremely high level of ^{35}S relative to the maternal blood level following intraarterial injection of the dose, which implicated the liver as an important site for removal of the administered dose from the circulation. When the dose was given intragastrically both maternal and fetal blood levels of ^{35}S remained relatively low, possibly as a result of retention of ^{35}S by the gastric mucosa and passage of the absorbed ^{35}S through the liver prior to entering the peripheral blood. Elwyn et al. (1968) observed that perfusion of the liver with a solution designed to increase the plasma methionine concentration 2 to 8 fold over base-line values resulted in liver uptake of approximately 80% of the methionine in the perfusate within 90 minutes. In

the present studies, a single injection of methionine caused an apparently extensive liver uptake of maternal plasma methionine resulting in a rapid return to baseline values. There would be little stimulus for placental transport of methionine caused by an increase in maternal plasma methionine level in this instance.

Homeostatic mechanisms in the maternal system may act as an efficient buffer to regulate the supply of methionine available to the fetus, by maintaining the maternal blood methionine concentration at close to normal levels in spite of fluctuations in supply of methionine reaching the maternal blood. This effect would likely occur through interchange of methionine between blood and tissues, although the role of excretion should not be overlooked. While the results of the present study are inconclusive, others (Kerr et al., 1968) have suggested that the placenta functions as a regulatory or metering system for transfer of amino acids to the fetus. The extent of placental regulation of the fetal methionine level is as yet unclear.

Sturman et al. (1971) reported that intravenous injection of ^{35}S-methionine into pregnant Rhesus monkeys caused an accumulation of ^{35}S in fetal blood to approximately 3 times that of maternal values. Kerr (1968) observed fetal methionine concentrations twice those of maternal blood for Rhesus monkeys, while other workers have shown F/M ratios close to

1 for methionine in several species (page 19). In the present investigations the F/M ratio for methionine (Table 2.6) varied to some extent but appeared to favor the fetus slightly in most cases.

Hopkins et al. (1971b) indicated that the extent of transport of amino acids across the placenta of the sheep depended upon the particular amino acid injected intravenously into the ewe. For amino acids belonging to the A preferring system (Oxender and Christensen, 1963), little change in fetal blood concentration occurred when maternal levels were increased. Amino acids of the L preferring system readily crossed the placenta and increased fetal levels when the maternal level was raised. Methionine has a high affinity for both systems (Oxender and Christensen, 1963) thus an intermediate effect might have been expected. In present studies with the sow the effects observed for the ewe by the above workers were not seen.

Hopkins et al. (1971b) suggested that the placental membranes transported amino acids from maternal to fetal blood at a relatively constant rate, regardless of maternal blood level. Transport systems for amino acids across biological membranes have been shown (Christensen, 1969) to exhibit kinetic behavior similar in many respects to those of enzymic reactions. Placental membranes have been

suggested (Curet, 1970) to function in a manner similar to other membranes. The contention of Hopkins et al. (1971b) would therefore be true only if the maternal plasma amino acid level was sufficiently high as to saturate the transport mechanism and reach Vmax. At maternal blood levels which were not sufficiently elevated to saturate the transport process, the transport velocity would be less and a smaller proportion of the administered dose would be transferred to the fetus over a given period of time. In such a case, dilution of injected methionine by maternal blood, and the proportion of maternal tissue uptake might be expected to be higher. Together these factors may have resulted in a condition where an injection of methionine which would not elevate maternal plasma methionine level to any great extent or maintain an increased level for any substantial period of time would not stimulate transfer of methionine across the placenta to any significant degree. The results of studies with swine reported herein would support such a proposal.

In the investigation of Sturman et al. (1971) approximately 10 mg/kg of ^{35}S-methionine was injected into the Rhesus monkey whereas in the present swine studies a level of 1.6 mg/kg was employed. The apparent contradiction in the data of Sturman et al. (1971) and the present study would be resolved if a threshold effect occurred, above which ^{35}S-

methionine was rapidly transported to the fetus, and below which transfer was much less. The "threshold" hypothesized would be the ability of the maternal system to regulate the blood methionine level within physiological limits. Above this threshold methionine would accumulate rapidly in the maternal blood and be transferred to the fetus at a rapid rate. The overall transfer of methionine to the fetus would then be dependent upon the duration of the elevated maternal blood level. Below the threshold level the amount of methionine transported to the fetus would be a consequence of the relative velocity of the placental uptake process at any particular time. The net effect of such a scheme would be that the placenta may act as the primary regulator of fetal blood methionine levels under normal conditions. In situations where the homeostatic mechanisms of the mother have been overwhelmed by the administration of a large amount of methionine, the maternal plasma level may become the primary regulatory factor in determining fetal blood methionine concentrations. This hypothesis has been examined further in studies with the ewe reported herein.

Concentrations of ^{35}S which accumulated in fetal blood following maternal intravenous injection of ^{35}S-methionine appeared to decrease more slowly than did the maternal level with time after injection. Intravenous injection of the fetus with ^{35}S-methionine confirmed that the fetus reduced

its plasma ^{35}S level at a slow rate. A similar observation was reported by Sturman et al. (1971) for the Rhesus monkey. Mechanisms by which the fetus would remove the accumulated ^{35}S from blood are unknown. Liver uptake by the fetus as a means of removal of ^{35}S from blood did not appear to be of great significance (Table 2.1). Dancis et al. (1968) reported that fetal liver uptake of ^{14}C-AIB was much lower than that of the maternal liver in studies with guinea pigs. Excretion into amniotic fluid did not occur over the experimental periods studied herein, suggesting that if excretion into the amniotic fluid was a major route of elimination of ^{35}S from fetal plasma, such excretion did not occur rapidly. Several authors (Sturman et al., 1971; Dancis et al., 1968) have suggested a slow transfer of amino acids from fetus to maternal blood, thus this route may have been of limited importance in ^{35}S clearance. In most species little activity of the enzymes concerned with methionine degradation has been observed prior to birth (Sturman et al., 1970; Volpe and Laster, 1972) thus fetal metabolism of methionine was not likely a major route of removal of ^{35}S methionine from fetal blood in these studies.

Regardless of possible mechanisms of clearance of methionine from fetal blood, the observation that the fetal blood ^{35}S decreased more slowly than maternal implied that an increase in fetal blood methionine level would be main-

tained for a prolonged period of time, and that if this level was elevated to a toxic range then the fetus would be exposed to a potentially hazardous regimen for an extended period of time.

F/M ratios for plasma amino acids indicated that fetal plasma did not accumulate all amino acids to higher levels than observed in maternal plasma. Those amino acids belonging to the L preferring system of Oxender and Christensen (1963) ie. valine, isoleucine, leucine, phenylalanine appeared to be present in lower concentrations in fetal plasma than in maternal, while those of the A preferring group (alanine, glycine, threonine, proline) were at higher concentrations in fetal blood. These results may be interpreted in light of the predisposition of the L system for exchange while the A system is apparently a strictly uphill transport system. In contrast, other studies have shown higher concentration of almost all amino acids in the fetal plasma of several species (page 19). No explanation can be given for the present results.

Post-operative abortion in these surgical preparations was very common (Table 2.1). A further 7 commercial sows were later subjected to the cannulation procedures and all aborted. The sow at this point was abandoned as a model for chronic in utero studies due to its apparent poor tolerance of the surgical procedures. Perhaps the timing of the surgery was inappropriate since the sow was approaching term, however,

fetal size at earlier periods of gestation made cannulation impractical.

The data obtained in these studies were in themselves insufficient to draw firm conclusions. A variety of technical difficulties were overcome as a result of these investigations, however, and several avenues for additional research were opened. Several of these avenues were explored in greater depth in studies with the ewe as an experimental model. Results of these studies are reported in the following section.

Studies with Ewes

The pregnant ewe has been extensively used as a model to study placental transport. Procedures for the chronic *in utero* cannulation of the ovine fetus have been developed by Willes et al. (1970) and Mellor and Slater (1971). The pregnant ewe as an experimental model in the present study appeared to be advantageous from several points of view. Firstly, the ewe is a relatively easily handled animal. Secondly, the ewe appears to tolerate surgical manipulation and fetal venous cannulation as indicated by the above mentioned workers. Thirdly, venous cannulation of the ovine fetus during the last trimester of pregnancy would be relatively simple since the fetus is relatively large by this period of gestation.

A major disadvantage of the ewe in nutritional studies is that she is a ruminant and the validity of extrapolating data from this species to the monogastric may be open to some question. If the rumen was bypassed in administration of the nutrient, however, the ewe may react in a manner similar to that of the monogastric.

The ovine fetus appears to accumulate amino acids from the maternal circulation in much the same manner as do monogastric species, and there is little reason to suspect differences in transport characteristics.

Six crossbred ewes of various ages were surgically

prepared for *in utero* transport studies. The animals were housed on wood shavings, fed good quality mixed hay *ad libitum* and were given approximately 250 g oats daily. Water was provided *ad libitum*. The ewe was clipped closely 3 to 4 days prior to surgery and was fasted for a period of 40 to 48 hours prior to induction of anesthesia. Atropine sulfate (1.8 mg) was injected intramuscularly 30 minutes prior to anesthesia. Following induction with thiopental, anesthesia was maintained with halothane via endotrachial intubation. Strict aseptic procedure was followed throughout surgery. The surgical techniques employed were similar to those described for the sow (page 75) and by Willes et al. (1970). Post surgical care and handling was similar to that described for the sow (page 78).

The first ewe aborted before any experiments could be conducted. All preparations remained viable following experiments and eventually lambed normally. One ewe gave birth to a live lamb of normal weight immediately following the experiment.

Placental transport of ^{35}S following intravenous injection of ^{35}S-methionine into the ewe

Objectives:

1. To study transport of ^{35}S from maternal blood to fetal blood and amniotic fluid following intravenous injection

of ^{35}S-methionine at a level which would elevate maternal plasma methionine concentration to a limited extent.

2. To assess the effect of administering non-labelled carrier methionine on stimulation of transport of ^{35}S across the placenta.

3. To examine free amino acid concentrations in the maternal and fetal circulations and in amniotic fluid following intravenous injection of methionine to the ewe.

4. To determine the distribution of ^{35}S in metabolites of methionine in the ewe and fetus following ^{35}S-methionine injection to the ewe.

5. To confirm observations obtained with the sow.

Procedures:

Two ewes were prepared surgically as described on the previous page. Fortyeight hours postsurgery one ewe was given 100 μCi ^{35}S-L-methionine (3.3 mg/kg) intravenously via the maternal cannula. The remaining ewe was administered with 100 μCi ^{35}S-L-methionine (carrier-free, contained 28 μg L-methionine) intravenously. Samples of maternal and fetal blood and amniotic fluid were collected at the time intervals indicated in Fig. 7. Total ^{35}S activity in each sample was determined by liquid scintillation spectrometry (Appendix A). Amino acid analyses to determine free amino acid concentrations and distribution of ^{35}S in methionine

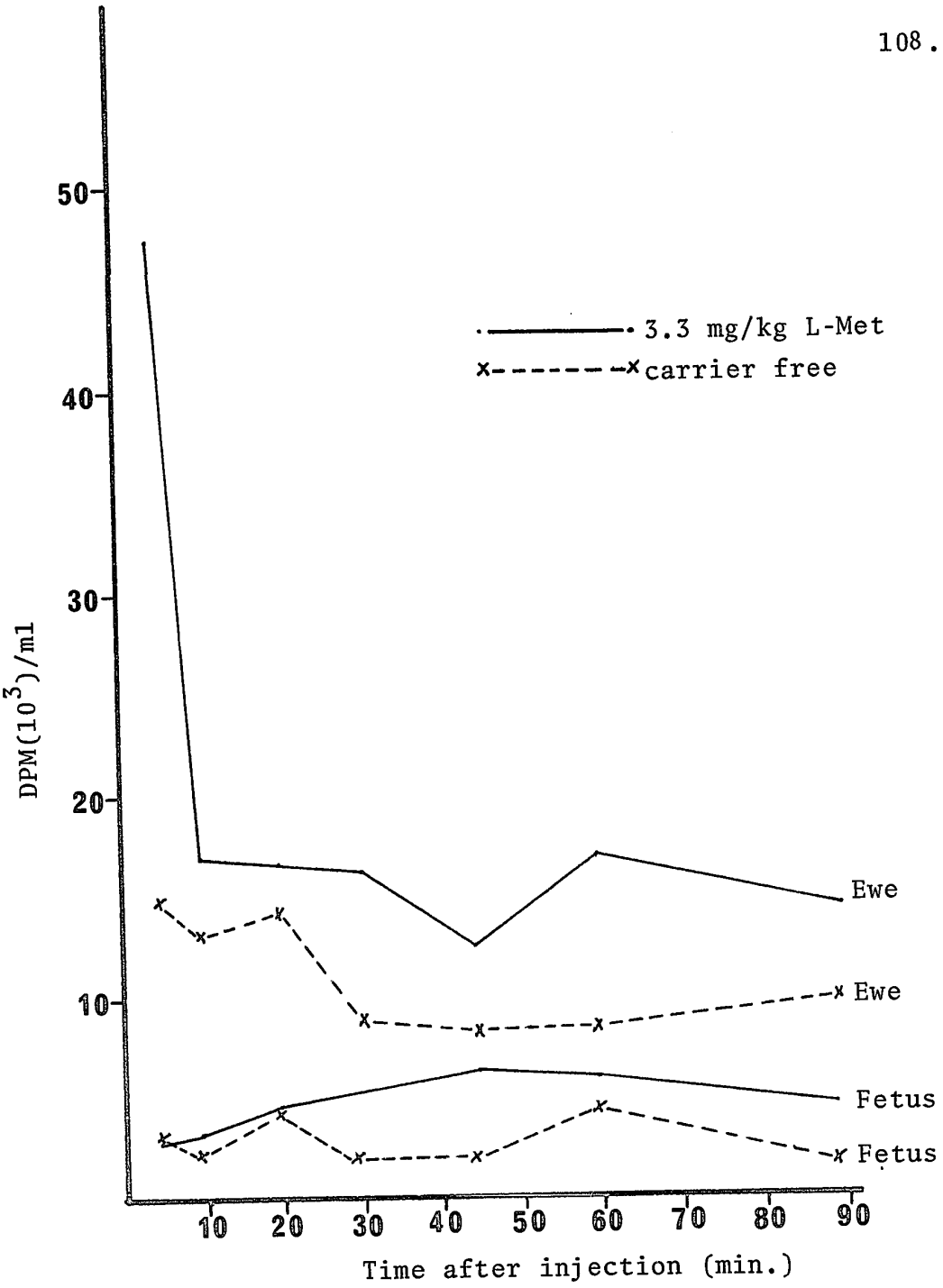


Fig. 7. Distribution of ^{35}S Between Maternal and Fetal Plasma Following Intravenous Injection of ^{35}S-Methionine into Ewe

and its metabolites were undertaken using an amino acid analyzer - liquid scintillation spectrometer (Appendix A). Amniotic fluid from the ewe given carrier free ^{35}S methionine was analyzed for free amino acid concentrations by standard procedure (Appendix A).

Results:

Following intravenous injection of 3.3 mg/kg L-methionine labelled with ^{35}S into the ewe, the maternal plasma ^{35}S level rapidly decreased over the first 10 minutes, then remained relatively constant for the duration of the experiment (Fig. 7). The fetal blood ^{35}S level tended to increase over the first 60 minutes of the test period, however, the change was not pronounced (Fig. 7). A relatively small proportion of the injected label was transferred to fetal blood and at no time during the study did the fetal ^{35}S activity approach the concentrations observed in the ewe's blood (Fig. 7). When carrier free ^{35}S-methionine was injected intravenously into the ewe the initial drop in ^{35}S activity in maternal blood over the first 10 minutes post injection was much less pronounced than was the case when carrier methionine was included in the dose given (Fig. 7). Fetal blood ^{35}S activity was considerably lower than that of the ewe and did not show any apparent tendencies to change with time after injection. However, the fact that ^{35}S was detectable in fetal blood

indicated that some transfer had occurred. No changes were apparent in the plasma amino acid concentrations of either maternal or fetal blood which could be attributed to the difference in amount of methionine injected, therefore plasma amino acid data for the two preparations were pooled and mean values presented in Tables 2.8 and 2.9. While changes in maternal plasma concentrations of a number of amino acids occurred during the test period (Table 2.8), these changes did not appear to follow any particular pattern. Fetal plasma amino acid concentrations also tended to fluctuate randomly (Table 2.9). In general each amino acid appeared to exhibit a characteristic ratio between the fetal and maternal blood (F/M ratio) although variation in the ratio occurred with time following injection of methionine (Table 2.10) for most amino acids. This variation was a result of changes in both maternal and fetal plasma concentrations (Tables 2.8, 2.9). In all cases plasma amino acid concentrations were higher in the fetus than in the ewe (Table 2.10).

There was no detectable ^{35}S activity observed in the amniotic fluid up to 90 minutes post administration. Concentrations of amino acids in amniotic fluid (Table 2.11) did not resemble either the maternal or fetal plasma concentrations. Levels of some amino acids approached those of the ewe, while others were at concentrations close to those of the fetus. Cystine concentrations in amniotic fluid were considerably

TABLE 2.8 Free Amino Acid Concentrations in Ewe Plasma Following Intravenous Injection of ^{35}S-Methionine to Ewe

Amino Acid	Time After Injection (min.)							
	0	5	10	20	30	45	60	90
	(concentrations in μM/100 ml)							
Asp	1.4	1.4	1.1	2.9	0.9	1.1	1.3	1.0
Thr	2.1	2.1	3.3	5.3	2.9	3.3	3.2	3.5
Glu	6.0	4.7	3.8	4.2	4.3	4.3	3.6	4.2
Gly	16.0	16.7	12.4	13.1	12.5	12.3	13.6	13.7
Ala	5.2	4.5	3.9	4.1	4.2	4.5	4.9	3.8
Val	5.0	7.6	5.0	4.0	4.2	3.7	3.4	3.3
Cys	0.9	T[1]	T	T	1.2	T	T	0.7
Met	0.8	1.7	0.8	2.1	2.0	1.5	0.9	0.8
Ileu	3.1	4.7	2.9	3.3	2.7	2.9	2.7	2.6
Leu	4.2	6.2	3.9	4.0	3.3	3.4	3.9	3.1
Tyr	2.0	3.9	2.0	2.4	1.6	1.4	1.9	1.9
Phe	5.8	7.2	5.3	5.3	4.0	4.6	5.3	4.8
Cit	9.8	8.0	11.5	10.9	8.5	8.3	9.5	9.2

[1] Trace present

TABLE 2.9 Free Amino Acid Concentrations in Fetal Plasma Following Intravenous Injection of ^{35}S-Methionine to Ewe

Amino Acid	Time After Injection (min.) 0	5	10	20	30	45	60	90
	(concentrations in µM/100 ml)							
Asp	7.5	4.7	4.2	5.0	2.7	5.1	4.9	5.0
Thr	8.3	8.3	8.7	10.7	9.3	7.0	10.5	10.2
Glu	13.2	7.3	8.1	8.4	5.4	8.6	7.7	9.6
Gly	41.1	24.6	22.3	22.5	17.5	20.8	22.1	25.3
Ala	19.9	11.0	10.2	10.9	9.5	9.7	11.1	12.4
Val	18.0	15.7	17.7	16.5	13.0	16.9	17.7	14.4
Cys	4.5	3.9	3.7	3.7	1.6	5.2	4.4	7.8
Met	2.2	2.5	2.4	2.3	1.6	2.1	2.4	2.1
Ileu	5.5	4.7	4.0	4.1	3.7	3.3	5.0	5.3
Leu	9.5	7.1	5.9	6.3	5.3	5.2	6.8	7.2
Tyr	8.7	9.5	8.4	9.2	6.1	8.1	7.7	12.0
Phe	14.8	14.1	13.6	14.2	12.3	13.0	14.2	16.0
Cit	12.4	10.5	10.6	10.6	10.0	10.3	10.8	12.8

TABLE 2.10 F/M Ratios for Free Amino Acids Following Intravenous Injection of ^{35}S-Methionine to Ewe

Amino Acid	Time After Injection (min.)							
	0	5	10	20	30	45	60	90
Asp	5.4	3.4	3.8	1.7	3.0	4.7	3.8	5.0
Thr	4.0	4.0	2.6	2.0	3.2	2.1	3.3	2.9
Glu	2.2	1.6	2.1	2.0	1.3	2.0	2.1	2.3
Gly	2.6	1.5	1.8	1.7	1.4	1.7	1.6	1.9
Ala	3.8	2.5	2.6	2.7	2.3	2.2	2.3	3.3
Val	3.6	2.1	3.5	4.1	3.1	4.6	5.2	4.4
Cys	5.0	–[1]	–	–	1.3	–	–	11.2
Met	2.8	1.5	3.0	1.1	0.8	1.4	2.7	2.6
Ileu	1.8	1.0	1.4	1.3	1.4	1.1	1.9	2.0
Leu	2.3	1.2	1.5	1.6	1.6	1.5	1.8	2.3
Tyr	4.4	2.4	4.2	3.8	3.8	5.8	4.1	6.3
Phe	2.6	2.0	2.6	2.7	3.1	2.8	2.7	3.3
Cit	1.3	1.3	0.93	1.0	1.2	1.2	1.1	1.4

[1] Unable to calculate

TABLE 2.11 Free Amino Acid Concentrations in Amniotic Fluid Following Intravenous Injection of ^{35}S-Methionine to Ewe

Amino Acid	Time After Injection (min.) 0	5	10	20	30	45	60	90
	(concentrations in µM/100 ml)							
Asp	3.5	T[1]	T	T	T	T	T	T
Thr	13.2	12.7	9.9	10.4	5.3	6.9	5.6	6.0
Glu	5.6	10.0	8.1	7.5	9.1	10.8	10.0	10.2
Gly	19.7	26.0	22.0	23.6	24.6	27.7	24.1	25.3
Ala	10.8	17.3	14.3	16.4	17.3	18.0	15.7	15.5
Val	1.8	1.6	T	T	T	1.2	1.3	1.4
Cys	25.8	26.4	17.3	13.2	14.4	24.5	21.4	16.9
Met	1.6	1.3	0.9	1.0	1.1	1.3	1.0	0.8
Ileu	T	3.0	2.8	2.4	2.8	3.1	2.8	2.8
Leu	T	5.7	5.1	4.5	5.1	5.8	5.4	4.7
Tyr	T	4.6	3.3	3.3	4.0	4.5	4.5	4.7
Phe	T	5.9	–[2]	5.2	5.5	5.6	5.3	5.4
Cit	9.5	11.1	8.9	8.6	10.1	12.6	10.2	10.4

[1] Trace present

[2] Amino acid analyzer malfunction - no data

higher than those of either maternal or fetal blood. The data from intravenous injection of low doses of methionine to the ewe indicated that under conditions of normal maternal plasma methionine concentrations the overall transport to the fetus was relatively small. A similar observation was made in studies with the sow discussed previously. The amount of ^{35}S transported to the fetus was higher when carrier methionine was administered with the label, suggesting that transfer of ^{35}S to the fetus was dependent upon the maternal methionine concentration to some degree.

Methionine has been shown (Meister, 1965) to be very active in metabolism, thus question arises as to whether the ^{35}S activity at early times post injection represented the same compound(s) as did the activity at later times. No estimates of this possibility could be made in these studies as the level of ^{35}S was insufficient to allow determination of radioactivity in metabolites by the combined amino acid analyzer - liquid scintillation counter (Appendix A). Further investigation of this area appears warranted.

The low proportion of ^{35}S transport observed in these studies confirmed the data from studies with sows (page 91) given small doses of ^{35}S-methionine intravenously. The question of a "threshold" in placental transport remained unanswered.

In order to establish the validity of the "threshold"

suggested earlier, amounts of methionine would have to be administered which would overwhelm the homeostatic capabilities of the ewe to regulate her blood methionine level within physiological limits. Methionine was found to be soluble only to the extent of about 50 mg/ml in aqueous solution. The volume of fluid required for injection of much larger doses than given previously would, under intravenous injection, be impractical from a single dose standpoint. Therefore in subsequent studies abomasal dosing was adopted to allow administration of fluid volumes of 100 ml or more over a minimum time interval.

An additional advantage of abomasal injection was that the duration of exposure of the maternal system to a high level of methionine might be prolonged as a result of absorption of the dose over an extended period of time.

Placental transport of ^{35}S following abomasal injection of ^{35}S-methionine into the ewe

Objectives:

1. To study transport of ^{35}S from maternal to fetal system following abomasal injection of ^{35}S-methionine at a level comparable to that suggested therapeutically for oral intake.

2. To establish the effects of a sustained elevation of the maternal blood methionine concentration on the

distribution of ^{35}S-containing metabolites in the blood plasma of the ewe and fetus and in amniotic fluid.

3. To study the effects of elevation of the maternal blood methionine level concentrations of amino acids in maternal and fetal blood plasma and amniotic fluid.

Procedures:

A. Three ewes were surgically prepared as previously outlined with the exception that an abomasal fistula, as shown in Fig. 6, was included in each preparation. The ewe was allowed 48 hours to recover from surgical stress before any experiments were conducted. Each study involved the abomasal injection of 160 mg/kg L-methionine into the ewe. In the first preparation studied 180 μCi ^{35}S-methionine was given with the carrier methionine injection. Samples of maternal blood, fetal blood, and amniotic fluid were obtained at intervals over a four hour period immediately following administration of the test dose. Additional samples were taken 24, 51 and 96 hours after injection. Sample preparation, amino acid analyses and ^{35}S determination were carried out by usual procedures (Appendix A).

The second ewe was given 1 mCi ^{35}S-methionine with the carrier load in order to elevate the levels of ^{35}S in the body fluids to a greater extent than occurred in previous experiments. Following abomasal injection, samples of maternal and fetal blood and amniotic fluid were collected

at 30 minute intervals for 6.5 hours. An additional sample of these fluids was taken 13 hours after injection of the dose into the ewe. Parturition occurred between 13 and 29 hours after methionine was injected. Samples of blood were obtained from the ewe and lamb at 29 and 52 hours after the methionine injection. ^{35}S activity in these samples was determined (Appendix A) and amino acid analyses of maternal and fetal plasma were undertaken (Appendix A).

The third ewe was given 1 mCi ^{35}S-methionine with the carrier methionine and samples of maternal blood, fetal blood and amniotic fluid taken at 2 hour intervals from time of injection for a 10 hour period. Additional samples of each fluid were collected 19, 21 and 24 hours after injection. ^{35}S activity in the various fluids, distribution of ^{35}S in metabolites and amino acid analyses were performed as described in Appendix A. Changes in total ^{35}S activity were expressed as percentages of maternal ^{35}S levels for fetal and amniotic fluid in order that differences in actual ^{35}S dose given would not confound the results. These data were plotted against time after maternal abomasal injection. Free ^{35}S activity in methionine and its metabolites in fetal plasma and amniotic fluid were plotted as percentages of maternal plasma activities. Specific activities of free methionine and its metabolites (DPM/μM) in maternal and fetal blood and amniotic fluid were plotted.

B. One ewe was anesthetized by subdural injection of 5 ml 2% Xylocaine (Lidocaine HCl, Pharmaceutical Division, Astra, Mississauga, Ont.). An abomasal fistula was implanted as described earlier (Fig. 6). The ewe was injected daily with 160 mg/kg L-methionine at 0800 hours. A blood sample was obtained by jugular puncture from the ewe immediately prior to the daily methionine injection. Following 6 days on this regimen, the maternal jugular vein was cannulated by insertion of tubing into the vein through a 14 gauge needle following jugular puncture. The needle was then withdrawn over the tubing leaving the cannula in place in the vein. The cannula was coupled to a stopcock by a 21 guage hypodermic needle. One mCi ^{35}S-methionine, in addition to the daily methionine dose, was introduced via the abomasal fistula. Blood samples were collected via the jugular cannula every 30 minutes for 360 minutes. The ewe was then anesthetized by intravenous injection of thiopental to effect, the fetus and amniotic sac were exposed and samples of maternal jugular blood, fetal venous blood (via heart puncture) and amniotic fluid at 390 minutes post-abomasal injection of methionine were obtained. The ewe was euthanized by pentobarbital overload. Analyses of blood and amniotic fluid samples were performed according to Appendix A to determine ^{35}S activities and free amino acid concentrations.

Where more than one observation was obtained at a

sampling interval the mean plus standard error was calculated (Steele and Torrie, 1960).

Results:

Effects of a single abomasal administration of methionine

Following abomasal injection of L-methionine (160 mg/kg) the maternal plasma methionine concentration increased to a level approximately 10 fold higher than the pre-dose level within 120 minutes and remained at that level for at least 390 minutes after the injection (Table 2.12). Fetal blood methionine began to increase between 30 and 60 minutes after injection and eventually reached levels considerably higher than those of maternal blood (Table 2.13). Elevated fetal plasma methionine concentrations were observed for at least 24 hours post administration (Table 2.13) even though the maternal level of plasma methionine had returned to a value approximating the preinjection concentration by 480 minutes post administration (Table 2.12). An initial decrease in the F/M ratio for methionine from preinjection to 30 minutes post injection occurred (Table 2.14) caused by the increase in the maternal plasma methionine concentration (Table 2.12) and little change in the fetal plasma methionine concentration (Table 2.13). From 30 minutes to 120 minutes, the fetal blood accumulated methionine at a more rapid rate than did that of the ewe, as shown by a progressive increase

TABLE 2.12 Free amino acid concentrations in maternal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe

Amino Acid	Time After Injection (min.) 0	30	60
	(concentrations in μM/100 ml)		
Asp	1.5 ± 1.9(2)[1]	T[2]	1.5[3]
Thr	5.4 ± 2.6(4)	3.4 ± 0.6(3)	4.2 ± 1.8(3)
Ser	10.0 ± 5.1(4)	9.3 ± 5.2(3)	10.3 ± 3.8(3)
Glu	4.7 ± 0.8(4)	5.7 ± 0.3(3)	8.1 ± 1.9(3)
Gly	19.7 ± 4.8(4)	21.9 ± 6.1(3)	26.0 ± 7.2(3)
Ala	10.0 ± 3.1(4)	11.1 ± 2.7(3)	12.3 ± 3.7(3)
Val	11.4 ± 2.1(4)	9.5 ± 1.2(3)	6.8 ± 1.4(3)
Cys	2.6 ± 0.8(4)	2.8 ± 0.5(2)	4.6
Met	3.5 ± 2.4(4)	14.1 ± 7.8(3)	26.0 ± 11.2(3)
Ileu	6.7 ± 1.5(4)	6.1 ± 1.4(3)	6.0 ± 2.8(3)
Leu	10.0 ± 1.7(4)	9.2 ± 3.6(3)	10.0 ± 5.2(3)
Tyr	3.2 ± 0.5(4)	3.1 ± 0.8(3)	2.5 ± 0.4(3)
Phe	7.4 ± 2.8(4)	9.0 ± 3.4(3)	11.1 ± 5.5(3)
Tau	9.6 ± 7.6(4)	9.9 ± 5.7(3)	11.8 ± 6.7(3)
Met Sulfox	4.0 ± 2.8(4)	1.6 ± 0.6(3)	5.5 ± 1.7(3)
Cit	10.8 ± 9.0(4)	10.1 ± 7.9(3)	12.3 ± 8.3(3)
AAB	2.1 ± 0.4(4)	3.5 ± 0.6(2)	2.1 ± 0.8(2)

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Trace present

[3] Single observation

. . . continued

TABLE 2.12 Free amino acid concentrations in maternal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.) 90	120	150
	(concentrations in μM/100 ml)		
Asp	1.1	1.4	T
Thr	4.6 ± 1.5(3)	4.9 ± 1.4(3)	3.5 ± 0.1(2)
Ser	12.1 ± 7.4(3)	10.1 ± 7.0(2)	10.9 ± 7.1(2)
Glu	7.7 ± 1.2(3)	4.4 ± 0.8(3)	5.0 ± 0.5(2)
Gly	25.3 ± 10.3(3)	20.4 ± 5.4(3)	20.9 ± 8.8(2)
Ala	10.9 ± 1.2(3)	9.9 ± 3.2(3)	9.7 ± 1.8(2)
Val	9.3 ± 2.9(3)	7.5 ± 1.5(3)	7.3 ± 1.9(2)
Cys	3.9 ± 1.8(2)	6.7 ± 4.2(3)	6.0 ± 0.4(2)
Met	27.2 ± 2.1(3)	35.6 ± 6.1(3)	42.5 ± 13.2(2)
Ileu	4.7 ± 2.7(3)	4.4 ± 0.8(3)	3.5 ± 0.5(2)
Leu	7.7 ± 5.2(3)	6.6 ± 1.2(3)	5.3 ± 0.4(2)
Tyr	2.7 ± 0.8(3)	2.8 ± 0.7(3)	2.1 ± 0.4(2)
Phe	9.8 ± 5.2(3)	7.3 ± 2.9(3)	7.4 ± 4.2(2)
Tau	13.1 ± 7.7(3)	11.0 ± 9.5(3)	13.1 ± 12.3(2)
Met Sulfox	6.5 ± 2.9(3)	5.8 ± 2.7(3)	7.3 ± 4.3(2)
Cit	12.0 ± 9.2(3)	9.7 ± 5.8(3)	12.3 ± 9.6(2)
AAB	1.6 ± 0.9(3)	1.7 ± 0.3(3)	1.5 ± 0.6(2)

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Trace present

[3] Single observation

. . . continued

TABLE 2.12 Free amino acid concentrations in maternal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.) 180	210	240
	(concentrationa in µM/100 ml)		
Asp	4.2 ± 4.1(2)	T	1.2 ± 0.3(2)
Thr	5.2 ± 3.9(3)	4.8 ± 1.7(2)	3.7 ± 0.7(4)
Ser	10.9 ± 1.4(3)	9.9 ± 4.6(2)	7.1 ± 2.0(3)
Glu	8.3 ± 2.4(3)	4.8 ± 0.0(2)	5.1 ± 1.4(4)
Gly	24.9 ± 6.0(3)	25.9 ± 7.8(2)	22.3 ± 4.5(4)
Ala	15.3 ± 6.1(3)	13.7 ± 6.6(2)	10.9 ± 5.6(4)
Val	10.0 ± 2.7(3)	7.0 ± 0.0(2)	7.2 ± 1.1(4)
Cys	6.3 ± 5.0(3)	4.8	5.4 ± 4.7(4)
Met	29.4 ± 15.7(3)	36.7 ± 9.2(2)	46.2 ± 33.3(4)
Ileu	5.8 ± 2.8(3)	3.9 ± 1.1(2)	3.3 ± 0.5(4)
Leu	8.5 ± 2.1(3)	5.8 ± 0.6(2)	5.3 ± 1.0(4)
Tyr	4.5 ± 2.9(3)	2.5 ± 0.6(2)	1.8 ± 0.4(4)
Phe	9.8 ± 4.2(3)	7.4 ± 4.4(2)	8.2 ± 2.4(4)
Tau	12.5 ± 7.5(3)	12.9 ± 10.3(2)	10.8 ± 8.0(4)
Met Sulfox	12.6 ± 8.2(3)	14.2 ± 11.5(2)	12.9 ± 8.5(4)
Cit	10.6 ± 8.7(3)	15.6 ± 13.7(2)	8.4 ± 5.8(4)
AAB	4.1 ± 2.9(3)	2.1	1.6 ± 0.4(4)

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Trace present

[3] Single observation

. . . continued

TABLE 2.12 Free amino acid concentrations in maternal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.) 270	300	330
	(concentrations in μM/100 ml)		
Asp	T	T	
Thr	3.5 ± 0.4(2)	3.4 ± 0.0(2)	2.9 ± 0.1(2)
Ser	5.4 ± 0.7(2)	5.0 ± 0.1(2)	5.7 ± 0.4(2)
Glu	5.3 ± 0.9(2)	5.6 ± 1.1(2)	4.4 ± 0.6(2)
Gly	24.0 ± 0.4(2)	23.3 ± 1.6(2)	20.1 ± 2.6(2)
Ala	14.9 ± 10.3(2)	13.0 ± 7.4(2)	13.1 ± 8.5(2)
Val	7.8 ± 1.0(2)	6.1 ± 0.6(2)	7.0 ± 0.3(2)
Cys	5.7 ± 1.6(2)	4.6 ± 2.5(2)	5.0 ± 1.5(2)
Met	36.9 ± 8.7(2)	30.0 ± 13.2(2)	29.7 ± 12.6(2)
Ileu	3.3 ± 0.5(2)	3.1 ± 0.6(2)	3.3 ± 0.2(2)
Leu	5.6 ± 0.1(2)	5.2 ± 0.3(2)	5.1 ± 0.1(2)
Tyr	2.2 ± 0.1(2)	1.9 ± 0.8(2)	2.1 ± 0.1(2)
Phe	6.4 ± 1.9(2)	6.2 ± 3.1(2)	5.9 ± 2.3(2)
Tau	12.7 ± 7.1(2)	10.9 ± 7.5(2)	11.3 ± 8.6(2)
Met Sulfox	18.6 ± 17.5(2)	16.5 ± 15.1(2)	15.6 ± 12.5(2)
Cit	10.6 ± 5.9(2)	11.9 ± 4.5(2)	10.3 ± 7.8(2)
AAB	1.1 ± 0.2(2)	1.8	1.5 ± 0.9(2)

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Trace present

[3] Single observation

. . . continued

TABLE 2.12 Free amino acid concentrations in maternal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.) 360	390
	(concentration in μM/100 ml)	
Asp	1.6 ± 1.6(2)	T
Thr	5.2 ± 4.1(3)	2.2 ± 0.8(2)
Ser	8.0 ± 4.5(3)	4.5 ± 0.1(2)
Glu	5.5 ± 3.4(3)	4.1 ± 0.9(2)
Gly	20.6 ± 4.0(3)	20.6 ± 2.9(2)
Ala	10.5 ± 6.1(3)	11.3 ± 6.9(2)
Val	6.5 ± 1.5(3)	7.0 ± 0.8(2)
Cys	4.5 ± 2.1(3)	5.5 ± 2.7(2)
Met	28.6 ± 14.1(3)	31.1 ± 7.0(2)
Ileu	3.2 ± 0.9(3)	3.1 ± 0.6(2)
Leu	5.2 ± 1.3(3)	5.3 ± 0.7(2)
Tyr	2.2 ± 0.6(3)	2.1 ± 1.1(2)
Phe	6.8 ± 1.1(3)	8.0 ± 2.8(2)
Tau	8.5 ± 8.1(3)	13.7 ± 12.1(2)
Met Sulfox	16.7 ± 8.7(3)	12.8 ± 9.5(2)
Cit	8.8 ± 5.5(3)	9.3 ± 6.4(2)
AAB	2.1 ± 1.5(3)	1.4 ± 1.0(2)

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Trace present

[3] Single observation

TABLE 2.12 Free Amino Acid Concentrations in Maternal Plasma Following Abomasal Injection of ^{35}S-Methionine (160 mg/kg) to Ewe (continued)

Amino Acid	Time After Injection (min.)					
	480	600	780	1140	1260	1440
	(concentrations in μM/100 ml)					
Asp	T[1]	3.4	T	T	T	T
Thr	3.7	2.9	2.6	3.0	3.8	3.7
Ser	16.8	12.3	9.3	9.6	12.2	9.4
Glu	3.1	2.5	3.7	2.9	3.3	2.4
Gly	17.7	16.2	12.4	21.2	11.3	17.3
Ala	6.1	4.4	14.9	4.7	5.1	4.3
Val	7.0	5.1	7.3	6.1	8.4	7.5
Cys	3.6	3.3	5.5	9.6	3.6	2.0
Met	10.9	8.1	9.4	3.5	2.6	1.5
Ileu	4.3	3.0	5.3	3.5	4.8	4.3
Leu	4.6	5.0	7.7	4.4	6.3	5.2
Tyr	2.7	2.2	2.7	2.0	3.8	2.5
Phe	7.1	8.0	6.8	7.4	8.1	6.0
Tau	2.3	2.0	2.4	3.3	4.5	4.5
Met Sulfox	22.1	17.8	11.3	4.8	4.9	3.0
Cit	8.7	6.8	5.6	8.5	10.9	7.9
AAB	1.3	2.2	1.9	1.5	1.8	0.7

[1] Trace present

TABLE 2.13 Free amino acid concentrations in fetal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe

Amino Acid	Time After Injection (min.) 0	30	60
	(concentrations in μM/100 ml)		
Asp	5.1 ± 1.0(2)[1]	4.2 ± 0.6(2)	4.6 ± 0.9(2)
Thr	14.4 ± 3.7(2)	20.1 ± 5.0(2)	22.1 ± 7.8(2)
Ser	112.5 ± 1.5(2)	107.2 ± 4.0(2)	110.0[2]
Glu	11.6 ± 4.7(2)	17.4 ± 13.5(2)	15.2 ± 7.7(2)
Gly	105.1 ± 53.5(2)	72.5 ± 2.2(2)	70.0 ± 11.3(2)
Ala	35.3 ± 9.7(2)	27.2 ± 4.7(2)	26.5 ± 0.4(2)
Val	18.6 ± 5.3(2)	18.1 ± 4.6(2)	19.0 ± 10.0(2)
Cys	23.8 ± 6.7(2)	21.3 ± 0.1(2)	22.2 ± 1.1(2)
Met	3.9 ± 1.5(2)	4.4 ± 3.9(2)	22.7 ± 8.9(2)
Ileu	4.6 ± 1.5(2)	6.0 ± 3.2(2)	5.8 ± 4.9(2)
Leu	10.6 ± 3.3(2)	11.3 ± 4.7(2)	13.3 ± 10.7(2)
Tyr	7.3 ± 3.1(2)	7.8 ± 3.7(2)	8.5 ± 3.9(2)
Phe	13.7 ± 9.4(2)	14.0 ± 10.0(2)	13.3 ± 7.9(2)
Tau	4.5 ± 3.2(2)	5.7 ± 4.3(2)	7.3 ± 5.8(2)
Met Sulfox	7.9 ± 0.9(2)	5.6 ± 1.8(2)	7.4 ± 1.3(2)
Cit	12.5 ± 4.4(2)	11.2 ± 1.3(2)	11.5 ± 1.9(2)
AAB	4.7 ± 4.1(2)	9.3 ± 3.2(2)	10.6 ± 4.9(2)

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Single observation

. . . continued

TABLE 2.13 Free amino acid concentrations in fetal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.) 90	120	150
	(concentrations in μM/100 ml)		
Asp	3.5	5.3 ± 2.5(3)	7.0
Thr	18.2	18.7 ± 1.8(3)	20.7
Ser	111.2	142.6 ± 3.9(3)	143.2
Glu	9.0	17.0 ± 6.7(3)	12.7
Gly	85.9	108.2 ± 53.3(3)	90.5
Ala	33.3	39.1 ± 12.2(3)	38.9
Val	13.9	22.3 ± 7.8(3)	14.2
Cys	26.3	27.7 ± 7.7(3)	30.7
Met	35.0	80.6 ± 81.9(3)	65.5
Ileu	2.4	7.1 ± 4.2(3)	2.8
Leu	6.7	14.4 ± 7.0(3)	6.8
Tyr	4.4	10.6 ± 4.4(3)	5.5
Phe	6.6	18.7 ± 11.0(3)	7.6
Tau	5.4	6.6 ± 4.9(3)	4.3
Met Sulfox	8.6	8.6 ± 3.1(3)	11.9
Cit	11.0	14.9 ± 2.3(3)	13.9
AAB	9.3	11.3 ± 1.9(3)	10.9

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Single observation

. . . continued

TABLE 2.13 Free amino acid concentrations in fetal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.) 180	210	240
	(concentrations in μM/100 ml)		
Asp	5.2	6.7	5.3 ± 0.5(3)
Thr	24.1 ± 4.6(2)	21.7	19.9 ± 7.7(3)
Ser	172.0	174.0	151.2 ± 20.9(2)
Glu	14.7 ± 0.5(2)	13.3	20.6 ± 7.8(3)
Gly	86.9 ± 12.6(2)	101.8	107.6 ± 28.9(3)
Ala	36.9 ± 10.0(2)	43.5	40.6 ± 7.0(3)
Val	18.4 ± 11.3(2)	16.8	19.1 ± 6.3(3)
Cys	26.4 ± 8.1(2)	32.6	27.6 ± 6.4(3)
Met	67.0 ± 21.7(2)	90.9	122.7 ± 60.3(3)
Ileu	4.0 ± 1.5(2)	4.3	5.7 ± 1.6(3)
Leu	9.1 ± 5.2(2)	8.7	7.7 ± 4.1(3)
Tyr	7.9 ± 4.1(2)	6.6	8.6 ± 2.1(3)
Phe	16.7 ± 12.3(2)	9.4	16.4 ± 6.5(3)
Tau	5.7 ± 3.0(2)	4.3	6.8 ± 5.1(3)
Met Sulfox	13.1 ± 2.8(2)	21.3	18.7 ± 8.6(3)
Cit	13.8 ± 1.8(2)	15.1	17.2 ± 2.6(3)
AAB	13.9 ± 2.5(2)	12.9	13.1 ± 2.3(3)

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Single observation

[3] Trace present

. . . continued

TABLE 2.13 Free amino acid concentrations in fetal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)		
	300	330	360
	(concentrations in μM/100 ml)		
Asp	4.6	5.7	5.1
Thr	20.8	22.2	17.3 ± 0.6(2)
Ser	176.0	0.5	136.6 ± 7.7(2)
Glu	16.3	13.1	19.8 ± 2.6(2)
Gly	104.2	95.9	113.3 ± 36.0(2)
Ala	45.3	40.7	48.9 ± 10.3(2)
Val	12.8	11.9	14.0 ± 5.0(2)
Cys	36.3	41.8	28.8 ± 9.6(2)
Met	145.2	171.4	164.2 ± 91.0(2)
Ileu	4.0	3.2	4.7
Leu	5.9	3.2	6.3 ± 0.6(2)
Tyr	6.3	7.9	8.4 ± 3.7(2)
Phe	9.4	11.3	14.4 ± 4.1(2)
Tau	4.7	6.2	7.1 ± 3.2(2)
Met Sulfox	25.6	28.9	29.7 ± 13.4(2)
Cit	16.0	13.9	17.3 ± 5.9(2)
AAB	13.5	12.3	13.9 ± 4.8(2)

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Single observation

[3] Trace present

. . . continued

TABLE 2.13 Free amino acid concentrations in fetal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.) 390	480	600	780
	(concentration in μM/100 ml)			
Asp	4.2	T[3]	5.6	T
Thr	16.1	21.0	21.7	10.8
Ser	118.2	158.8	155.6	80.2
Glu	13.6	21.9	18.9	24.0
Gly	76.2	163.4	151.7	50.0
Ala	39.7	59.0	57.6	56.8
Val	10.0	11.8	17.3	13.2
Cys	31.3	29.9	27.0	55.9
Met	97.6	282.5	271.3	110.0
Ileu	3.1	T	T	T
Leu	5.5	6.3	11.0	7.4
Tyr	5.2	12.1	12.0	6.4
Phe	7.6	20.2	21.7	8.1
Tau	4.1	3.9	3.8	7.1
Met Sulfox	31.7	60.1	64.3	33.4
Cit	11.5	28.4	29.8	10.6
AAB	10.4	22.7	23.0	6.1

[1] Mean ± standard error. Number in brackets indicates number of observations.

[2] Single observation

3 Trace present

. . . continued

TABLE 2.13 Free amino acid concentrations in fetal plasma following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)			
	1140	1260	1440	5760
	(concentrations in µM/100 ml)			
Asp	5.0	T	5.4	T
Thr	24.9	22.4	18.5	12.0
Ser	141.1	117.5	97.6	71.4
Glu	13.5	17.4	8.2	5.6
Gly	127.6	117.7	94.6	38.0
Ala	41.8	40.1	28.2	21.1
Val	18.7	25.1	20.5	26.9
Cys	28.7	24.3	22.7	2.6
Met	163.2	123.9	81.5	5.7
Ileu	T	11.2	12.6	9.2
Leu	6.4	10.7	6.7	10.2
Tyr	16.3	23.8	20.9	15.4
Phe	22.7	25.6	23.0	9.9
Tau	5.6	5.1	4.0	8.3
Met Sulfox	44.8	22.7	22.7	2.2
Cit	28.3	24.6	22.2	14.9
AAB	21.3	21.1	14.8	1.5

TABLE 2.14 Mean F/M ratios for free amino acids following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe

Amino Acid	Time After Injection (min.)						
	0	30	60	90	120	150	180
Asp	3.40	—[1]	3.07	3.19	3.79	—	1.24
Thr	2.67	5.92	5.27	3.96	3.82	5.92	4.64
Ser	11.25	11.53	10.68	9.19	14.12	13.14	15.78
Glu	2.47	3.06	1.88	1.17	3.87	2.54	1.78
Gly	5.34	3.31	2.70	3.40	5.31	4.33	3.49
Ala	3.53	2.45	2.16	3.06	3.95	4.01	2.42
Val	1.64	1.91	2.80	1.50	2.98	1.95	1.84
Cys	9.16	7.61	4.83	6.75	4.14	5.12	4.19
Met	1.12	0.32	0.88	1.29	2.27	1.55	2.28
Ileu	0.69	0.99	0.97	0.51	1.62	0.80	0.69
Leu	1.06	1.23	1.33	0.87	2.19	1.29	1.07
Tyr	2.29	2.52	3.40	1.63	3.79	2.62	1.76
Phe	1.86	1.56	1.20	0.67	2.57	1.03	1.71
Tau	0.47	0.58	0.62	0.42	0.60	0.33	0.46
Met Sulfox	1.98	3.50	1.35	1.33	1.49	1.63	1.04
Cit	1.16	1.11	0.94	0.92	1.54	1.13	1.31
AAB	2.24	2.66	5.05	5.82	6.65	7.27	3.39

[1]Unable to calculate

. . . continued

TABLE 2.14 Mean F/M ratios for free amino acids following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)						
	210	240	270	300	330	360	390
Asp	–[1]	4.42	–	–	–	3.19	–
Thr	4.52	5.38	–	6.12	7.66	3.33	7.32
Ser	17.58	21.30	–	35.20	–	17.08	26.27
Glu	2.77	4.04	–	2.91	2.98	3.60	3.32
Gly	3.93	4.83	–	4.48	4.78	5.50	3.70
Ala	3.18	3.73	–	3.49	3.11	4.66	3.52
Val	2.40	2.66	–	2.10	1.70	2.16	1.43
Cys	6.80	5.12	–	7.90	8.36	6.40	5.69
Met	2.48	2.66	–	4.84	5.78	5.75	3.14
Ileu	1.11	1.73	–	1.29	0.97	1.47	1.00
Leu	1.50	1.46	–	1.14	0.63	1.22	1.04
Tyr	2.64	4.78	–	3.32	3.77	3.82	2.48
Phe	1.27	2.00	–	1.52	1.92	2.12	0.95
Tau	0.34	0.63	–	0.44	0.55	0.84	0.30
Met Sulfox	1.50	1.45	–	1.56	1.86	1.78	2.48
Cit	0.97	2.05	–	1.35	1.35	1.97	1.24
AAB	6.15	8.19	–	7.50	8.20	6.62	7.43

[1] Unable to calculate

. . . continued

TABLE 2.14 Mean F/M ratios for free amino acids following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)						
	480	600	780	1140	1260	1440	5760
Asp	-[1]	1.65	-	-	-	-	-
Thr	5.68	7.49	4.16	8.30	5.90	5.00	3.34
Ser	9.46	12.65	21.68	14.70	9.64	10.39	7.14
Glu	7.07	7.56	2.58	4.66	5.28	3.42	0.99
Gly	9.24	9.37	4.04	6.02	10.42	5.47	2.29
Ala	9.68	13.09	3.82	8.90	7.87	6.56	3.20
Val	1.69	3.40	1.81	3.07	2.99	2.74	3.17
Cys	8.31	8.19	10.17	2.99	6.75	11.35	-
Met	25.92	33.50	11.71	46.63	47.66	54.34	1.79
Ileu	-	-	-	-	2.34	2.93	1.59
Leu	1.37	2.20	0.97	1.46	1.70	1.29	1.53
Tyr	4.49	5.46	2.37	8.15	6.27	8.36	5.31
Phe	2.85	2.72	1.20	3.07	3.16	3.84	3.96
Tau	1.70	1.90	0.58	1.70	1.14	0.89	1.00
Met Sulfox	2.72	3.62	2.96	9.34	4.64	7.57	1.47
Cit	3.27	4.39	1.90	3.33	2.26	2.81	1.64
AAB	17.47	10.46	3.21	14.20	11.73	21.15	-

[1] Unable to calculate

in the F/M ratio with time after injection (Table 2.14) to 120 minutes while the maternal plasma methionine concentration was also increasing. From 120 minutes to 390 minutes post injection, although the maternal plasma methionine concentration had plateaued (Table 2.12), that of the fetus continued to increase (Table 2.13) and resulted in a steadily increasing F/M ratio (Table 2.14). From 8 hours to 24 hours post injection the F/M ratio was extremely high (Table 2.14) as a result of a decrease in the maternal plasma methionine concentration to the preinjection level (Table 2.12) and a much less pronounced decrease in the fetal plasma methionine concentration (Table 2.13). No increase in the amniotic fluid methionine concentration was apparent until 6 hours after injection of the dose (Table 2.15) and did not reach levels equal to those of fetal blood during the experimental period. The F/A ratio for methionine (Table 2.16) rose as a result of increased fetal plasma methionine concentration (Table 2.13) and little change in amniotic fluid methionine concentration prior to 6 hours post injection (Table 2.15). At sampling times from 6 to 10 hours, the F/A ratio decreased as a result of increased amniotic fluid methionine concentrations (Table 2.15) and after 600 minutes some decrease in the fetal plasma methionine concentration (Table 2.13).

The A/M methionine ratio decreased sharply (Table 2.17) as a result of the increased maternal plasma methionine concen-

TABLE 2.15 Free amino acid concentrations in amniotic fluid following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe

Amino Acid	Time After Injection (min.) 0	60	90
	(concentrations in μM/100 ml)		
Asp	3.3[1]	3.3 ± 1.1(2)	3.6 ± 0.3(2)
Thr	12.7 ± 3.8(2)[2]	11.7 ± 0.8(2)	10.5 ± 0.6(2)
Ser	42.7 ± 5.4(2)	48.0	50.8
Glu	7.1 ± 0.8(2)	7.5 ± 0.7(2)	7.9 ± 0.1(2)
Gly	35.9 ± 11.9(2)	22.1 ± 3.3(2)	22.5 ± 6.9(2)
Ala	17.3 ± 5.6(2)	10.9 ± 1.3(2)	11.6 ± 3.1(2)
Val	6.0 ± 2.2(2)	3.8 ± 0.4(2)	2.9 ± 1.8(2)
Cys	32.2 ± 2.4(2)	18.5 ± 6.5(2)	18.1 ± 11.9(2)
Met	2.9	4.0 ± 1.8(2)	1.9 ± 0.7(2)
Ileu	2.5 ± 0.4(2)	1.9 ± 0.3(2)	1.4
Leu	5.1 ± 2.3(2)	3.9	2.1 ± 1.3(2)
Tyr	2.5 ± 0.4(2)	1.6 ± 0.2(2)	2.1 ± 0.3(2)
Phe	3.8 ± 0.8(2)	5.7 ± 1.9(2)	4.9 ± 1.8(2)
Tau	14.3 ± 3.5(2)	14.0 ± 6.9(2)	12.4 ± 4.5(2)
Met Sulfox	26.5 ± 11.1(2)	18.9 ± 7.6(2)	22.2 ± 9.2(2)
Cit	5.2 ± 1.5(2)	3.7 ± 0.2(2)	3.9 ± 2.1(2)
AAB	1.2 ± 0.9(2)	1.7	1.0 ± 0.3(2)

[1] Single observation

[2] Mean ± standard error. Number in brackets indicates number of observations.

[3] Trace present

. . . continued

TABLE 2.15 Free amino acid concentrations in amniotic fluid following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.) 90	120	150
	(concentrations in μM/100 ml)		
Asp	3.6	4.2 ± 0.6(3)	4.5
Thr	10.8	11.4 ± 1.5(3)	12.4
Ser	30.1	31.5 ± 10.5(3)	41.3
Glu	4.6	7.6 ± 1.7(3)	7.1
Gly	26.6	29.2 ± 14.0(3)	34.2
Ala	11.5	15.8 ± 5.6(3)	17.2
Val	3.6	5.2 ± 1.7(3)	3.5
Cys	16.8	24.6 ± 11.6(3)	27.6
Met	4.5	4.3 ± 2.7(3)	2.0
Ileu	T[3]	2.7 ± 1.3(3)	T
Leu	3.6	5.9 ± 2.1(3)	1.3
Tyr	1.9	2.4 ± 0.1(3)	1.9
Phe	5.7	5.8 ± 3.5(3)	3.2
Tau	18.8	11.3 ± 4.2(3)	16.0
Met Sulfox	21.9	19.0 ± 9.4(3)	27.3
Cit	3.4	4.2 ± 2.4(3)	5.5
AAB	1.2	1.0 ± 0.5(3)	1.1

[1] Single observation

[2] Mean ± standard error. Number in brackets indicates number of observations.

[3] Trace present

. . . continued

TABLE 2.15 Free amino acid concentrations in amniotic fluid following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.) 180	210	240
	(concentrations in μM/100 ml)		
Asp	4.3 ± 0.9(2)	T	5.3 ± 0.1(2)
Thr	11.8 ± 1.6(2)	10.7	8.9 ± 1.6(2)
Ser	45.0	23.0	11.0 ± 4.5(2)
Glu	8.1 ± 0.5(2)	7.0	7.9 ± 2.5(2)
Gly	28.1 ± 9.2(2)	32.7	40.6 ± 11.0(2)
Ala	13.1 ± 4.0(2)	16.3	15.7 ± 1.9(2)
Val	3.0 ± 0.5(2)	2.6	5.0
Cys	21.2 ± 11.6(2)	27.0	25.2 ± 4.2(2)
Met	3.4 ± 1.3(2)	3.2	6.0 ± 1.7(2)
Ileu	T	T	2.3
Leu	0.9	T	5.3
Tyr	1.9 ± 0.3(2)	1.3	1.7 ± 0.3(2)
Phe	6.1 ± 4.2(2)	2.9	3.4 ± 0.8(2)
Tau	12.9 ± 3.0(2)	14.8	14.9 ± 1.3(2)
Met Sulfox	21.2 ± 10.0(2)	26.3	20.2 ± 9.8(2)
Cit	3.7 ± 2.8(2)	4.2	4.9 ± 0.8(2)
AAB	1.4 ± 0.1(2)	0.8	1.3 ± 0.7(2)

[1] Single observation

[2] Mean ± standard error. Number in brackets indicates number of observations.

[3] Trace present

. . . continued

TABLE 2.15 Free amino acid concentrations in amniotic fluid following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)				
	270	300	330	360	390
	(concentrations in μM/100 ml)				
Asp	4.7	T	T	4.6 ± 0.4(2)	T
Thr	8.2	9.5	9.5	8.9 ± 1.7(2)	11.2
Ser	5.1	5.4	6.8	11.7 ± 7.0(2)	11.4
Glu	10.4	15.0	13.5	10.9 ± 3.0(2)	18.5
Gly	31.9	39.2	37.9	42.6 ± 1.1(2)	56.8
Ala	15.9	20.0	17.2	21.0 ± 5.4(2)	17.1
Val	T	1.3	T	6.1	T
Cys	27.4	28.0	25.8	26.6 ± 2.8(2)	28.1
Met	2.8	5.6	4.2	13.4 ± 10.5(2)	10.0
Ileu	T	T	T	3.2	T
Leu	T	T	T	5.2	T
Tyr	1.0	1.1	1.3	2.4 ± 1.2(2)	1.7
Phe	2.8	3.2	2.7	3.4 ± 0.2(2)	3.1
Tau	17.3	17.7	15.3	14.0 ± 0.8(2)	14.9
Met Sulfox	25.9	25.5	26.3	18.9 ± 3.4(2)	29.5
Cit	5.4	6.6	4.8	6.2 ± 1.5(2)	6.0
AAB	T	0.7	T	2.2	0.9

[1] Single observation

[2] Mean ± standard error. Number in brackets indicates number of observations.

[3] Trace present

. . . continued

TABLE 2.15 Free amino acid concentrations in amniotic fluid following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)				
	480	600	780	1140	1260
	(concentrations in μM/100 ml)				
Asp	5.6	4.0	T	6.2	5.0
Thr	10.8	7.5	12.3	12.5	11.8
Ser	18.9	11.1	11.0	18.6	17.6
Glu	7.3	7.5	22.8	5.6	8.3
Gly	71.0	56.9	49.8	10.7	10.5
Ala	23.1	19.9	33.5	28.3	28.3
Val	6.2	3.7	7.3	6.9	6.0
Cys	30.9	17.3	34.6	32.7	31.7
Met	19.1	16.7	22.9	29.5	27.3
Ileu	3.5	3.0	T	4.3	2.3
Leu	6.1	3.7	2.7	5.6	4.4
Tyr	3.0	2.3	3.6	4.1	3.6
Phe	4.4	5.0	6.3	4.6	5.1
Tau	14.2	11.9	12.3	14.6	15.7
Met Sulfox	20.7	26.5	36.1	41.5	40.2
Cit	7.0	5.7	5.6	6.1	5.7
AAB	2.3	1.8	4.9	4.8	4.3

TABLE 2.16 Mean F/A ratios for free amino acids following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe

Amino Acid	Time After Injection (min.)						
	0	30	60	90	120	150	180
Asp	1.55	1.28	1.28	0.98	1.27	1.56	1.21
Thr	1.14	1.72	2.11	1.69	1.64	1.67	2.05
Ser	2.64	2.24	2.17	3.70	4.53	3.47	3.83
Glu	1.64	2.32	1.93	1.96	2.24	1.79	1.82
Gly	2.93	3.28	3.12	3.23	3.71	2.65	3.10
Ala	2.04	2.50	2.29	2.90	2.48	2.27	2.82
Val	3.10	4.77	6.56	3.87	4.29	4.06	6.14
Cys	0.74	1.16	1.23	1.57	1.13	1.12	1.25
Met	1.35	1.10	11.95	7.78	18.75	32.8	19.71
Ileu	1.84	3.16	4.15	—[1]	2.63	—	—
Leu	2.08	2.90	6.34	1.87	2.44	5.23	10.12
Tyr	2.92	4.88	4.05	2.32	4.42	2.90	4.16
Phe	3.61	2.46	2.72	1.16	3.23	2.38	2.74
Tau	0.32	0.41	0.59	0.29	0.59	0.27	0.45
Met Sulfox	0.30	0.30	0.34	0.40	0.46	0.44	0.62
Cit	2.41	3.03	2.95	3.24	3.55	2.53	3.73
AAB	3.92	5.47	10.60	7.75	11.30	9.91	9.93

. . . continued

[1] Unable to calculate

TABLE 2.16 Mean F/A ratios for free amino acids following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)						
	210	240	270	300	330	360	390
Asp	–[1]	1.00	–	–	–	1.11	–
Thr	2.03	2.24	–	2.19	2.32	1.95	1.44
Ser	7.57	13.75	–	32.60	–	11.68	10.37
Glu	1.90	2.61	–	1.09	0.97	1.82	0.74
Gly	3.12	2.65	–	2.66	2.59	2.66	1.35
Ala	2.67	2.59	–	2.27	2.37	2.33	2.33
Val	6.47	3.82	–	9.85	–	2.30	–
Cys	1.21	1.10	–	1.30	0.81	1.09	1.12
Met	28.41	20.45	–	25.93	40.81	12.26	9.76
Ileu	–	2.48	–	–	–	1.47	–
Leu	–	1.46	–	–	–	1.22	–
Tyr	5.08	5.06	–	5.73	6.08	3.50	3.06
Phe	3.25	4.83	–	2.94	4.19	4.24	2.46
Tau	0.29	0.46	–	0.27	0.41	0.51	0.28
Met Sulfox	0.81	0.93	–	1.01	1.01	1.58	1.08
Cit	3.60	3.51	–	2.43	2.90	2.79	1.92
AAB	16.13	10.08	–	19.29	–	6.32	11.56

[1] Unable to calculate

. . . continued

TABLE 2.16 Mean F/A ratios for free amino acids following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)						
	480	600	780	1140	1260	1440	5760
Asp	_[1]	1.40	–	0.81	–		
Thr	1.95	2.90	0.88	2.00	1.90		
Ser	8.41	14.02	7.29	7.59	6.68		
Glu	3.00	2.52	1.06	2.41	2.10		
Gly	2.31	2.67	1.01	11.93	11.21		
Ala	2.56	2.90	1.70	1.48	1.42		
Val	1.91	4.68	1.89	2.71	4.19		
Cys	0.97	1.56	1.62	0.88	0.77		
Met	14.79	16.25	4.81	5.54	4.54		
Ileu	–	–	–	–	4.87		
Leu	1.04	2.98	2.74	1.15	2.44		
Tyr	4.04	5.22	1.78	3.98	6.62		
Phe	4.59	4.34	1.29	4.94	5.02		
Tau	0.28	0.32	0.58	0.39	0.33		
Met Sulfox	2.91	2.43	0.93	1.08	0.57		
Cit	4.06	5.23	1.90	4.64	4.32		
AAB	9.87	12.78	1.25	4.44	4.91		

[1] Unable to calculate

TABLE 2.17 Mean A/M ratios for free amino acids following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe

Amino Acid	Time After Injection (min.)						
	0	30	60	90	120	150	180
Asp	2.20	>1	2.40	3.27	3.00	>1	1.02
Thr	2.35	3.44	2.79	2.35	2.33	3.54	2.27
Ser	4.27	5.16	4.93	2.49	3.12	3.79	4.13
Glu	1.51	1.32	0.98	0.60	1.73	1.42	0.98
Gly	1.82	1.01	0.87	1.05	1.43	1.64	1.13
Ala	1.73	0.98	0.94	1.06	1.60	1.77	0.86
Val	0.53	0.40	0.43	0.39	0.69	0.48	0.30
Cys	12.42	6.61	3.93	4.31	3.67	4.60	3.37
Met	0.83	0.28	0.07	0.17	0.12	0.05	0.12
Ileu	0.37	0.31	0.23	<1	0.61	<1	<1
Leu	0.51	0.42	0.21	0.47	0.89	0.25	0.11
Tyr	0.78	0.52	0.84	0.71	0.86	0.90	0.42
Phe	0.51	0.63	0.44	0.58	0.79	0.43	0.62
Tau	1.49	1.41	1.05	1.44	1.03	1.22	1.03
Met Sulfox	6.63	11.81	4.04	3.37	3.28	3.74	1.68
Cit	0.48	0.37	0.32	0.28	0.43	0.45	0.35
AAB	0.57	0.49	0.48	0.75	0.59	0.73	0.34

. . . continued

TABLE 2.17 Mean A/M ratios for free amino acids following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)						
	210	240	270	300	330	360	390
Asp	–	4.42	>1	–	–	2.88	–
Thr	2.23	2.41	2.34	2.79	3.28	1.71	5.09
Ser	2.32	1.55	0.94	1.08	1.19	1.46	2.53
Glu	1.46	1.55	1.96	2.68	3.07	1.98	4.51
Gly	1.26	1.82	1.33	1.68	1.89	2.07	2.76
Ala	1.19	1.44	1.07	1.54	1.31	2.00	1.51
Val	0.37	0.69	<1	0.21	<1	0.94	<1
Cys	5.63	4.67	4.81	6.09	5.16	5.91	5.11
Met	0.09	0.13	0.08	0.19	0.14	0.47	0.32
Ileu	<1	0.70	<1	<1	<1	1.00	<1
Leu	<1	1.00	<1	<1	<1	1.00	<1
Tyr	0.52	0.94	0.45	0.58	0.62	1.09	0.81
Phe	0.39	0.41	0.44	0.52	0.46	0.50	0.39
Tau	1.15	1.38	1.36	1.62	1.35	1.65	1.09
Met Sulfox	1.85	1.57	1.39	1.55	1.69	1.13	2.30
Cit	0.27	0.58	0.51	0.55	0.47	0.70	0.65
AAB	0.38	0.81	<1	0.39	<1	1.05	0.64

. . . continued

TABLE 2.17 Mean A/M ratios for free amino acids following abomasal injection of ^{35}S-methionine (160 mg/kg) to ewe (continued)

Amino Acid	Time After Injection (min.)				
	480	600	780	1140	1260
Asp	>1	1.18	—	>1	>1
Thr	2.92	2.59	4.73	4.17	3.10
Ser	1.13	0.90	0.89	1.94	1.44
Glu	2.35	3.00	6.16	1.93	2.52
Gly	4.01	3.51	4.02	0.50	0.93
Ala	3.79	4.52	2.25	6.02	5.55
Val	0.89	0.73	1.00	1.13	0.71
Cys	8.58	5.24	6.29	3.41	8.81
Met	1.75	1.53	2.44	8.43	10.50
Ileu	0.81	1.00	<1	1.23	0.48
Leu	1.33	0.74	0.35	1.27	0.70
Tyr	1.11	1.05	1.33	2.05	0.95
Phe	0.62	0.63	0.93	0.62	0.63
Tau	6.17	5.95	5.13	4.42	3.49
Met Sulfox	0.94	1.49	3.19	8.65	8.20
Cit	0.80	0.84	1.00	0.71	0.52
AAB	1.77	0.82	2.58	3.20	2.39

tration following injection (Table 2.12). Increases in the A/M ratio occurring from 360 minutes to the end of the experimental period were a result of both a maternal plasma methionine decrease (Table 2.12) and an increase in amniotic fluid methionine concentration.

Methionine sulfoxide increased in maternal plasma within 3 hours following methionine injection (Table 2.12) and appeared to remain at an elevated level for 780 minutes. The fetal plasma concentration increase in methionine sulfoxide (Table 2.13) approximated that of the maternal during the first 3 hours post injection, but continued to increase to maximum concentrations from 8 to 10 hours after maternal injection. At later sampling intervals the fetal plasma methionine sulfoxide concentration appeared to decrease (Table 2.13), although relative to the preinjection concentration, the methionine sulfoxide concentration was still elevated 24 hours after injection. No pronounced changes in F/M ratios for methionine sulfoxide were observed (Table 2.14) although a tendency for an increase in the F/M ratio was apparent after approximately 6 hours post injection. Amniotic fluid methionine sulfoxide concentrations exhibited little tendency to increase until the later stages of the study ie. after 600 minutes post injection (Table 2.15). Initially the concentration of methionine sulfoxide was considerably higher in amniotic

fluid than fetal blood as indicated by the F/A ratio (Table 2.16), however, the gradual increase in fetal blood methionine sulfoxide with time after injection (Table 2.13) resulted in a steady increase of the F/A ratio to a value showing the fetal blood methionine sulfoxide concentration to be higher than that of amniotic fluid (Table 2.16). Between 10 and 24 hours after injection the F/A ratio decreased to a value approaching that of preinjection (Table 2.16). F/M ratios for methionine sulfoxide (Table 2.17) reflected increased maternal plasma concentrations (Table 2.15) following maternal injection of methionine, with the preinjection A/M ratio not re-established until between 780 and 1140 minutes after injection. The return to a high ratio was mainly a consequence of a return to close to preinjection methionine sulfoxide concentrations in ewe plasma (Table 2.12) but there appeared to be some increase in the amniotic fluid concentrations also (Table 2.15).

In maternal plasma no definite trends for fluctuations in taurine could be established; variation in the concentrations between animals was large (Table 2.12). In the fetal plasma (Table 2.13) and in amniotic fluid (Table 2.15) taurine concentrations fluctuated in a random manner. The F/M ratio for taurine may have increased to some extent at later sampling times (Table 2.14), however, the F/M ratios did not suggest taurine to be highly concentrated in fetal blood relative to

that of the ewe. Taurine distribution between fetal blood and amniotic fluid (Table 2.16) remained relatively constant with the amniotic fluid taurine concentration higher than that of the fetus. At earlier sampling intervals the A/M ratios for taurine indicated a somewhat higher amniotic fluid taurine concentration (Table 2.17). At later sampling times the ratio was much larger in favor of amniotic fluid (Table 2.17) primarily as a result of much lower maternal plasma taurine concentrations (Table 2.12).

Cysteine concentrations appeared to increase somewhat in maternal plasma following methionine injection (Table 2.12) but extremely high concentrations were not attained at any time. In fetal blood the plasma cysteine was considerably higher (Table 2.13) than that observed in maternal blood and may also have increased following maternal methionine injection. This increase in fetal plasma cysteine occurred over the first 5 to 6 hours post injection (Table 2.13). F/M ratios for cysteine (Table 2.14) fluctuated in a random manner but cysteine appeared to be highly concentrated in fetal blood relative to maternal. In amniotic fluid, cysteine concentrations did not show any definite trends (Table 2.15). Amniotic fluid and fetal plasma contained approximately equivalent concentrations of cysteine (Table 2.16), with only minor changes in the F/A ratios occurring with time after methionine injection. No definite pattern of behavior of the A/M ratios for cysteine

was established (Table 2.17).

The maternal plasma concentration of α-aminobutyric acid did not change markedly following methionine injection (Table 2.12). However, in fetal plasma the concentration of α-aminobutyric acid began to increase by 30 minutes post maternal injection (Table 2.13). Elevated fetal plasma α-aminobutyric acid concentrations were apparently maintained for 24 hours (Table 2.13). Some tendency for amniotic fluid α-aminobutyric acid concentration to increase late in the sampling period may have occurred (Table 2.15). The fetal plasma was characterized by a higher concentration of α-aminobutyric acid than maternal with the F/M ratio increasing throughout the experiment to very high values (Table 2.14). The F/A ratio increased considerably from the beginning of the study and maintained a high value for 780 minutes, following which a decrease in the F/A ratio (Table 2.16) occurred as a result of increased amniotic fluid α-aminobutyric acid concentrations (Table 2.15). The fetal plasma concentration was higher than that of amniotic fluid for α-aminobutyric acid at all sampling intervals (Table 2.16). α-Aminobutyric acid concentrations in amniotic fluid were initially lower than those of ewe plasma (Table 2.17) and gradually increased to levels higher than the ewe as a result of amniotic fluid concentration increases (Table 2.15).

An apparent increase in the plasma concentration of citrulline of the fetus occurred following methionine injection

(Table 2.13) which was not accompanied by changes in either maternal plasma (Table 2.12) or amniotic fluid (Table 2.15) concentrations.

The concentration of serine decreased markedly in amniotic fluid between 210 and 240 minutes post injection (Table 2.15) and remained low for the remainder of the experimental period. A similar tendency may have occurred for maternal plasma serine (Table 2.12). Conversely, the fetal plasma serine concentration appeared to increase after maternal methionine injection (Table 2.13).

Concentrations of most other amino acids in the maternal plasma (Table 2.12), fetal plasma (Table 2.13), and in amniotic fluid (Table 2.15) fluctuated randomly during the sampling period.

Most other amino acids were present at higher concentrations in fetal than in maternal plasma as indicated by F/M ratios greater than 1 (Table 2.14). Each amino acid appeared to have a characteristic F/M ratio which was subject to considerable variation. This variation was a result of both maternal (Table 2.12) and fetal (Table 2.13) plasma amino acid fluctuations. Amino acids transported primarily by the L system of Oxender and Christensen (1963), including valine, isoleucine, leucine, phenylalanine were not initially strongly concentrated in fetal plasma although a tendency for concentration to occur with time after methionine injection may have occurred (Table

2.14). Amino acids for which transport occurs primarily by the A system (Oxender and Christensen, 1963) were more strongly concentrated in the fetal blood, as indicated by the F/M ratios of threonine, serine, glycine, alanine (Table 2.14). A characteristic distribution pattern between fetal blood and amniotic fluid F/A ratio was apparent (Table 2.16), with some amino acids at higher concentrations in fetal blood and others elevated in amniotic fluid. No distinct groupings of amino acids could be made on the basis of previously established classifications regarding transport characteristics.

A/M ratios for valine, isoleucine, leucine and phenylalanine (Table 2.17) showed that amino acids transported by the L system (Oxender and Christensen, 1963) were at considerably lower concentrations in amniotic fluid than in maternal plasma. Threonine, serine, glycine and alanine (primarily transported by the A system) were present in higher concentrations in amniotic fluid (Table 2.17) than in the plasma of the ewe.

Following abomasal administration of ^{35}S-methionine to the ewe, the fetal plasma ^{35}S rose to a level exceeding that of maternal plasma within 3 to 4 hours post injection and continued to rise to a level in excess of twice that of the maternal ^{35}S concentration (Fig. 8). The fetal blood ^{35}S level appeared to plateau 24 hours after injection and was maintained or slightly increased over the remainder of the

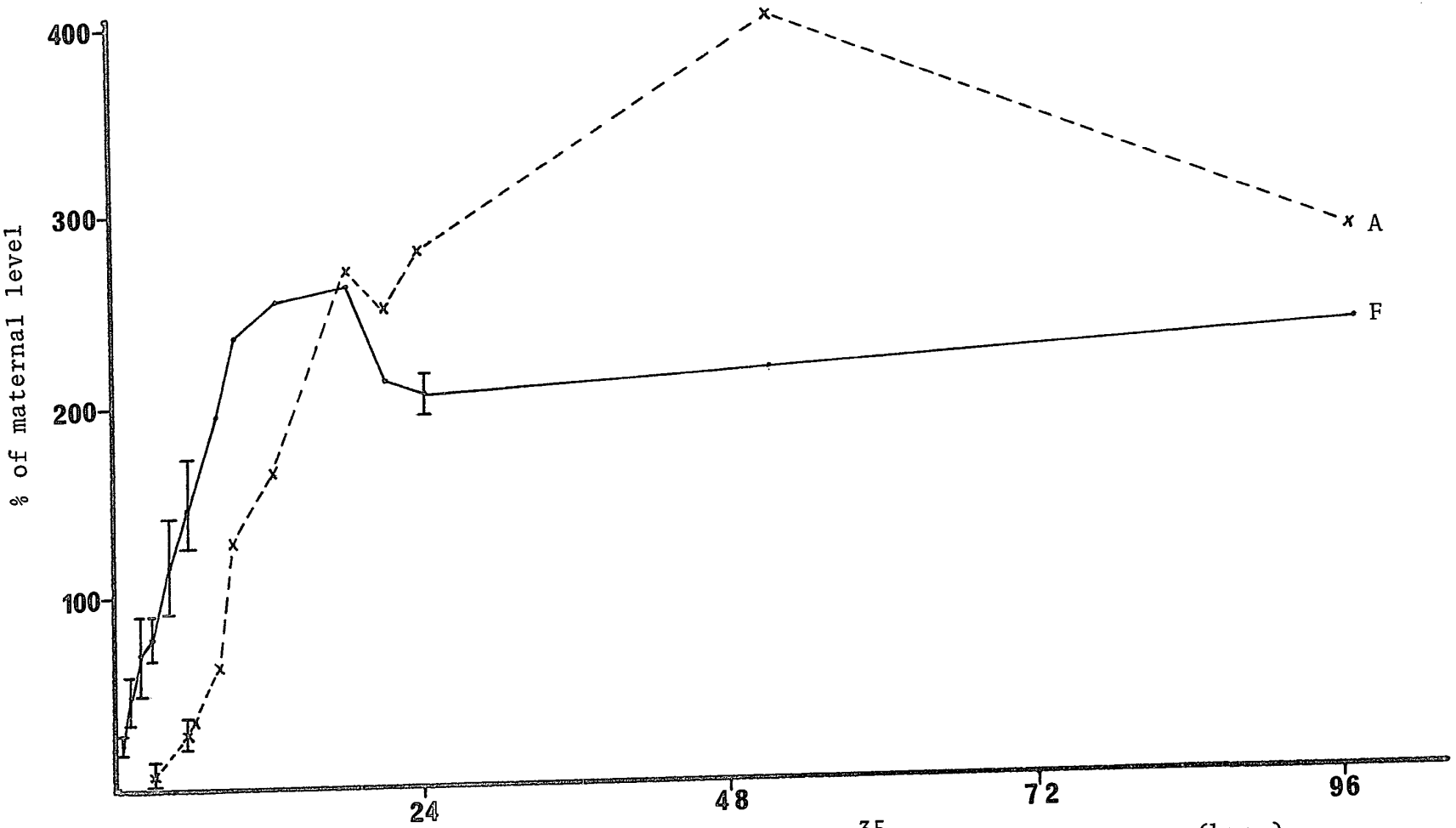


Fig. 8. Accumulation of ^{35}S in Fetal Plasma (F) and Amniotic Fluid (A) Following Abomasal Injection of ^{35}S-Methionine (160 mg/kg) to Ewe

experimental period (Fig. 8). Amniotic fluid accumulated ^{35}S more slowly than fetal plasma, reaching the maternal level approximately 9 hours after maternal injection (Fig 8). The amniotic fluid ^{35}S content continued to rise and was greater than that of fetal blood at 18 hours post injection (Fig. 8) with an apparent peak of ^{35}S activity at 48 hours post injection. The amniotic fluid ^{35}S content remained greater than that of maternal or fetal plasma for the duration of the study although the level tended to decrease after the 48 hour peak (Fig. 8). The ^{35}S contents of the different fluids may not have represented indentical compounds but rather a mixture of methionine and its metabolites. Changes in free amino acid concentrations were not parallel for maternal and fetal plasma and amniotic fluid (Tables 2.14, 2.16 and 2.17). In addition, incorporation of the label into plasma proteins may have been a significant factor in preservation of blood ^{35}S concentrations at later sampling times.

Fetal blood ^{35}S-methionine concentration increased beyond the maternal level by 2 hours post injection and continued to rise to an extremely elevated concentration relative to that of the ewe during the experimental period (Fig. 9). ^{35}S-taurine increased more slowly in fetal blood and became greater than that of the ewe between 6 and 8 hours after methionine injection to the ewe (Fig. 9). ^{35}S-labelled

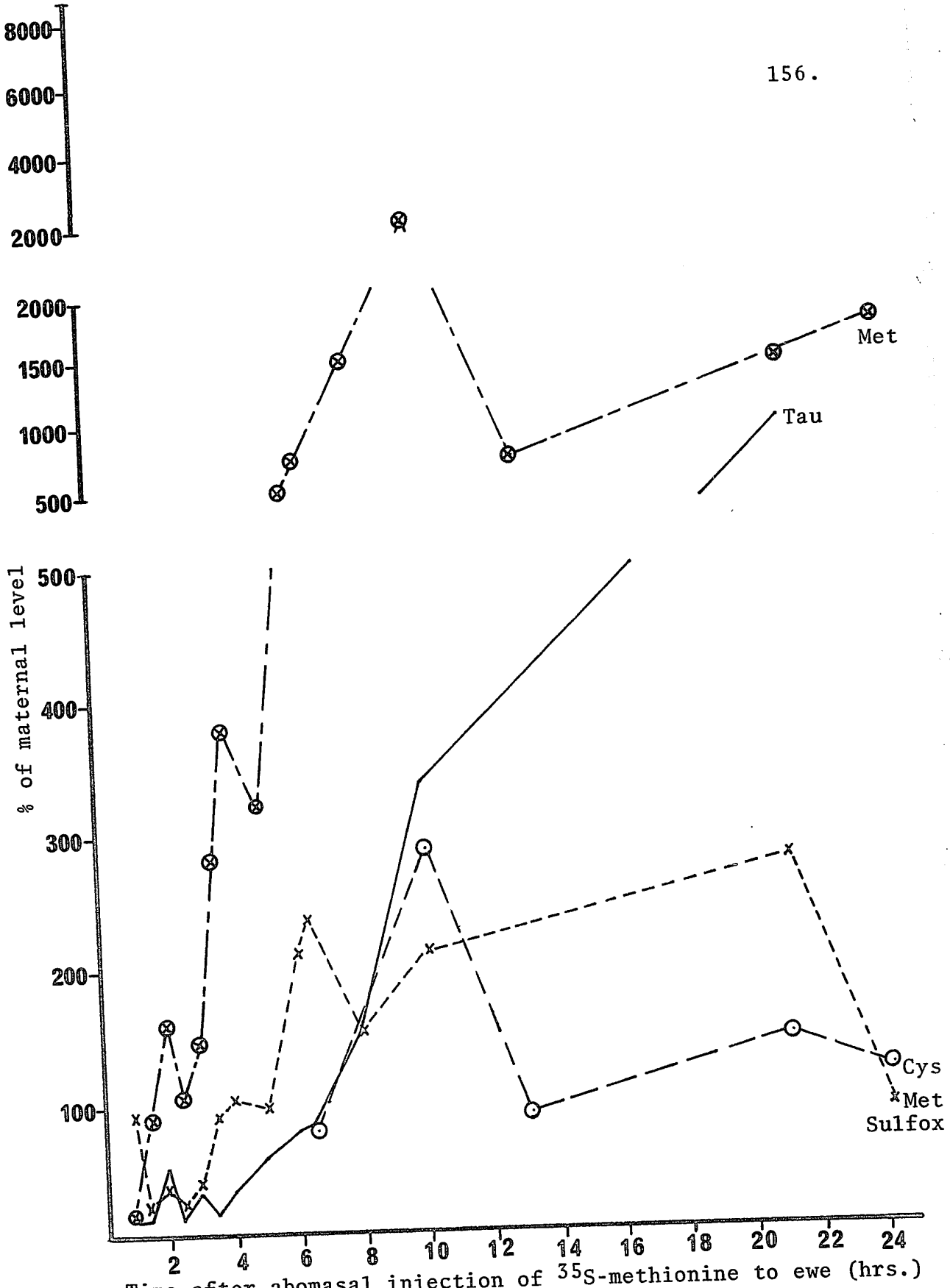


Fig. 9. Accumulation in Fetal Plasma of ^{35}S-Methionine and Metabolites Following Abomasal Injection of ^{35}S-Methionine (160 mg/kg) to

taurine in fetal blood relative to maternal continued to increase for the duration of the period studied. The ^{35}S content of methionine sulfoxide increased in fetal blood to approximately twice that of the maternal blood in the first 6 hours following maternal injection of ^{35}S-methionine and had fallen to the maternal level by 24 hours after injection (Fig.9). The fetal blood level of ^{35}S-cystine also increased to a peak at 10 hours declining thereafter (Fig. 9).

In amniotic fluid the accumulation of free ^{35}S in methionine, taurine and methionine sulfoxide continued throughout the experimental period starting at approximately 6 hours post maternal injection (Fig. 10). ^{35}S-Cystine increased from 6 to 10 hours post injection to about three fold the maternal concentration, then decreased to levels approximately the same as maternal by 24 hours post injection (Fig. 10). Total accumulation of free ^{35}S in amniotic fluid methionine, taurine and methionine sulfoxide was considerably greater than that of the fetal or maternal blood (Figs. 9 and 10).

The specific activity of methionine in maternal blood plasma was highest during the initial 3 hours following maternal abomasal injection of methionine and tended to decrease to some extent over the remainder of the experimental period (Fig. 11). This behavior approximated that of the

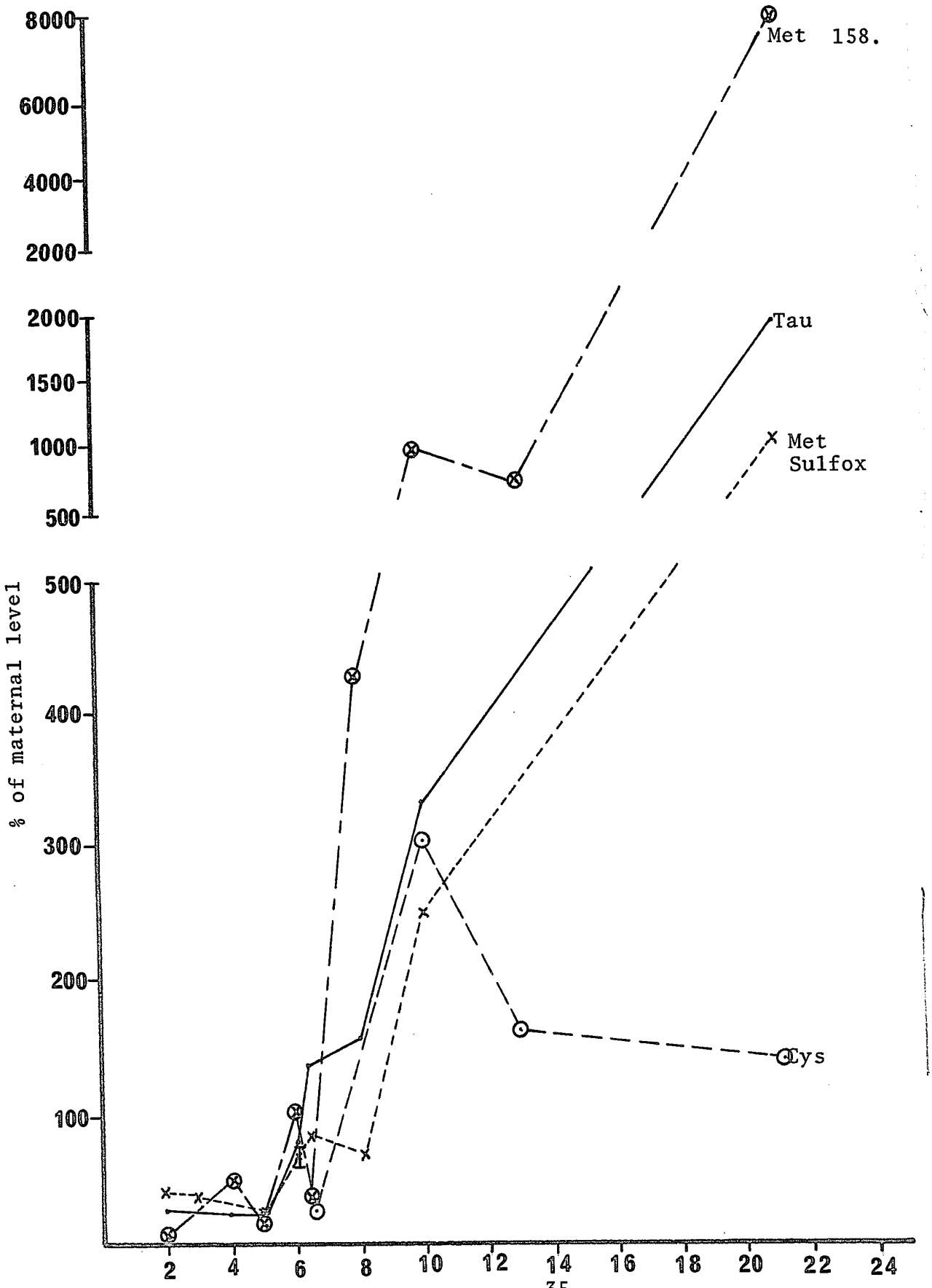


Fig. 10. Accumulation in Amniotic Fluid of ^{35}S-Methionine and Metabolites Following Abomasal Injection of ^{35}S-Methionine (160 mg/kg) to Ewe

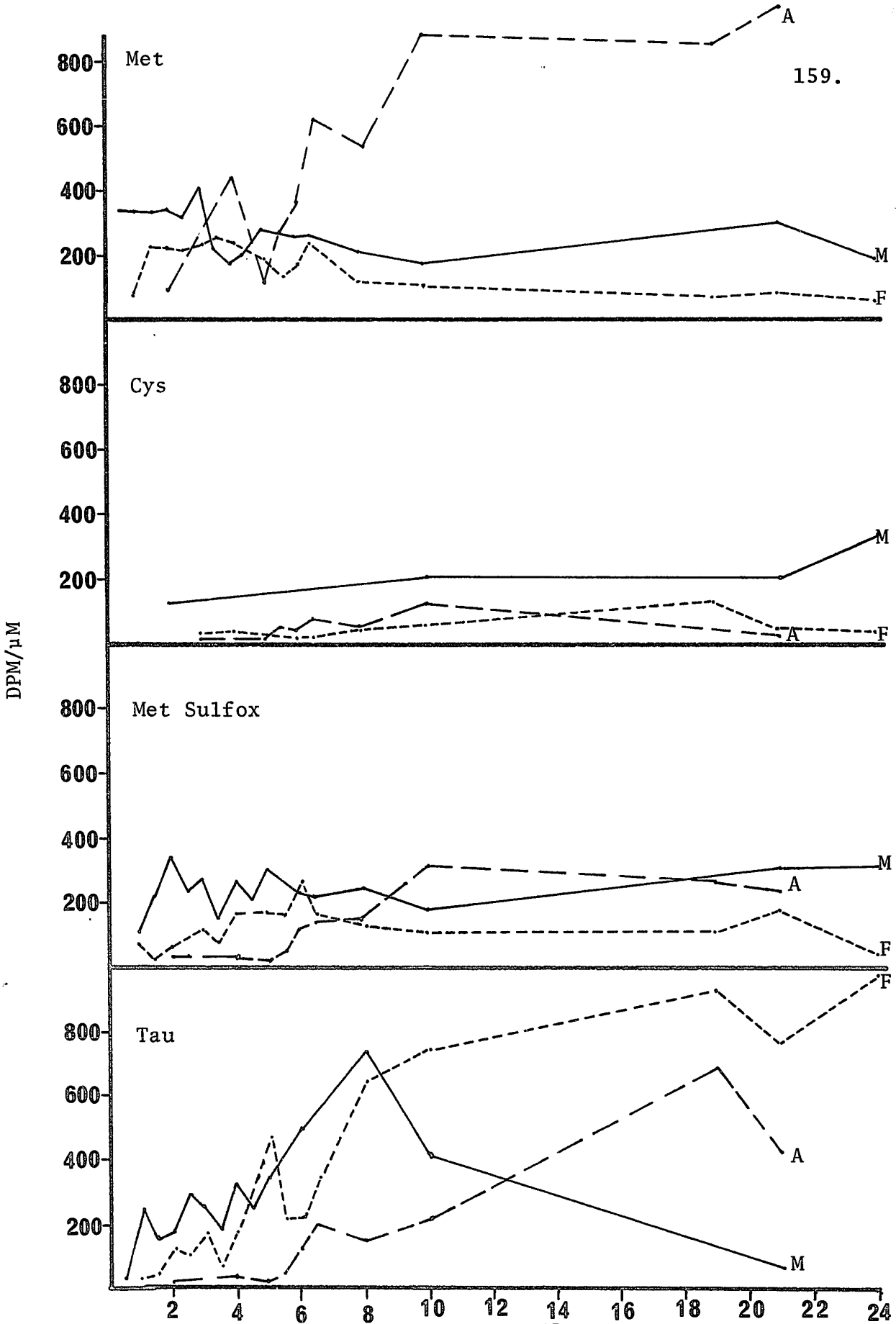


Fig. 11. Specific Activity (DPM/μM) in ^{35}S-Containing Metabolites Following Abomasal Injection of ^{35}S-Methionine (160 mg/kg) to Ewe

maternal plasma methionine level (Table 2.12) up until approximately 390 minutes post injection. At later time intervals, however, the maternal plasma methionine concentration decreased considerably while the specific activity changed only to a minor extent.

The specific activity of fetal plasma methionine was lower than that of maternal plasma except for a brief period between 3.5 and 4.5 hours post injection when it became slightly greater than maternal (Fig. 11).

The specific activity of methionine in fetal blood appeared to increase within 90 minutes of maternal abomasal injection of ^{35}S-methionine (Fig. 11), following which the level remained relatively stable for several hours and then gradually decreased during the remainder of the experiment.

In amniotic fluid the specific activity of methionine began to rise several hours after injection and continued to rise for the duration of the experimental period to much higher values than either maternal or fetal plasma had exhibited (Fig. 11).

Maternal plasma cystine showed higher specific activity than that of the fetus (Fig. 11). While the maternal cystine specific activity gradually increased through the test period, the fetal plasma and amniotic fluid specific activities remained low (Fig. 11). There appeared to be little difference in the specific activities of cystine in the fetal plasma

or amniotic fluid.

A tendency for the specific activity of maternal plasma methionine sulfoxide to increase was not pronounced (Fig. 11). Fetal blood methionine sulfoxide exhibited a similar behavior pattern. The specific activity of amniotic fluid methionine sulfoxide increased only slowly (Fig. 11). Fetal plasma methionine sulfoxide showed consistently lower specific activity than maternal, while the amniotic fluid specific activity for methionine sulfoxide approached that of ewe plasma.

The specific activity of taurine in maternal blood plasma increased to a maximum value at 8 hours post injection (Fig. 11). In fetal plasma the increase in specific activity of taurine appeared to follow that of maternal plasma for the initial 8 hours post injection (Fig. 11) with a slight time lag evident. The specific activity of fetal plasma taurine continued to rise throughout the experiment, while that of the ewe decreased from 8 hours post injection.

The taurine pool of amniotic fluid increased in specific activity (Fig. 11) beginning at approximately 5 hours post injection and increasing for at least a further 14 hours. By 21 hours post injection the specific activity of taurine in fetal plasma was considerably higher than that of amniotic fluid, which was in turn considerably higher than that of the maternal plasma (Fig. 11).

Effects of repeated abomasal administration of methionine

With repeated daily loading of the ewe by abomasal injection of methionine, the plasma taurine concentration of the ewe increased (Table 2.18) indicating that catabolism of methionine had increased. A tendency for increases in concentration of plasma methionine sulfoxide and cystathionine also occurred (Table 2.18). Fluctuations in the plasma concentrations of many other amino acids occurred also but these did not appear to follow any consistent pattern (Table 2.18). Since the maternal blood sample was drawn 24 hours after loading, changes in plasma amino acid concentrations which may have occurred in the interval between loading and sampling may not have been detected.

When 160 mg/kg of ^{35}S-methionine was injected abomasally into the ewe following 6 previous daily injections, fetal plasma methionine concentration was markedly increased 390 minutes post injection (Table 2.19). The maternal plasma methionine concentration (Table 2.19) was comparable to that observed at the same time interval following single abomasal dose (Table 2.12). The F/M ratio for methionine (Table 2.19) was therefore considerably higher than that obtained following a single injection of methionine (Table 2.14). Following repeated daily loading with methionine the F/M specific activity ratio (Table 2.19) was 0.63 whereas following a single injection (Fig. 11), the specific activities of

TABLE 2.18 Maternal Plasma Amino Acid Concentrations as Affected by 6 Consecutive Daily Abomasal Injections of L-Methionine (160 mg/kg) to Ewe

Amino Acid	Time After Initial Methionine Injection (hrs.)					
	24	48	72	96	120	144
	(concentrations in μM/100 ml)					
Asp	2.7	0.5	0.6	0.9	T[1]	T
Thr	6.9	2.7	5.4	4.2	2.9	6.7
Ser	6.9	4.4	6.6	6.4	4.4	8.1
Glu	7.0	6.1	4.9	6.0	6.4	5.6
Gly	21.4	20.0	17.2	20.6	25.2	21.1
Ala	8.7	8.5	6.1	5.4	7.4	7.2
Val	10.9	11.0	10.0	6.6	10.0	11.8
Cys	2.7	T	T	T	T	3.1
Met	3.8	2.7	6.8	3.8	2.6	6.3
Ileu	7.4	5.7	5.4	6.1	4.2	6.2
Leu	9.5	9.7	8.0	9.8	7.0	9.0
Tyr	4.6	2.3	2.8	3.8	2.8	3.1
Phe	4.3	2.1	2.5	2.8	2.3	5.6
Tau	6.9	5.0	7.6	10.9	12.7	20.8
Met Sulfox	2.8	1.8	2.9	2.8	2.0	5.7
Cit	14.5	16.8	21.4	31.0	20.3	23.7
AAB	2.7	0.7	1.0	1.4	T	T
Cys Ac	2.8	T	T	T	T	0.7
Cystat	0.6	1.2	1.0	2.0	1.9	2.0

[1] Trace present

TABLE 2.19 Free amino acid concentrations in maternal and fetal plasma and in amniotic fluid following 6 consecutive daily abomasal injections of methionine (160 mg/kg) to ewe[1]

Amino Acid	Maternal Plasma	Fetal Plasma	Amniotic Fluid	F/M ratio	F/A ratio	A/M ratio	Specific Activity Ratio F/M	F/A	A/M
			(concentrations in μM/100 ml)						
Asp	T[2]	0.6	2.5	1	0.24	1			
Thr	3.3	24.0	30.1	7.27	0.80	9.16			
Ser	4.5	83.9	158.1	18.64	0.53	35.16			
Glu	3.4	6.1	4.6	1.79	1.33	1.34			
Gly	22.6	79.4	104.1	3.51	0.76	4.62			
Ala	6.4	55.7	39.0	8.70	1.43	6.10			
Val	6.4	27.9	13.8	4.36	2.02	2.16			
Cys	3.6	+[3]	+	—[4]	—	—	3.56*	20.00*	.18*
Met	36.1	374.6	114.0	10.38	3.29	3.72	0.63	28.80	.02
Ileu	2.7	11.7	5.2	4.33	2.25	1.92			
Leu	4.8	14.5	9.5	3.02	1.51	2.00			
Tyr	1.3	12.3	10.6	9.46	1.16	8.16			
Phe	9.9	18.3	12.6	1.84	1.45	1.28			
Tau	22.2	23.8	17.8	1.07	1.34	0.80	0.70	21.34	.04
Met sulfox	6.0	45.6	88.9	7.60	0.51	14.84	0.25	56.66	.01
Cit	13.8	28.5	40.4	2.07	0.71	2.92			
AAB	0.7	7.5	2.9	10.71	2.50	4.28			
Cys Ac	1.2	2.0	3.7	1.67	0.56	3.00	0.34	17.52	.02
Cystat	1.1	4.3	9.0	3.91	0.48	8.18	0.40	35.14	.01

1 Samples of maternal and fetal plasma and amniotic fluid 390 minutes after abomasal injection
2 Trace present
3 Unable to quantitate
4 Unable to calculate
* Ratio of radioactivity in each fluid

methionine in maternal and fetal plasma appeared to be closer to 1.

The amniotic fluid methionine concentration was considerably higher after repeated methionine loadings (Table 2.19) than when a single injection was given to the ewe (Table 2.15). The F/A specific activity ratio for methionine was very high (Table 2.19) while the A/M ratio for methionine was very low (Table 2.19). Thus little of the radioactive dose was transferred to the amniotic fluid in this time interval.

Cystine apparently accumulated rapidly in the fetal blood as indicated by the F/M radioactivity ratio for cystine which was considerably greater than 1 (Table 2.19). In amniotic fluid ^{35}S-cystine was observed (Table 2.19) although the ratio of radioactivities did not indicate extensive accumulation.

Taurine appeared to be at similar levels in maternal and fetal plasma and in amniotic fluid (Table 2.19). Transfer of taurine from fetal plasma to amniotic fluid had not occurred to an appreciable extent 390 minutes post injection (Table 2.19) as indicated by the F/A specific activity ratio for taurine. A similar situation was observed with taurine following a single injection of ^{35}S-methionine (Fig. 11).

Elevated methionine sulfoxide concentrations were observed in both the fetus and amniotic fluid relative to the maternal level (Table 2.19). The F/M specific activity

ratios (Table 2.19), however, were very low. In addition, very little of the fetal accumulation of ^{35}S-methionine sulfoxide had been transferred to amniotic fluid by 390 minutes post injection (Table 2.19).

Other metabolites of methionine also accumulated in fetal blood and amniotic fluid. Cysteic acid and cystathionine showed a similar behavioral pattern (Table 2.19) with each at a higher concentration in fetal plasma than in the maternal plasma. In spite of this ratio favoring the fetal circulation in terms of concentration, low specific activity F/M ratios were observed (Table 2.19). The transfer of ^{35}S-cysteic acid and cystathionine from fetal blood to amniotic fluid was relatively low as indicated by the high F/A ratio for specific activity of the two compounds.

The pattern of other amino acids in fetal plasma (Table 2.19) was not strikingly different from patterns observed in studies following a single abomasal injection of methionine to the ewe (Table 2.13).

Amniotic fluid concentrations of most amino acids following repeated abomasal injections of methionine (Table 2.19) were much higher than those observed after a single injection (Table 2.15), perhaps indicative of a situation analagous to a generalized amino aciduria.

Effects of preparturant injection on postnatal plasma amino acid levels

Twenty-nine hours after a single abomasal injection of methionine (160 mg/kg) into the ewe and 8 to 16 hours after parturition, the plasma methionine concentration in the lamb was at a higher level than that in the ewe (Table 2.20). The lamb plasma methionine concentration had decreased to some extent by 52 hours post injection but was still elevated considerably over that observed in the ewe (Table 2.20). From 29 to 52 hours post injection the concentration of cystine in lamb plasma increased (Table 2.20). A similar tendency was apparent in the case of methionine sulfoxide, alpha-aminobutyris acid and cysteic acid (Table 2.20). Plasma taurine concentration was considerably lower in the lamb than in the mother (Table 2.20).

The pattern of other amino acids in lamb plasma did not closely resemble either that of maternal plasma (Table 2.20) or that of fetal plasma (Table 2.13).

TABLE 2.20 Free Amino Acid Concentrations in Ewe and Lamb Plasma Post Partum Following Abomasal Injection of ^{35}S-Methionine (160 mg/kg) to Ewe Pre Partum

Amino Acid	Time After Injection (hrs.)			
	29		52	
	Ewe plasma	Lamb plasma	Ewe plasma	Lamb plasma
	(concentrations in µM/100 ml)			
Asp	0.8	2.5	1.1	2.0
Thr	3.6	4.8	3.1	6.2
Ser	4.9	18.8	4.2	27.1
Glu	5.7	4.1	9.4	11.2
Gly	8.7	13.4	8.3	16.7
Ala	11.6	17.0	13.4	21.8
Val	6.7	9.0	6.3	10.0
Cys	14.4	10.4	3.4	14.6
Met	8.3	54.9	5.6	40.4
Ileu	2.1	2.6	3.2	3.2
Leu	4.4	4.6	5.0	5.2
Tyr	2.7	4.5	3.3	6.0
Phe	5.7	4.9	5.2	5.9
Tau	18.0	4.5	17.0	3.9
Met Sulfox	6.4	31.6	8.7	40.8
Cit	7.5	5.9	5.3	7.8
AAB	1.3	1.2	1.4	1.5
Cys Ac	7.0	1.0	5.1	1.6

Discussion of Studies with the Ewe

When the maternal blood plasma methionine concentration was markedly increased by abomasal injection of a high level of methionine, transport of methionine to the fetus was extensive. As a result, the fetal plasma methionine concentration was elevated to levels considerably higher than those in maternal plasma. This observation, and those made when low levels of methionine were injected intravenously to the ewe, confirmed the existence of a "threshold effect" for placental transport of methionine. This threshold is suggested to be the plasma methionine level above which the maternal system can no longer maintain its blood methionine concentration within physiological limits. The homeostasis of blood amino acids is accomplished in large part through interchange of the amino acid between blood and tissues (Christensen, 1964). Increases in the blood level of an amino acid have been shown to be reflected to an even greater extent in tissues (Christensen, 1964). There is little reason to suspect the placenta to respond differently in this respect from any other tissue. An increase in maternal blood methionine level would therefore by expected to result in increased placental uptake of methionine and consequently an increased transfer of methionine into the fetal circulation.

Hopkins et al. (Fig. 12) showed that an elevation in

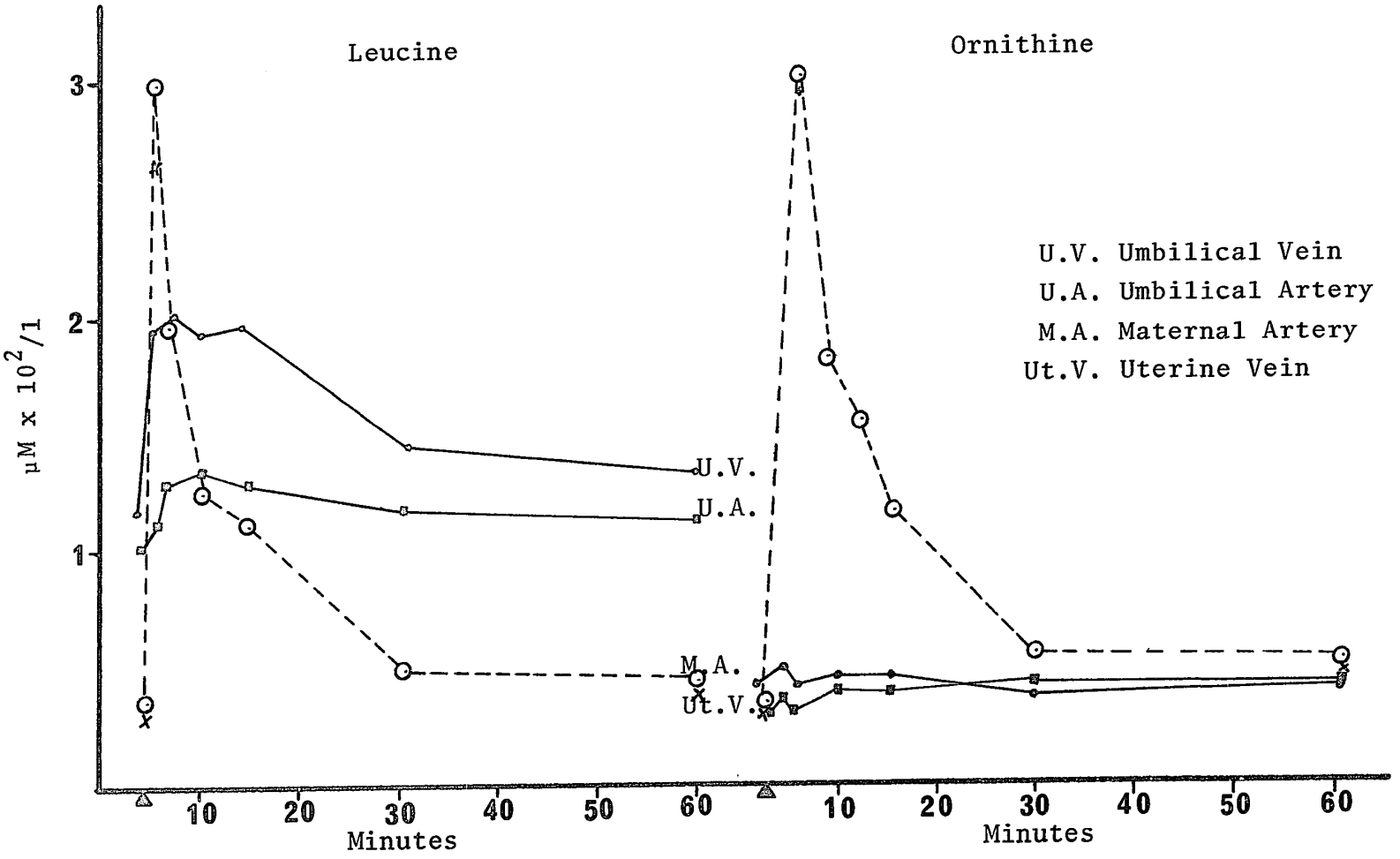


Fig. 12. Effect of Intravenous Injection of 0.4 g L-Leucine or 0.4 g L-Ornithine into Ewe on Plasma Levels of the Amino Acid in the Ewe and Fetus. From Hopkins et al. (1971b).

maternal plasma leucine concentration of about 5 fold resulted in less than a 50% increase in the fetal plasma leucine concentration. For ornithine (Fig. 12), a similar increase in maternal level was reflected to only a minor extent in fetal blood. The maternal level decreased to the preinjection level rapidly, however, which may have been a result of removal of these two amino acids from maternal plasma through uptake by tissues other than the placenta. Lines and Waisman (1970) suggested that following phenylalanine loading of rats, elevation of the maternal plasma phenylalanine for 30 to 60 minutes was required to result in phenylalanine concentrations in the fetal plasma which were higher than maternal concentrations. In the present investigations, although the fetal plasma methionine level began to increase in the 30 to 60 minutes post injection interval (Table 2.13), peak levels of fetal plasma methionine were not observed until considerably later. However, the maternal plasma methionine concentration remained elevated for a much longer interval (Table 2.12) than did maternal levels of leucine and ornithine in the studies of Hopkins et al. (1971b) and phenylalanine in the studies of Lines and Waisman (1970). Therefore the time period over which the maternal plasma methionine remains elevated may be of more importance than the magnitude of the elevation at any one time, in determining the extent of transport when the threshold was exceeded. If

the maternal level of methionine was elevated for an extended period of time, the transfer of methionine to the fetus via the placenta would then be expected to be that much greater.

Clearance of methionine from fetal blood has been shown (Sturman et al., 1971) to be considerably slower than from that of the mother. In the present study a similar observation was made (Tables 2.12, 2.13) after a single injection of methionine. Following repeated loading of the ewe with methionine (Table 2.18), extremely high methionine concentrations were recorded in fetal blood, further suggesting a reduced ability of the fetus to decrease its plasma methionine. The F/M specific activity ratio following repeated loading (Table 2.18) was lower than that observed when single doses of methionine were administered (Fig. 11). This observation indicated that there may have been dilution of the transported ^{35}S-methionine by a high level of methionine existing in fetal blood prior to administration of the labelled dose. Such a high fetal blood methionine level would occur if the fetus was unable to reduce its blood methionine concentration back to normal levels between loads, thus creating a "staircase" effect, where repeated loading would result in a gradual elevation of the fetal blood methionine concentration relative to that occurring following a single load.

Transport of methionine from fetal to maternal blood has been shown to be a relatively slow process in the Rhesus

monkey (Sturman et al., 1971) and in the sow (Table 2.3). The major routes by which the fetus may dispose of an accumulation of methionine may therefore be by tissue uptake and/or excretion via the kidney into amniotic fluid. Amniotic fluid has been shown (Tyson et al., 1968) to have a slow turnover rate, a result which was confirmed in the present investigations (Table 2.15). The observed increase in specific activity of ^{35}S in amniotic fluid (Fig. 11) indicated that a steady prolonged excretion of methionine into amniotic fluid did occur, although at a relatively slow rate. Nevertheless, the relatively high levels of methionine and its metabolites which had accumulated in amniotic fluid following repeated loading of methionine to the ewe (Table 2.19) implicate excretion into amniotic fluid as an important route by which the fetus may clear these compounds from its circulation. The total ^{35}S activity in the fetal plasma was approximately twice that of maternal plasma (Fig. 8) while free methionine and its metabolites exhibited a much greater increase in fetal blood relative to maternal (Fig. 9). The proportion of ^{35}S associated with protein was therefore much larger in the maternal than in fetal plasma. Similarly, considerably more protein associated ^{35}S was observed in fetal plasma than in amniotic fluid. Since plasma proteins are known to be synthesized largely in the liver (Bocci, 1970), these observations may indicate that the fetal liver had a

reduced ability to incorporate methionine into protein relative to the capacity of the maternal liver for such incorporation. Further investigation of this aspect of the problem is warranted. Amniotic fluid would be expected to contain little protein bound ^{35}S in view of a questionable amount of protein transport from extra-amniotic sites.

The specific activity of free ^{35}S-methionine in the maternal and fetal plasma indicated that a state of equilibrium was rapidly achieved in which the absorbed dose was uniformly distributed in the maternal and fetal blood and tissues. That the label was not diluted in blood by efflux of amino acids from tissues to any great extent seems clear since the specific activity remained relatively stable. The lowered specific activity of methionine in fetal plasma relative to maternal (Fig. 11) might be accounted for by dilution of the label with cold methionine as it passed through the placenta. Placental tissue has previously been shown to have a high content of free amino acids relative to the maternal or fetal circulations (Dancis et al., 1968).

A gradual increase in specific activity of cysteine in maternal plasma occurred (Fig. 11). No such increase occurred in the fetal plasma or amniotic fluid, which suggested a low rate of metabolism of methionine to cysteine in the fetus as previously suggested by others (Volpe and Laster, 1972; Gaull et al., 1972). The low activity of ^{35}S-cysteine observed

in fetal blood and amniotic fluid may have been a result of transport from the maternal circulation. Sturman et al. (1971) suggested that cystine was not readily transferred across the placenta of the Rhesus monkey. In the present studies a high level of cysteine was observed in fetal blood relative to maternal. This may not have been a consequence of a rapid transfer, however, but rather an accumulation due to a limited transfer of cysteine from fetus to mother relative to that occurring in the reverse direction over an extended time period.

The biological significance of methionine sulfoxide has not been fully established (Meister, 1965); accumulation of this compound in the blood following methionine loading of humans has been reported (Block et al., 1969). In this study methionine sulfoxide apparently crossed the placenta and was excreted into amniotic fluid (Fig. 10).

While the concentration of taurine did not change markedly in maternal plasma (Table 2.12), fetal plasma (Table 2.13) or amniotic fluid (Table 2.15), the specific activity of taurine increased considerably in both fetal plasma and amniotic fluid (Fig. 11) relative to that of the mother, indicating that taurine was removed from these two pools at about the same rate as it was entering.

The accumulation of methionine and its metabolites in fetal plasma and amniotic fluid observed after either single

or repeated loading of the ewe with methionine suggest that such metabolites did not readily cross the placenta from the fetal to the maternal circulation. Sturman et al. (1971) observed a similar phenomenon for methionine and cysteine in the Rhesus monkey. These metabolites may have occurred as a result of fetal metabolism and/or transport from the ewe. The newborn lamb appeared to have a limited capability to reduce an elevated plasma methionine level relative to the capability of the ewe (Table 2.20). In view of the limited capacity of other fetal species (Volpe and Laster, 1972; Gaull et al., 1972) to metabolize methionine, the limited capability of the neonatal lamb was not surprising and appeared to be a result of reduced metabolic activity toward methionine in the neonate. If such were the case, the ovine fetus would have been expected to have a low metabolic activity toward methionine also. Accumulation of methionine and its metabolites in the fetal blood and amniotic fluid could then have been ascribed to placental transport from the ewe rather than metabolic activity of the fetus. The post natal period required for the lamb to achieve adult methionine metabolizing activity remains unknown.

Transport systems in the placenta appeared to be similar to those of other tissues in that a distinct separation of amino acids transported by the L system from those transported by the A system could be made on the basis of F/M ratios

(Table 2.14). Whether the characteristics of the placental transport systems for amino acids are identical to those of other systems remains unknown, however, these data strongly suggest a common basis.

Results of studies with the ewe have shown that administration of high levels of methionine to the ewe during pregnancy resulted in accumulation of methionine and its metabolites in fetal blood and amniotic fluid, and that these compounds, once accumulated, are less readily removed from the fetal blood and amniotic fluid than from the maternal circulation. While the consequences of such accumulations upon the development of the fetus and its post natal performance have not been established, there appeared to be potential for adverse effects to occur.

Further studies were therefore undertaken using the rat to attempt to establish whether fetal development and post partum performance would be affected as a result of the maternal dietary methionine level.

Studies with the Rat

Investigations with the ewe indicated that elevation of the maternal plasma methionine concentration by administration of a loading dose of methionine resulted in elevation of fetal plasma methionine concentrations to levels higher than the elevated levels observed in the ewe. The degree to which such an elevated plasma methionine concentration might affect the development of the fetus *in utero* has not been established. There is, however, evidence of adverse effects of high levels of methionine in the diet of the pregnant female upon the fetal rat (Viau et al., 1969; Leatham et al., 1968). The possibility that adverse effects of excess methionine upon fetal development might be expressed postnatally cannot be overlooked. Kerr et al. (1968) reported adverse effects of high maternal levels of phenylalanine upon post natal development in the Rhesus monkey. Chow et al. (1967) reported that the congenitally malnourished female rat gave birth to stunted offspring despite the fact that the female had received an adequate diet throughout her post natal life. The possibility of adverse effects of excess methionine during pregnancy on fetal development and of "carry over" effects into succeeding generations cannot be overlooked. The consequences of feeding high levels of methionine to the pregnant female rat on the post natal performance and reproductive capacity of her offspring was investigated in the studies reported herein.

Effects of maternal dietary methionine level on maternal and fetal body and organ weights, and the distribution of ^{35}S between mother and fetuses following ^{35}S-methionine loading of the mother

Objectives:

1. To assess the effects of maternal dietary methionine level on growth and organ weights of the dam and her fetuses.

2. To determine if changes in maternal and fetal tissue ^{35}S uptake occurred as a result of chronic exposure to differing maternal dietary methionine levels.

Procedures:

Wistar strain rats purchased from Woodlyn Farms, Inc., Guelph, Ontario were employed in these studies. Females were purchased at 150-175 g body weight and were quarantined for 7 days in the Health Protection Branch Animal Colony. From arrival at the Health Protection Branch facilities until pregnancy was confirmed, the rats received a standard laboratory ration (Maple Leaf Mills, Ltd., Montreal, Quebec). All rats were inspected by a veterinarian prior to use. All females were bred in the Health Protection Branch Animal Colony using 250-300 g males. Two females were confined overnight with one male. The presence of sperm in the vaginal tract of the female(s) the following morning was taken as an indication of successful breeding. This was designated day 1

of pregnancy. The bred female was then allotted to one of the test diets shown in Table 2.21, 15 females per diet, and maintained on that diet for the duration of the study. The amino acid composition of the basal diet is given in Appendix D. Body weight gains and food intakes were determined for the test period for 6 animals on each diet.

On day 20 of pregnancy the pregnant female was fasted for 16 hours and then given a loading dose of methionine (50 mg L-methionine, containing 2.5 μ Ci ^{35}S-L-methionine). The loading dose was administered by stomach tube in 1 ml Physiological saline. The food can was replaced immediately following loading with methionine to eliminate effects due to prolonged fasting when the interval between loading and sacrifice was extended. At the intervals indicated in Table 2.24, 3 animals from each dietary group were lightly anesthetized with ether, weighed and decapitated. Maternal blood was collected in heparinized tubes. The maternal brain, liver and kidney were rapidly removed, blotted clear of blood, weighed, homogenized and sampled. A sample of muscle tissue was excised from the rear leg. The entire uterus was removed from the animal. The uterus was incised and three intact fetal compartments were pooled, weighed, homogenized and sampled. The remaining fetuses were removed from the uterus following which the uterus was homogenized and sampled. The total number of

TABLE 2.21 % Composition of Test Diets Employed in Studies with Rats

Constituent	Diet				
	0.20	0.55	0.80	1.60	3.20
Mineral mix[1]	4.0	4.0	4.0	4.0	4.0
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0
Cellulose[3]	5.0	5.0	5.0	5.0	5.0
Cornstarch[4]	63.7	63.7	63.7	63.7	63.7
Corn Oil[5]	5.0	5.0	5.0	5.0	5.0
Soya Assay Protein[6] (84 % C.P.)	18.1	18.1	18.1	18.1	18.1
L-methionine	0.04	0.4	0.64	1.44	3.04
L-glutamic acid	3.16	2.8	2.56	1.76	0.16

[1]USP.14 General Biochemicals, Chagrin Falls, Ohio

[2]A.M. Mix General Biochemicals, Chagrin Falls, Ohio

[3]Alphacel Nutritional Biochemicals Corp., Cleveland, Ohio

[4]Nutritional Biochemicals Corp., Cleveland, Ohio

[5]Mazola Canada Starch Co., Montreal, Quebec

[6]General Biochemicals, Chagrin Falls, Ohio

fetuses and the number of resorption sites in the uterus were recorded. The remaining fetuses were handled as follows: the umbilical cord was severed close to the placenta. Amniotic membranes were discarded. The fetal throat was cut and blood was collected from the wound by aspiration with a pasteur pipette. The fetal liver was removed. The remainder of the fetal body was denoted fetal carcass. The placentas, blood, livers and carcasses were pooled for each litter, weighed, homogenized and sampled. Mean values for fetal compartments and organ weights were calculated. ^{35}S activity was determined as described in Appendix A. The DPM/ml were calculated as a proportion of the dose administered since the radioactive decay rate of ^{35}S was sufficient to cause significant decreases in the actual dose of ^{35}S-methionine given over the period of the study. Where missing data occurred, missing values were calculated as described by Steele and Torrie (1960). Statistical analysis of the data were performed by standard procedures (Steele and Torrie, 1960) with components of variance partitioned as outlined in Appendix B. Mean values were compared by Tukey's ω-procedure (Steele and Torrie, 1960) for statistically significant differences.

Results:

Performance and organ weights of mother and fetus

The body weight gain of pregnant rats fed either 1.6 or 3.2% of methionine was significantly ($P < 0.05$) lower than

that of the animals fed lower dietary methionine levels (Table 2.22). This effect was not a result of reduced food intake alone since at the 1.6% dietary methionine content, the food intake was not significantly lower than that of rats receiving the two lowest dietary methionine levels (Table 2.22). For rats receiving the 3.2% methionine diet, reduced food intake was obviously responsible for much of the reduction in weight gain, however, when gain was expressed in terms of food intake (gain/g intake, Table 2.22), it became apparent that at the two highest dietary methionine levels, methionine exerted a specific growth depressing effect.

No significant changes were noted in litter size attributable to dietary methionine level (Table 2.22). However, the percentage of pregnant animals with resorption sites was highest amongst females receiving the two highest methionine levels (Table 2.22).

Dietary methionine level did not cause changes in relative liver or kidney weights (Table 2.22). The relative brain weights of animals fed 3.2% of methionine in the diet were significantly ($P < 0.05$) higher than those of animals receiving 0.55% methionine but were not significantly different from those of rats receiving the other dietary methionine levels (Table 2.22).

Pregnant females fed either 1.6% or 3.2% dietary methionine tended to have lower uterus weights than those observed in rats receiving the lower methionine levels (Table

TABLE 2.22 Maternal Performance and Organ Weight Distributions for Mother and Fetus as Influenced by Maternal Dietary Methionine Content

Criterion	Dietary Methionine Content (% of Diet)											
	0.20		0.55		0.80		1.60		3.20			
Maternal												
Body gain (g)[1]	72.2^{a}	± 33.9	75.4^{a}	± 16.6	91.7^{a}	± 10.2	34.4^{b}	± 11.6	17.8^{b}	± 8.3		
Food intake (g)	278^{ab}	± 24	261^{b}	± 16	311^{a}	± 22	259^{b}	± 42	186^{c}	± 42		
Litter size	10.8	± 2.7	9.9	± 3.9	10.0	± 3.2	10.1	± 3.8	9.2	± 4.1		
% of Litters with Resorptions	5.9		13.3		12.5		22.2		37.5			
Gain/g intake	0.25^{a}	± 0.11	0.29^{a}	± 0.05	0.30^{a}	± 0.02	0.13^{b}	± 0.03	0.10^{b}	± 0.0		
$\frac{\text{Liver wt}}{\text{body wt}} \times 100$	3.76	± 0.38	3.96	± 0.54	4.07	± 0.56	3.82	± 0.25	3.99	± 0.5		
$\frac{\text{Kidney wt}}{\text{body wt}} \times 100$	0.70	± 0.12	0.63	± 0.12	0.64	± 0.12	0.64	± 0.12	0.73	± 0.1		
$\frac{\text{Brain wt}}{\text{body wt}} \times 100$	0.73^{ab}	± 0.18	0.64^{b}	± 0.13	0.68^{ab}	± 0.13	0.70^{ab}	± 0.18	0.80^{a}	± 0.1		
$\frac{\text{Uterus wt}}{\text{body wt}} \times 100$	1.69^{a}	± 0.39	1.46^{ab}	± 0.43	1.44^{ab}	± 0.59	1.25^{b}	± 0.21	1.34^{ab}	± 0.3		

[1] Means with same or no superscript not significantly different ($P < 0.05$)

. . . continued

TABLE 2.22 Maternal Performance and Organ Weight Distributions for Mother and Fetus as Influenced by Maternal Dietary Methionine Content (continued)

Criterion	Dietary Methionine Content (% of Diet) 0.20	0.55	0.80	1.60	3.20
Fetal					
Compartment (g)	4.16^{a} ± 0.75	3.96^{ab} ± 0.75	4.50^{a} ± 0.83	3.96^{ab} ± 0.67	3.29^{b} ± 0.84
Placenta (g)	0.48^{a} ± 0.12	0.44^{ab} ± 0.10	0.38^{b} ± 0.07	0.35^{b} ± 0.04	0.31^{b} ± 0.07
Carcass (g)	2.55 ± 0.83	2.49 ± 1.02	2.83 ± 0.47	2.62 ± 0.67	1.93 ± 0.76
Liver (g)	0.16 ± 0.05	0.16 ± 0.05	0.21 ± 0.04	0.17 ± 0.05	0.13 ± 0.06
Amniotic Fluid (g)[2]	0.97	0.87	1.09	0.82	1.02

[2] Difference in weight between compartment and placenta, carcass, liver

2.22). These differences were statistically significant ($P < 0.05$) only for the 1.6% methionine level compared with the 0.2% methionine fed rats.

Pregnant females receiving 3.2% dietary methionine had significantly ($P < 0.05$) reduced fetal compartment weights compared to those fed 0.2% or 0.8% methionine (Table 2.22). Placental weight appeared to decrease steadily as the methionine content of the diet increased, with placentas from mothers fed 0.2% methionine significantly heavier ($P < 0.05$) than those from mothers fed 0.8% or higher of methionine (Table 2.22). A tendency toward lowered fetal liver and carcass weights was evident at the highest dietary methionine level fed (Table 2.22), however, at lower maternal dietary methionine levels (1.6% and less) no effects upon growth of the fetus were noted.

^{35}S distribution in mother and fetus

Among females receiving 1.6% and 3.2% dietary methionine the maternal liver retained significantly ($P < 0.05$) less of the administered dose than observed in those receiving 0.55% dietary methionine. This trend, although not significant, was noted in females receiving 0.2% or 0.8% (Table 2.23).

The percentage of the dose taken up by maternal liver increased significantly ($P < 0.05$) from 4 to 8 hours post loading and then decreased to a lower percentage by 24 hours after administration of the load (Table 2.24). However, the

TABLE 2.23 ^{35}S Content (% of Dose) in Maternal Tissues as Affected by Maternal Dietary Methionine Level

% Dose in	Dietary Methionine Level (% of Diet)				
	0.20	0.55	0.80	1.60	3.20
Liver[1]	$9.4^{ab} \pm 1.9$	$10.5^{a} \pm 2.9$	$9.4^{ab} \pm 3.9$	$6.8^{b} \pm 3.6$	$7.2^{b} \pm 4.5$
Kidney	1.07 ± 0.33	0.96± 0.27	0.95 ± 0.32	0.78± 0.50	0.85± 0.45
Brain	0.19 ± 0.08	0.20± 0.13	0.17 ± 0.04	0.16± 0.08	0.19± 0.09
Muscle	8.0 ± 5.2	10.4 ± 4.6	8.0 ± 4.5	10.8 ± 8.4	9.5 ± 8.4
Uterus	1.26 ± 0.73	1.08± 0.56	0.97 ± 0.27	0.86± 0.37	0.87± 0.39
Blood	$2.66^{b} \pm 1.32$	$4.54^{a} \pm 1.86$	$3.60^{ab} \pm 1.64$	$2.44^{b} \pm 2.12$	$2.56^{b} \pm 1.20$

[1] Means with same or no superscript not significantly different ($P < 0.05$)

TABLE 2.24 ^{35}S Content (% of Dose) in Maternal Tissues as Affected by Time After Administration of Loading Dose to Mother

% Dose in	Time After Loading (hrs.)				
	1	2	4	8	24
Liver[1]	$9.2^{ab} \pm 3.4$	$9.7^{ab} \pm 3.8$	$7.7^{bc} \pm 3.5$	$11.3^{a} \pm 3.0$	$5.5^{c} \pm 1.9$
Kidney	0.79 ± 0.38	1.01 ± 0.51	0.99 ± 0.42	1.05 ± 0.25	0.77 ± 0.26
Brain	0.15 ± 0.06	0.23 ± 0.15	0.15 ± 0.06	0.20 ± 0.06	0.19 ± 0.08
Muscle	$12.3^{ab} \pm 7.4$	$13.4^{a} \pm 6.6$	$8.10^{ab} \pm 5.3$	$7.1^{ab} \pm 7.2$	$5.8^{b} \pm 3.6$
Uterus	$0.61^{b} \pm 0.25$	$0.99^{ab} \pm 0.44$	$0.99^{ab} \pm 0.20$	$1.17^{a} \pm 0.43$	$1.27^{a} \pm 0.72$
Blood	$2.30^{bc} \pm 1.15$	$2.75^{bc} \pm 1.14$	$3.54^{b} \pm 2.23$	$5.17^{a} \pm 1.38$	$2.03^{c} \pm 1.08$

[1] Means with same or no superscript not significantly different ($P < 0.05$)

percentage of the dose in maternal liver at 1 or 2 hours was not significantly different from that at 8 hours (Table 2.24).

No significant differences in ^{35}S activity/g liver were observed for the 4 lowest dietary methionine contents from 1 to 8 hours, however, for rats fed 3.2% dietary methionine, the uptake of ^{35}S per g liver at 8 hours post administration was higher than at any other sampling time (Table 2.25). The maternal liver ^{35}S (DPM/g) was significantly lower at 24 hours for animals fed either 0.55% or 3.2% dietary methionine than at 8 hours while no significant alterations occurred with the other diets (Table 2.25).

Mothers fed 0.55% dietary methionine exhibited higher ($P < 0.05$) percentages of the dose in blood than were seen with 0.2%, 1.6% or 3.2% methionine (Table 2.23). However, no significant changes in ^{35}S activity (DPM/ml) of maternal blood due to dietary methionine content effects were evident (Table 2.26). The ^{35}S activity per unit of maternal blood reached a maximum at 8 hours post dosing and by 24 hours had decreased to the 1 hour level of activity (Table 2.27).

No other tissues showed statistically significant changes in total tissue uptake of ^{35}S as dietary methionine content increased (Table 2.23).

Muscle uptake of ^{35}S was rapid (Table 2.24) with the highest percentage of the dose in muscle observed at 2 hours post injection followed by a gradual decrease to a significantly lower percentage by 24 hours (Table 2.24).

TABLE 2.25 Effect of Maternal Dietary Methionine Content on Accumulation in Maternal Liver of ^{35}S (% of Dose/g) with Time after Methionine Administration

Time After Load (hrs.)	Dietary Methionine Level (% of Diet)				
	0.20	0.55	0.80	1.60	3.20
1[1]	1.05	0.90^{ab}	1.06	0.51	0.79^{b}
2	1.21	1.08^{ab}	1.05	0.80	0.43^{b}
4	1.04	1.13^{ab}	0.95	0.64	0.57^{b}
8	1.37	1.23^{a}	0.88	1.02	1.65^{a}
24	0.89	0.54^{b}	0.70	0.42	0.69^{b}

[1] Means with same or no superscripts not significantly different ($P < 0.05$) in the same column.

TABLE 2.26 Effect of Gestational Dietary Methionine Level on Distribution of ^{35}S in Mother and Fetus Following Loading with ^{35}S-Methionine

Criterion[1]	Dietary Methionine Level (% of Diet)		
	0.20	0.55	0.80
DPM/ml Maternal Blood	2.57 ± 1.21	2.64 ± 1.20	2.74 ± 1.73
DPM/ml Fetal Blood	2.90 ± 1.47	2.75 ± 0.81	2.82 ± 1.02
Blood F/M Ratio (/ml)	1.61 ± 1.13	1.01 ± 0.41	1.38 ± 0.74
F/M Ratio (Total ^{35}S in M,F)	0.37 ± 0.15	0.30 ± 0.18	0.36 ± 0.22
DPM/g Fetal Liver	0.56 ± 0.11	0.54 ± 0.08	0.51 ± 0.15
% Dose in Fetal Compartment	16.3^{a} ± 7.1	12.5^{ab} ± 6.0	16.1^{a} ± 4.5
% Dose Absorbed	74.5 ± 18.5	75.0 ±17.0	75.6 ±16.0
% Absorbed Transferred to Fetus	20.6^{ab} ± 8.0	17.9^{ab} ± 9.8	22.2^{a} ± 7.4
% of Fetal Activity in Placenta	11.4 ± 3.6	11.9 ± 3.3	9.4 ± 2.0
% Fetal Activity in Blood	1.44 ± 0.53	1.88 ± 0.58	1.96 ± 0.58
% Fetal Activity in Carcass	61.5 ± 14.6	66.0 ±14.1	68.4 ± 8.7
% Fetal Activity in Liver	5.3^{b} ± 1.4	6.8^{ab} ± 2.1	7.3^{a} ± 1.0

[1] Means with same or no superscript not significantly different ($P < 0.05$)

...continued

TABLE 2.26 Effect of Gestational Dietary Methionine Level on Distribution of ^{35}S in Mother and Fetus Following Loading with ^{35}S-Methionine (continued)

Criterion	Dietary Methionine Level (% of Diet)	
	1.60	3.20
DPM/ml Maternal Blood	2.08 ± 1.47	2.62 ± 0.92
DPM/ml Fetal Blood	2.35 ± 0.74	3.20 ± 1.16
Blood F/M Ratio (/ml)	1.56 ± 0.74	1.35 ± 0.51
F/M Ratio (Total ^{35}S in M,F)	0.36 ± 0.28	0.34 ± 0.18
DPM/g Fetal Liver	0.44 ± 0.17	0.54 ± 0.19
% Dose in Fetal Compartment	10.6^{b} ± 6.0	10.4^{b} ± 3.8
% Dose Absorbed	84.8 ± 10.0	79.1 ±13.3
% Absorbed Transferred to Fetus	12.5^{b} ± 6.9	14.0^{ab} ± 6.2
% of Fetal Activity in Placenta	10.8 ± 3.2	11.6 ± 2.0
% Fetal Activity in Blood	1.69 ± 0.36	2.04 ± 0.61
% Fetal Activity in Carcass	73.4 ± 9.6	69.9 ±11.7
% Fetal Activity in Liver	5.9^{ab} ± 1.8	6.5^{ab} ± 1.3

TABLE 2.27 Effect of Time after Methionine Loading on Distribution of ^{35}S in Mother and Fetus

Criterion[1]	Time After Loading of Mother (hrs.)		
	1	2	4
DPM/ml Maternal Blood	$1.68^{b} \pm 0.61$	$2.34^{b} \pm 0.50$	$2.46^{b} \pm 1.22$
Blood F/M Ratio (/ml)	$1.75^{ab} \pm 0.73$	$1.40^{ab} \pm 0.36$	$1.18^{b} \pm 0.72$
F/M Ratio (Total ^{35}S in M,F)	0.34 ± 0.12	0.26 ± 0.13	0.30 ± 0.11
DPM/g Fetal Liver	0.46 ± 0.14	0.54 ± 0.19	0.45 ± 0.19
% Dose Absorbed	$68.2^{b} \pm 15.3$	$75.5^{ab} \pm 14.6$	79.6 ± 16.4^{ab}
% Dose Transferred to Fetus	14.5 ± 7.2	12.1 ± 6.6	13.8 ± 4.7
% Absorbed Transferred to Fetus	20.5 ± 10.0	17.0 ± 10.1	18.0 ± 7.1
% of Fetal Activity in Placenta	9.6 ± 2.8	12.5 ± 2.6	10.6 ± 3.2
% Fetal Activity in Blood	1.97 ± 0.50	1.91 ± 0.43	1.49 ± 0.69
% Fetal Activity in Carcass	70.9 ± 7.5	67.6 ± 14.7	66.6 ± 13.9
% Fetal Activity in Liver	$6.0^{ab} \pm 0.9$	$6.4^{ab} \pm 1.6$	$5.3^{b} \pm 1.8$

[1] Means with same or no superscript not significantly different ($P < 0.05$)

...continued

TABLE 2.27 Effect of Time after Methionine Loading on Distribution of ^{35}S in Mother and Fetus (continued)

Criterion	Time After Loading of Mother (hrs.)	
	8	24
DPM/ml Maternal Blood	$4.33^{a} \pm 1.36$	$1.83^{b} \pm 0.84$
Blood F/M Ratio (/ml)	$0.57^{c} \pm 0.19$	$2.01^{a} \pm 0.75$
F/M Ratio (Total ^{35}S in M,F)	0.32 ± 0.21	0.32 ± 0.31
DPM/g Fetal Liver	0.56 ± 0.14	0.58 ± 0.11
% Dose Absorbed	76.0 ± 14.5^{ab}	$89.8^{a} \pm 8.2$
% Dose Transferred to Fetus	12.6 ± 4.9	13.0 ± 6.7
% Absorbed Transferred to Fetus	17.1 ± 6.6	12.3 ± 7.3
% of Fetal Activity in Placenta	10.8 ± 3.3	11.4 ± 4.0
% Fetal Activity in Blood	1.70 ± 0.65	1.94 ± 0.61
% Fetal Activity in Carcass	60.6 ± 8.2	73.6 ± 12.8
% Fetal Activity in Liver	$7.2^{a} \pm 1.5$	$6.6^{ab} \pm 1.8$

The uterus appeared to accumulate ^{35}S more slowly and retain that ^{35}S taken up, so that at 8 and 24 hours post injection the percentage of the dose in uterine tissue was significantly higher ($P < 0.05$) than that at 1 hour post injection (Table 2.24).

In the fetal blood, however, the accumulation was dependent upon both diet and time after dosing (Table 2.28). The methionine deficient diet (0.20% methionine) and the high methionine diet (3.2%) appeared to affect accumulation of ^{35}S in fetal blood to a similar extent; peak levels of ^{35}S activity were observed in fetal blood 2 hours after dosing (Table 2.28). With 0.55% or 0.8% dietary methionine in the maternal diet, the increase in fetal blood ^{35}S was more gradual with highest activities occurring 24 hours post dosing (Table 2.28). Changes in fetal blood ^{35}S with time were not significant when 1.6% dietary methionine was fed (Table 2.28).

The percent of total dose transported to the fetus did not appear to be time dependent (Table 2.27). Therefore there must have been changes in the fetal distribution pattern. The percentage of total fetal ^{35}S activity in the liver showed an increase from 4 to 8 hours post dosing (Table 2.27). No other fetal tissues changed in proportion of total activity with time (Table 2.27).

The F/M ratio of ^{35}S/ml increased from 8 to 24 hours (Table 2.27). The activity of ^{35}S in maternal plasma decreased

TABLE 2.28 Effects of Maternal Dietary Methionine Level and Time after Administration of a Loading Dose of Methionine on ^{35}S Accumulation (DPM/ml) in Fetal Blood

Time After Load (hrs.)	Dietary Methionine Content (% of Diet)				
	0.20	0.55	0.80	1.60	3.20
1[1]	3.12^{ab}	2.03^{b}	3.21^{ab}	1.35	3.22^{ab}
2	4.07^{a}	2.20^{b}	2.50^{ab}	2.62	4.84^{a}
4	2.07^{b}	2.90^{ab}	2.72^{ab}	2.30	2.52^{b}
8	2.15^{b}	2.52^{ab}	1.67^{b}	2.37	2.55^{b}
24	3.06^{b}	4.10^{a}	4.01^{a}	3.10	2.87^{b}

[1] Means with same or no superscript not significant ($P < 0.05$) in the same column.

significantly ($P < 0.05$) during the same period (Table 2.27), while the fetal ^{35}S did not appear to change (Table 2.27). The F/M ratio was not affected by dietary methionine content, whether expressed on either a per unit or a total accumulation basis (Table 2.26). Dietary methionine had no effect on the accumulation of ^{35}S/g fetal liver (Table 2.26). No significant effects of dietary methionine level were observed on the percentage of dose absorbed (Table 2.26).

Feeding of the pregnant mother with 1.6% or 3.2% dietary methionine resulted in a significant ($P < 0.05$) decrease in the amount of ^{35}S transferred to the fetal compartment, whether expressed as percentage of dose given to the mother or on the basis of percentage of the dose absorbed by the mother (Table 2.26).

Examination of various portions of the fetal compartment showed that only the fetal liver was affected by maternal dietary methionine level, with the fetuses of mothers fed a methionine deficient diet (0.2%) taking up significantly ($P < 0.05$) less ^{35}S than was the case when 0.8% dietary methionine was fed (Table 2.26).

The significance of these results is discussed following the remaining studies with rats.

Effects of gestational dietary methionine level over three generations of female rats on reproductive performance, and on post weaning performance and response to methionine loading by the male offspring

Objectives:

1. To study the reproductive performance of succeeding generations of female rats exposed to the same dietary methionine level during pregnancy as were their dams.

2. To determine if the gestational dietary methionine level of the female rat would affect the post natal growth of the offspring during the pre-weaning and early post weaning periods.

3. To assess whether the ability of the post weaning male rat to dispose of a loading dose of methionine was affected by the prenatal dietary methionine level of his dam.

4. To establish whether the effects which might occur on post natal performance as a result of gestational dietary methionine level would be repeated in succeeding generations.

Procedures:

Wistar strain females were utilized throughout these studies. Procedures for breeding and the diets fed during pregnancy (Table 2.21) were identical to those of the previous study (page 179). Each female was allowed to terminate her pregnancy naturally and litter size was recorded. Each litter

was reduced to 8 pups as suggested by Chow et al. (1967). The pups were weaned at 21 days of age. Female pups from mothers fed a particular dietary methionine level during pregnancy were placed in a community cage and were fed the standard laboratory ration until they reached breeding weight (175 g). The breeding procedure previously described was then repeated, using Wistar males of 250-300 g which were replaced by new purchases in each generation.

Three male pups from each litter were weighed and placed in individual screened bottom cages for a 21 day period. Each animal was fed laboratory ration ad libitum with water provided at all times. Food intakes and weight gains over the experimental period were recorded. On day 21 the food can was removed at 1700 hours. At 0800 hours the following day each animal was given 50 mg L-methionine (containing 2.5 μCi ^{35}S-methionine) in a total normal saline volume of 1.0 ml by stomach tube. The rat was then replaced in its cage for a 24 hour period and allowed free access to the laboratory ration. Urine was collected for the entire 24 hour period. The rat was lightly anesthetized with ether, decapitated and blood and tissues (Table 2.32) were collected. Tissues were weighed, homogenized and sampled. ^{35}S activity in the samples was determined by the usual procedure (Appendix A). The percentage of administered dose of ^{35}S appearing in the blood after 24 hours was calculated by assuming blood to represent 5.0% of

body weight. For muscle weight, 40% of body weight was adopted. For the remaining tissues, the % dose was calculated using actual weights. Statistical analyses of performance and ^{35}S-distribution data were performed by standard procedures (Steele and Torrie, 1960) with variance components partitioned as outlined in Appendix C. Mean values were compared by Tukey's ω-procedure (Steele and Torrie, 1960).

Results:

No significant differences were noted for litter size, weaning weights, weight gains, or protein efficiency (g gain/ g protein intake) in the F_1 offspring attributable to dietary methionine level fed the pregnant female rat (Table 2.29). The total food intake of the F_1 offspring was significantly ($P < 0.05$) higher for rats whose mothers were fed 1.6% methionine during gestation than for those whose mothers were fed lower dietary methionine levels (Table 2.29). There was also a tendency toward higher food intake among F_1 offspring from mothers fed 3.2% dietary methionine during pregnancy (Table 2.29).

No significant differences were noted in performance in the F_2 generation attributable to maternal gestational methionine level (Table 2.30).

In the F_3 generation a trend toward decreases at the two highest maternal dietary methionine levels was seen for most

TABLE 2.29 Post Natal Performance of Growing Rats as Affected by Prenatal Maternal Dietary Methionine Level. F_1 Generation

Criteria	Dietary Methionine Level (% of Diet)				
	0.2	0.55	0.80	1.60	3.20
Litter Size	9.8 ± 3.3	9.6 ± 3.1	9.9 ± 3.4	9.7 ± 1.6	11.9 ± 1.5
Weaning Wt (g)	46.8 ± 10.0	44.4 ± 6.5	38.9 ± 10.7	46.3 ± 7.2	42.3 ± 7.3
Weight Gain (g)	79.4 ± 18	87.2 ± 20	88.9 ± 24	98.2 ± 25	88.9 ± 17
Total Food Intake (g)	257^{b} ± 45	247^{b} ± 39	239^{b} ± 33	307^{a} ± 60	263^{ab} ± 43
Protein Efficiency (gain/g protein intake)	1.34 ± 0.18	1.60 ± 0.40	1.61 ± 0.42	1.45 ± 0.26	1.50 ± 0.20

TABLE 2.30 Post Natal Performance of Growing Rats as Affected by Prenatal Maternal Dietary Methionine Level. F_2 Generation

Criteria	Dietary Methionine Level (% of Diet)				
	0.20	0.55	0.80	1.60	3.20
Litter Size	9.8 ± 2.5	11.0 ± 1.8	10.0 ± 2.9	9.0 ± 2.8	9.2 ± 1.9
Weaning Wt (g)	39.1 ± 3.3	41.5 ± 5.1	46.9 ± 5.1	43.4 ± 7.5	40.7 ± 6.9
Weight Gain (g)	94.1 ± 24.6	91.7 ± 26.4	104.1 ± 19.7	72.4 ± 14.6	87.0 ± 16.1
Total Food Intake (g)	256 ± 41.8	258 ± 44.7	292 ± 37.2	244 ± 22.4	255 ± 39.5
Protein Efficiency (gain/g protein)	1.62 ± 0.23	1.51 ± 0.23	1.55 ± 0.17	1.28 ± 0.21	1.45 ± 0.21

TABLE 2.31 Post Natal Performance of Growing Rats as Affected by Prenatal Maternal Dietary Methionine Level. F_3 Generation

Criteria	Dietary Methionine Level (% of Diet)				
	0.20	0.55	0.80	1.60	3.20
Litter Size	7.5 ± 2.1	8.0 ± 3.6	11.0 ± 1.9	8.0 ± 2.5	7.6 ± 1.6
Weaning Wt (g)	44.3 ± 6.6	42.6 ± 3.9	45.7 ± 8.6	35.8 ± 5.2	37.5 ± 3.5
Weight Gain (g)	104.4 ± 14.8	97.7 ± 13.9	97.8 ± 12.8	91.1 ± 14.4	85.1 ± 13.4
Total Food Intake (g)	281 ± 16.5	263 ± 20.6	268 ± 37.2	245 ± 28.9	243 ± 23.3
Protein Efficiency (gain/g protein intake)	1.61 ± 0.16	1.61 ± 0.11	1.63 ± 0.11	1.60 ± 0.11	1.50 ± 0.12

criteria of performance, however, none of the criteria showed statistically significant differences among diets (Table 2.31).

No significant changes in tissue uptake or excretion of ^{35}S were observed as a result of maternal dietary methionine level (Table 2.32) in the F_1 generation.

In F_2 generation animals absorption of ^{35}S from the gut apparently increased as methionine content of the maternal diet increased (Table 2.33) as evidenced by lower gut ^{35}S activity 24 hours after loading with ^{35}S-methionine. Of the amount absorbed, those F_2 animals whose mothers were fed either 1.6% or 3.2% methionine in their diets during pregnancy tended to excrete more of the absorbed dose into urine than did those at 0.55% or 0.8% dietary methionine during pregnancy (Table 2.33). In the remaining tissues examined (Table 2.33), the level of ^{35}S activity tended to decrease at the two highest levels of methionine fed, with F_2 generation males whose mothers had been fed 3.2% dietary methionine during pregnancy showing the lowest retention of ^{35}S in those tissues (Table 2.33).

Absorption of ^{35}S from the gut was lowest for F_3 animals whose mothers were fed 1.6% dietary methionine during pregnancy (Table 2.34). The amount of ^{35}S activity in other tissues examined was also highest for those animals on the 1.6% dietary methionine regimen.

Significant differences among litters within diet groups

TABLE 2.32 Effects of Gestational Dietary Methionine Content on Distribution of ^{35}S (% of Dose) in F_1 Generation Tissues 24 Hours after ^{35}S-Methionine Loading

% Dose in	Dietary Methionine Level (% of Diet)				
	0.20	0.55	0.80	1.60	3.20
Gut	8.9 ± 3.9	6.4 ± 3.6	6.5 ± 3.2	6.7 ± 2.2	8.8 ± 3.6
Urine	37.1 ± 15.2	33.5 ± 12.3	37.7 ± 14.1	37.4 ± 15.4	39.7 ± 14.7
Whole Blood	0.99 ± 0.46	0.58 ± 0.34	0.77 ± 0.32	0.80 ± 0.47	1.03 ± 0.33
Brain	0.33 ± 0.16	0.18 ± 0.10	0.24 ± 0.11	0.23 ± 0.09	0.25 ± 0.11
Liver	5.62 ± 3.02	3.86 ± 1.42	4.73 ± 2.40	4.63 ± 2.10	5.42 ± 2.01
Kidney	1.00 ± 0.20	1.16 ± 0.26	0.79 ± 0.34	0.84 ± 0.39	0.96 ± 0.39
Muscle	13.0 ± 5.0	9.2 ± 5.4	10.6 ± 4.6	10.6 ± 3.9	11.1 ± 6.2

TABLE 2.33 Effects of Gestational Dietary Methionine Content on Distribution of ^{35}S (% of Dose) in F_2 Generation Tissues 24 Hours after ^{35}S-Methionine Loading

	Dietary Methionine Level (% of Diet				
% Dose in	0.20	0.55	0.80	1.60	3.20
Gut	8.1^{ab} ± 2.5	9.4^{a} ± 3.4	7.3^{abc} ± 3.2	5.5^{bc} ± 2.7	4.4^{c} ± 0.8
Urine	41.7^{ab} ± 8.0	30.3^{c} ± 9.4	32.8^{bc} ± 11.8	48.1^{a} ± 13.5	44.8^{ab} ± 4.7
Whole Blood	0.98 ± 0.63	1.21 ± 0.54	1.06 ± 0.54	1.32 ± 0.61	0.60 ± 0.41
Brain	0.42^{a} ± 0.21	0.36^{ab} ± 0.15	0.39^{a} ± 0.08	0.23^{ab} ± 0.16	0.17^{b} ± 0.08
Liver	5.48^{ab} ± 2.25	5.85^{a} ± 2.71	4.76^{ab} ± 2.64	4.73^{ab} ± 2.33	2.48^{b} ± 0.80
Kidney	1.05^{a} ± 0.43	1.01^{a} ± 0.36	1.00^{a} ± 0.18	0.65^{ab} ± 0.35	0.39^{b} ± 0.12
Muscle	14.9^{a} ± 5.6	13.2^{ab} ± 5.9	14.7^{a} ± 4.2	6.4^{ab} ± 5.6	5.5^{b} ± 2.5

TABLE 2.34 Effects of Gestational Dietary Methionine Content on Distribution of ^{35}S (% of Dose) in F_3 Generation Tissues 24 Hours after ^{35}S-Methionine Loading

	Dietary Methionine Level (% of Diet)				
% Dose in	0.2	0.55	0.80	1.60	3.20
Gut	7.0^{b} ± 2.2	5.0^{b} ± 2.7	7.9^{ab} ± 0.9	10.3^{a} ± 1.6	7.3^{ab} ± 1.5
Urine	34.2 ± 7.1	29.0 ± 9.6	27.4 ± 6.0	32.7 ± 5.3	30.6 ± 7.7
Whole Blood	1.05^{b} ± 0.11	1.03^{b} ± 0.34	1.31^{b} ± 0.16	1.76^{a} ± 0.34	1.22^{b} ± 0.41
Brain	0.25^{b} ± 0.05	0.24^{b} ± 0.10	0.33^{ab} ± 0.06	0.41^{a} ± 0.05	0.34^{ab} ± 0.06
Liver	4.90^{b} ± 0.8	4.21^{b} ± 1.6	4.41^{b} ± 0.5	7.26^{a} ± 1.5	5.75^{ab} ± 1.7
Kidney	0.90^{b} ± 0.17	0.69^{b} ± 0.24	0.76^{b} ± 0.09	1.20^{a} ± .11	0.86^{b} ± 0.19
Muscle	11.4^{ab} ± 1.9	9.7^{b} ± 3.5	12.8^{ab} ± 1.6	14.5^{a} ± 3.4	13.8^{ab} ± 3.9

were observed for most criteria of performance and in ^{35}S accumulations in the tissues, blood and urine (Appendix C) which may have confounded any effects occurring as a result of maternal dietary methionine level.

Discussion of Studies with the Rat

Depression of growth in the pregnant rat was evident when 1.6% of dietary methionine was fed while food intake depression was not apparent until 3.2% dietary methionine was fed (Table 2.22). A direct toxic effect of methionine in the pregnant rat was therefore evident in the present studies. Similar observations were reported by Beaton (1967) with growing rats.

Leatham et al. (1968) did not observe a reduction in litter size when 4.0% methionine was fed to rats. In the present study, adverse effects resulting from methionine toxicity in the mother did not appear to result in reduced litter size although there was a suggestion of an increased number of resorption sites at the two highest dietary methionine levels (Table 2.22).

At the 1.6% and 3.2% maternal dietary methionine levels, both uterine tissue and placental weights appeared to be reduced (Table 2.22). Placental weight has been shown (Dawes, 1968) to correlate well with fetal growth for humans, thus placental size may indicate placental sufficiency. In the present studies, however, placental weight decreases were not reflected in reduced weights of the fetal compartment until 3.2% methionine was fed. At the highest maternal dietary level the trend toward lowered fetal carcass and liver weights suggested a possible intrauterine growth retardation perhaps

caused by placental insufficiency. Whether this effect was due to methionine per se remains questionable since the food intake of the mother at 3.2% was reduced considerably and this reduction may have caused the decreases in uterine and fetal weights observed. Leatham et al. (1968) reported that 4% methionine in the diet of pregnant rats caused a reduction in fetal weight in pair-fed animals. Both lowered food intake and the toxic effects of methionine may therefore have been involved in the reductions in fetal weight observed (Table 2.22).

The decreases in fetal weight as a result of feeding 3.2% methionine in the diet of the pregnant rat (Table 2.22) did not represent a permanent post natal growth stunting such as observed (Table 1.1) when the maternal dietary protein adequacy was restricted. Rather, some compensatory growth must have occurred in the period between birth and weaning so that at weaning no effects of the prenatal dietary methionine level were observed in weaning weights (Table 2.29). Prenatal dietary methionine level also had no effect on the post weaning performance of male rats, although higher food intakes were apparent for animals whose mothers were fed 1.6% or 3.2% dietary methionine during pregnancy. In succeeding generations no significant effects of maternal dietary methionine on post natal performance were observed. There was, however, a trend toward lowered performance in the third generation. Methionine ingestion during pregnancy in the rat did not therefore have

any obvious lasting adverse effects upon fetal performance.

Following a loading dose of ^{35}S-methionine to the pregnant female, a significant uptake of the label by maternal tissues occurred and only a small proportion of the labelled dose was transported to the fetus (Tables 2.26, 2.27), confirming suggestions made in studies with the sow and ewe regarding the importance of maternal tissue uptake in reduction of plasma methionine elevations.

The clearance of ^{35}S from the fetus was considerably slower than clearance from the mother (Table 2.27) as previously suggested in studies with the sow and ewe. The maternal dietary methionine content prior to dosing appeared to affect the amount of transport of ^{35}S to the fetus but such effects could not be related to the performance changes. Although many tendencies and significant effects were apparent, these effects did not follow any discernable pattern. The distribution patterns for ^{35}S in blood, urine and tissues of growing male rats whose mothers had been exposed to varying dietary methionine concentrations during pregnancy were inconsistent from generation to generation.

In the studies with rats, considerable variation was routinely encountered within groups of animals treated in supposedly identical fashions (Appendix C). Such observations suggested that larger treatment groups may be necessary in studies to allow more statistical control, particularly in long term, multi-generation studies.

General Discussion

Newberne et al. (1970) stated that "There is no ..... evidence to support the assumption that the fetus is immune to nutritional deficiencies ..... the results of numerous clinical and experimental investigations provide evidence that the embryo can be a very sensitive indicator of defects in maternal nutrition and metabolism ..... evidence of a deficit may appear late in the life of the offspring when a reasonably accurate appraisal of cause and effect relationships is tenuous if not impossible." The administration of excess quantities of amino acids to the pregnant female may be considered a maternal nutritional defect which could result in fetal and/or post natal abnormalities. The statement of Newberne et al. (1970) implies that the supply of nutrients crossing the placenta to the fetus may be altered as a result of altered maternal nutrient status.

In order for placental transport of a nutrient to occur, the nutrient must be present in the maternal blood. Maternal blood concentrations of methionine may, therefore, be of considerable importance in controlling the extent of transport of methionine across the placenta to the fetus.

The existence of a "threshold" concentration above which methionine accumulated rapidly in the fetal blood, if a consequence of overburdening of the maternal homeostatic

mechanisms for maintenance of a stable blood methionine level, should be demonstratable in other membrane transport systems of the body also.

McLaughlan (unpublished observations) found that following feeding of a protein to rats, the extent and duration of the elevation of plasma amino acid concentrations increased as the quantity of protein fed increased. In order for the plasma amino acid concentrations to increase, the rate of entry of amino acids into the blood from the intestinal lumen must have been greater than the rate of removal of amino acids from the blood by tissues. If the transport mechanisms for amino acids across the intestinal mucosa were saturated, then the total amount of transport would depend on the interval over which saturation occurred. Since other tissues appear to have a reduced capacity to take up amino acids from blood compared to the transport capacity of the gastrointestinal mucosa, the blood level of amino acids would remain elevated, even after intestinal absorption had been reduced, until the tissues could remove the accumulated amino acids from blood. Data obtained with the pregnant ewe (Table 2.12) indicated that a loading dose of methionine was initially absorbed from the intestine at a more rapid rate than body tissues could dispose of the accumulation in blood, resulting in an elevation of the maternal plasma methionine concentration, ie. exceeding the threshold. For an extended time period following the

initial increase, an elevated stable concentration of maternal blood methionine was maintained, indicating that an equilibrium between entry and removal of methionine from maternal blood had been established. As a result of the elevated blood methionine concentration, transport of methionine into tissues would likely occur at maximal velocity. The amount of transport of methionine into tissues would therefore depend upon the duration of elevation of maternal blood methionine. The extent of transport of methionine into fetal plasma, as indicated by the elevated plasma methionine concentration observed in the fetus, suggested that the ovine placenta avidly accumulated methionine from maternal blood. Transfer of methionine to the fetus from the placenta occurred at a rate which was greater than the ability of the fetus to remove the accumulation from its blood. In this respect the placenta appeared to function in an analagous manner to that of the gastrointestinal tract in the ewe. The placenta may therefore function to effectively reduce fluctuations in fetal blood amino acid concentrations caused by transient fluctuations in maternal plasma level. However, when the maternal plasma level of an amino acid is elevated for an extended period of time, the placental transport systems may act to the detriment of the fetus. A similar proposal was advanced by Kerr et al. (1968).

The ultimate control of fetal blood plasma methionine concentrations appears to rest with the maternal plasma methionine concentration, with the placental role of secondary importance. The placenta may have a more active role under marginal or deficient amino acid supply, although the observations of Slater and Mellor (1972) would indicate that placental ability to regulate the supply of amino acids reaching the fetus is limited under conditions of inadequate protein (amino acid) intake.

Only a small proportion of the ^{35}S-methionine administered to the pregnant rat crossed the placenta. Maternal tissue uptake accounted for most of the ^{35}S absorbed from the gut (Tables 2.26, 2.27). In studies with the sow (Fig. 5), and following intravenous injections of ^{35}S-methionine to the ewe (Fig. 7), only limited amounts of the dose crossed the placenta. These observations, as well as the previous discussion, suggest that the placental amino acid transport mechanisms function in a similar fashion to those of other membrane systems. The similarity of placental amino acid transport systems to those of other tissues was further exemplified by ability to classify the neutral amino acids into two distinct groups on the basis of relative concentrations of these amino acids in maternal and fetal plasma. One group, including valine, isoleucine, leucine and phenylalanine were not markedly different in concentration in the two circulations, which would have been

expected on the basis of their inclusion in the L system of Oxender and Christensen (1963). The other group, including threonine, serine, glycine and alanine existed at considerably higher concentrations in fetal than in maternal blood as a result of their disposition toward uphill transport by the A system described by Oxender and Christensen (1963). Hopkins et al. (1971) and Young (1971) proposed that amino acids transported by the L system (Oxender and Christensen, 1963) when injected intravenously into the ewe at physiological levels resulted in a maternal plasma concentration increase which was reflected in the fetal plasma level (Fig. 12). The elevated plasma level observed in the fetus was maintained for a considerably longer period of time than was the maternal level. When an amino acid belonging to the A system was injected, the fetal level was suggested to not reflect the maternal elevation (Fig. 12). Exactly the opposite effect would have been expected on the basis of Oxender and Christensen's (1963) observations and the F/M ratio data of the present studies.

Close examination of the data of Hopkins et al. (1971b) revealed that an increase in maternal plasma leucine (representative of the L system) concentration of approximately 5 fold resulted in an increase in the fetal plasma level less than 2 fold, and that this response in the fetal blood returned to baseline value within 60 minutes of the maternal intravenous

injection (Fig. 12). Initially the fetal blood concentration was approximately 3 times as high as the maternal concentration, thus when the maternal level returned to baseline within 30 minutes, the slower return to baseline of the elevated leucine concentration in the fetus gave the appearance of an extensive transport of leucine across the placenta. When ornithine (representative of the A system) was injected intravenously, an approximately 5 fold increase in maternal plasma ornithine concentration occurred (Fig. 12). Fetal blood ornithine concentration increased approximately 2 fold, then decreased to the baseline level which was apparently maintained for the duration of the experiment. As the fetal plasma ornithine concentration was approximately equal to that of the maternal blood initially, the 2 fold increase and later fluctuations were not as apparent as those of leucine. In fact, the behavior of the two compounds in fetal blood was not markedly different on a net change basis following maternal intravenous injection. In the studies of Hopkins et al. (1971b) and Young (1971) the effects observed are suggested by this author to be the net result of placental transport and relative rates of extra-placental tissue uptake and release of amino acids. Their data do not therefore appear to support their hypothesis of differences in the relative concentrating abilities of the L and A transport systems in the placenta.

Following methionine loading in the ewe, a marked increase in the fetal plasma methionine concentration occurred which persisted for a much longer period than did the elevation of maternal blood methionine. Similarly in the rat, slower clearance of ^{35}S from the fetal compartment was observed. The limited capacity for transport of methionine from fetal to maternal blood observed in both the Rhesus monkey (Sturman et al., 1971) and the sow (Table 2.3), a relatively low uptake of methionine by the fetal liver (Table 2.1) and a low excretion of methionine into amniotic fluid (Table 2.15) could all contribute to the observed relatively slow clearance of methionine from fetal blood in both the pregnant ewe and rat. Therefore the homeostatic capability of the fetus to maintain its plasma methionine concentration within normal limits appears to be less than that of the mother.

Following administration of high levels of methionine to the pregnant ewe, ^{35}S appeared in taurine, methionine sulfoxide and cysteine as well as the originally labelled ^{35}S-methionine. Whether the appearance of these compounds in fetal plasma and amniotic fluid was a result of transport from the maternal circulation to the fetal circulation or a consequence of fetal metabolism of methionine is questionable. In the light of other investigations (Gaull et al., 1972; Volpe and Laster, 1972), demonstrating a very low activity of the major metabolic pathway responsible for methionine

degradation in the fetus, transport of these methionine metabolites from the maternal circulation to the fetal circulation would appear to be the major means of accumulation of ^{35}S-containing metabolites in the fetus. This hypothesis is reinforced by the apparent inability of the newborn lamb to metabolize methionine (Table 2.20). Changes in the specific activities of ^{35}S-containing metabolites with time varied widely in the fetus and suggested multiple amino acid transport systems in the placenta.

While growth depression caused by excess dietary methionine in the post-natal animal has been well documented (Harper, 1970), the adverse effects of methionine upon growth of the fetus have received less attention. Reductions in fetal weight were reported by Leatham et al. (1968) following feeding of 4% methionine to pregnant rats; later studies by the same workers (Viau et al., 1969) indicated that placental and uterine weights were also reduced when 4% methionine was fed to pregnant rats. In the present studies a dietary level of 1.60% methionine was sufficient to reduce placental and uterine weights. A reduction in fetal weight was not apparent until 3.20% dietary methionine was fed (Table 2.22). Placental weight correlated well with fetal growth (Dawes, 1968), which implies that the transport capacity of the placenta is a function of placental size. The effect of 3.20% dietary

methionine in reducing fetal weight (Table 2.22) may therefore have been a consequence of reduced transport of nutrients to the fetus as a result of reductions in transport capacity of the placenta through reduced size. In contrast to the results of Roeder and Chow (1970) who reported that prenatal methionine deficiency caused permanent growth stunting, the reduced intrauterine fetal weight caused by excess methionine in the present study was not reflected in the post natal period. The mechanism(s) by which intrauterine growth stunting, caused by an excess or a deficiency of methionine, may occur may therefore be different, as appears to be the case postnatally.

Chow et al. (1967) suggested that growth stunting caused by intrauterine protein malnutrition in rats carried over into succeeding generations. No such effects were observed when excess methionine was fed to several generations of pregnant rats in the present studies, further indicating that the fetal growth depression as a result of excess maternal dietary methionine was of a transient nature.

An accumulation of extremely high levels of methionine and its metabolites in fetal blood and amniotic fluid was observed (Table 2.19) following repeated loading of the pregnant ewe. A similar accumulation of methionine and its metabolites may have been responsible for the observed decreases in fetal weight (Table 2.22) in rats fed 3.20% dietary methionine during

pregnancy. Mechanisms by which this decrease might occur remain obscure. Kerr et al. (1968) reported a decrease in the birth weights of infant Rhesus monkeys born to mothers fed high dietary phenylalanine levels during pregnancy. In the post natal period, however, the growth rate of such animals was normal. A similar response pattern to high dietary levels of methionine was observed with rats in the present study.

Kerr et al. (1968) observed significant decreases in the learning ability of infant Rhesus monkeys as a result of feeding excess phenylalanine during gestation. No attempt to estimate possible behavioral changes was made in the present studies with rats. The lamb born immediately following study of placental transport of a large dose of methionine to the ewe (Table 2.20) appeared on visual observation to have altered behavior and, even though it was of normal weight at birth, died of unknown causes 3 days post partum.

The toxic effects of excess methionine administration in producing growth depression of the growing or pregnant rat appeared to occur in the fetus in utero also. As has previously been shown for the post natal animal (Harper, 1970), the effects of excess dietary methionine during pregnancy in depressing growth of the fetus appear to be reversible by removing the animal from exposure to the high methionine level during the post natal period.

No particular metabolic alterations in methionine tolerance were apparent postnatally. The possibility remains that other aspects of metabolism of the rat may have been affected.

In the light of behavioral pattern changes observed to be caused by gestational phenylalanine excess (Kerr et al., 1968), and a suggestion of similar behavior in one lamb in these studies, a re-examination of the toxic effects of methionine to assess possible effects on brain development and function seems to be warranted.

Summary and Conclusions

Under normal physiological conditions the transport of amino acids from maternal to fetal blood across the placenta occurred via transport mechanisms which appeared to be similar to those documented for other tissues (Young and Freedman, 1971). The velocity of the placental transport process for methionine appeared to be comparatively slow and may be saturated by high maternal levels of methionine. The result of this behavior of the placenta was to insure a relatively constant amount of methionine entering the fetal circulation in the face of short term, minor fluctuations in the maternal plasma methionine level.

The ability of the fetus to remove methionine from its blood is much lower than that of the mother, as a consequence of reduced fetal tissue uptake, limited metabolism of methionine, a slow excretion into amniotic fluid, and a limited capacity for transport of methionine from fetus to mother relative to that occurring in the reverse direction. This slower rate of clearance from fetal blood may represent the means by which fetal blood amino acid concentrations are maintained in an elevated state relative to the maternal blood, as shown by many investigators (cited by Curet, 1970).

Upon marked elevation of the maternal plasma methionine

level and maintenance of this maternal elevation for an extended interval, the rate of transport of methionine to the placenta may have increased to the saturation point of the transport mechanism(s). At this rate of transport, the rate of entry of methionine into the fetal circulation appeared to be much greater than the ability of the fetus to clear the methionine from its plasma, thus an accumulation of high levels of methionine in fetal blood occurred. Under conditions of prolonged elevation of maternal plasma methionine concentration, the maternal plasma methionine concentration was the primary regulator of the fetal plasma methionine level, with the placenta acting as a transport system which may be detrimental to fetal well being. The placenta therefore appears to function in a similar manner to other maternal body tissues, and does not provide an effective means by which the fetus may be protected from the effects of maternal nutritional stress.

Feeding pregnant rats high dietary methionine levels resulted in growth depression of both the mother and fetus, confirming earlier work (Viau et al., 1969; Leatham et al., 1968). The fetal growth depression was not a permanent stunting effect, however, and no post weaning effects of the prenatal dietary methionine level were seen. The possibility remains that toxic effects of prenatal exposure to high concen-

trations of methionine may not have been manifested in performance traits or ability to handle a methionine load. Further investigations of possible post natal behavioral effects of such a prenatal regimen would be in order, in view of the observation of impaired learning ability caused by excess gestational phenylalanine feeding (Kerr et al., 1968). The mechanism(s) by which methionine exerted growth depressing effects on the fetus and mother remain obscure. If the mechanism(s) of growth depression in the pre- and post natal animal are identical, direct injection of methionine to the fetus may provide another avenue of approach toward elucidation of these mechanisms. Since metabolic capabilities and ability to clear high methionine concentrations from blood appear to be much less in the fetus than in the adult, the fetus may represent a simpler *in vivo* system than the adult for such studies.

Although the administration of methionine to the pregnant rat did not result in permanent post natal growth stunting of the offspring, the possibility of other adverse effects such as behavioral abnormalities cannot be discounted. In view of the high accumulation of methionine and its metabolites in fetal blood, effects on the developing brain which were not detected in these studies may have occurred and should be investigated. Until such time as these areas have been investigated, the administration of large amounts

of methionine,as proposed by Downes et al. (1970) and the National Formulary (1970), may be a questionable practice for the pregnant female.

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APPENDICES

APPENDIX A Experimental Methods and Procedures

Collection of Samples

In both the sheep and the swine studies, blood and amniotic fluid samples were collected aseptically via the cannulas using a syringe and transferred to heparinized tubes. The cannulas were filled between samplings with sterile heparinized saline. Three times the volume of the cannula was withdrawn prior to sample withdrawal to minimize dilution of the sample with saline. The samples were prepared for analysis as described in the following section.

To facilitate collection of samples from rats, the animal was lightly anesthetized with ether and decapitated. Blood was collected in heparinized tubes following decapitation. Tissue samples were removed immediately, weighed and prepared for analysis.

During transport studies with the rat, the entire uterus was removed immediately upon decapitation and the fetal samples obtained as rapidly as possible. The maternal samples were obtained in minimum time and prepared for analysis.

Sample Preparation for Analyses

1. Blood and amniotic fluid samples for amino acid analysis combined with flowthrough liquid scintillation spectrometry.

Red blood cells were removed by centrifugation in a Model

CL clinical centrifuge (International Equipment Co., Needham, Mass.) run at maximum speed for 10 minutes. The plasma was removed by aspiration with the aid of a Pasteur disposable pipette (Fisher Scientific Co., Montreal, Quebec), using a separate pipette for each sample. One ml of plasma or amniotic fluid was mixed with 0.5 ml of 20%(w/v)aqueous trichloroacetic acid. This mixture was centrifuged at maximum velocity for 10 minutes in a clinical centrifuge. The supernatent was aspirated with a Pasteur pipette and was immediately stored at -30° C. Prior to analysis the sample was thawed and a 0.75 ml aliquot of sample combined with 0.075 ml of aqueous norleucine solution (1 μM/ml), 0.075 ml ^{14}C-DL-phenylalanine (ring-1-^{14}C labelled, New England Nuclear Canada Ltd., Dorval, Quebec), and 0.600 ml sodium citrate buffer (0.2 N, pH 2.2).

2. Body fluid (blood, amniotic fluid, urine) and tissue samples for discrete sample liquid scintillation spectrometry (counting).

All tissues were homogenized in a Lourdes tissue homogenizer (Canadian Laboratory Supplies, Ltd., Ottawa, Ont.) prior to sampling. Aliquots (0.2 ml) of body fluid were taken for analysis while for tissues the sample size was approximately 150 mg. Each sample was digested and prepared for liquid scintillation counting by the method of Mahin and Lofberg (1966) with the following modifications: the digestion period was

increased to overnight (approximately 18 hours) and the digestion temperature was maintained at 72°C. Perchloric acid was used at a concentration of 72%. The scintillation cocktail used was 1:1 v/v toluene-ethylene glycol monomethyl ether, containing 6 g PPO (Packard Instrument Co., Inc., Downers Grove, Ill.) per litre toluene.

Analytical Methods

1. Amino acid analyses

All amino acid analyses were performed using Beckman amino acid analyzers (Beckman Instruments, Inc., Palo Alto, Calif.). Most analyses were undertaken with the Model 116 analyzer, however, the Model 121 amino acid analyzer was used for analysis of samples in later stages of the investigations. Both amino acid analyzers were operated under standard procedures for analysis as outlined in the Beckman instruction manual. Except where otherwise presented, only the acidic and neutral amino acids were determined. Chromatograms were integrated manually by the HW method described in the instruction manual. Concentrations were calculated by comparing areas of sample peaks to those of known concentrations. Norleucine was utilized as an internal standard.

2. Amino acid analysis - flowthrough liquid scintillation counting

The usual transparent effluent line from the ion exchange

column of the amino acid analyzer was replaced with black teflon line of the same diameter to minimize transmission of light to the flow cell of the liquid scintillation counter. The exit line from the flow cell to the amino acid analyzer reagent mixing block was also black teflon. The flow cell was a commercially available (Packard Instrument Co., Inc., Downers Grove, Ill.) model. Details of construction of such have been discussed by Schram (1970). The flow cell was packed with anthracene as described by Schram (1970). The cell was inserted into the well of an automated Packard model 3320 liquid scintillation counter which printed radioactivity at 2 minute intervals. Efficiency of the system was determined from comparison of observed radioactivity of a known sample of ^{35}S-methionine with the true radioactivity determined from discrete sample liquid scintillation counting as described in the following method. ^{14}C-phenylalanine added to the blood and amniotic fluid as described under Sample Preparation (Page 247) was utilized as a reference standard to relate the position of ^{35}S as determined by liquid scintillation counting to the position of amino acids on the chromatogram.

3. Discrete sample liquid scintillation counting

Following preparation (page 247) samples were placed in the Packard model 3320 liquid scintillation spectrometer,

where they were allowed to cool for 1 hour prior to counting. The counting interval varied with the amount of radioactivity detected to allow reduction of variability due to low levels of radioactivity (Packard Instruction Manual). Disintegrations per minute (DPM) were calculated from counts per minute by the channels ratio method (Packard Instrument Manual).

APPENDIX B Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Maternal and Fetal Rat

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
Litter Size	Total	13.89	82			
	Diet	16.34	4	1.19	2.49	3.57
	Error	13.76	78			
Maternal						
Weight gain	Total	1207.46	29			
	Diet	5711.50	4	16.70**	2.76	4.18
	Error	341.92	25			
Food intake	Total	2654.19	29			
	Diet	12529.00	4	16.58**	2.76	4.18
	Error	755.72				
Gain/g intake	Total	0.0029	29			
	Diet	0.0505	4	14.85**	2.76	4.18
	Error	0.0034	25			
Liver wt.- % body wt.	Total	0.225	82			
	Diet	0.438	4	2.047	2.49	3.57
	Error	0.214	78			
Kidney wt.- % body wt.	Total	0.019	82			
	Diet	0.040	4	2.22	2.49	3.57
	Error	0.018	78			

* Statistically significant at $P < 0.05$

** Statistically significant at $P < 0.01$

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APPENDIX B Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Mother and Fetal Rat (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
Maternal (continued)						
Brain wt.- % body wt.	Total	0.025	82			
	Diet	0.066	4	3.00*	2.49	3.57
	Error	0.022	78			
Uterus wt.- % body wt.	Total	0.187	82			
	Diet	0.495	4	2.88*	2.49	3.57
	Error	0.172	78			
Fetal						
Compartment wt.	Total	0.92	82			
	Diet	2.55	4	3.11*	2.49	3.57
	Error	0.82	78			
Placenta wt.	Total	0.012	77			
	Diet	0.068	4	7.57**	2.49	3.57
	Error	0.009	73			
Carcass wt.	Total	0.999	75			
	Diet	2.02	4	2.15	2.49	3.57
	Error	0.941	71			
Liver wt.	Total	0.036	76			
	Diet	0.016	4	0.43	2.49	3.57
	Error	0.037	72			

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APPENDIX B Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on ^{35}S-Distribution in the Mother and Fetal Rat

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
DPM/ml/Dose in Maternal Blood	Total	2.02	68			
	Diet (D)	0.99	4	0.74	2.60	3.78
	Time (T)	16.87	4	12.57**	2.60	3.78
	DxT	0.45	16	0.34	1.90	2.48
	Error	1.342	44			
DPM/ml/Dose in Fetal Blood	Total	1.53	60			
	Diet (D)	1.39	4	2.14**	2.65	3.91
	Time (T)	3.90	4	6.01**	2.65	3.91
	DxT	2.94	16	4.52**	1.96	2.60
	Error	0.65	36			
Blood F/M Ratio	Total	0.76	60			
	D	0.81	4	2.19**	2.65	3.91
	T	4.62	4	12.49**	2.65	3.91
	DxT	0.65	16	1.76	1.96	2.60
	Error	0.37	36			
F/M ratio for ^{35}S total	Total	0.057	58			
	D	0.011	4	0.18	2.66	3.92
	T	0.140	4	2.33	2.66	3.92
	DxT	0.041	16	0.68	1.97	2.62
	Error	0.060	34			

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APPENDIX B Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
DPM/g/Dose in Maternal Liver	Total	0.143	69			
	D	0.396	4	5.43**	2.60	3.78
	T	0.652	4	8.93**	2.60	3.78
	DxT	0.149	16	2.04*	1.90	2.48
	Error	0.073	45			
DPM/g/Dose in Fetal Liver	Total	0.033	62			
	D	0.033	4	0.92	2.62	3.87
	T	0.052	4	1.44	2.62	3.87
	DxT	0.020	16	0.56	1.93	2.54
	Error	0.036	38			
% Dose in Fetal Compartment	Total	41.87	68			
	D	124.21	4	3.27*	2.60	3.78
	T	13.55	4	0.36	2.60	3.78
	DxT	38.92	16	1.02	1.90	2.48
	Error	37.98	44			
% Dose Absorbed	Total	269.78	69			
	D	279.5	4	1.19**	2.60	3.78
	T	929.4	4	3.94*	2.60	3.78
	DxT	198.0	16	0.84	1.90	2.48
	Error	235.8	45			

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APPENDIX B Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
% of Absorbed Dose Transported to Fetus	Total	81.27	68			
	D	258.67	4	3.97*	2.60	3.78
	T	69.19	4	1.06	2.60	3.78
	DxT	84.17	16	1.29	1.90	2.48
	Error	65.18	44			
% of Fetal Activity in Placenta	Total	13.26	63			
	D	14.76	4	1.04	2.62	3.87
	T	17.26	4	1.22	2.62	3.87
	DxT	9.86	16	0.70	1.93	2.54
	Error	14.17	39			
% of Fetal Activity in Blood	Total	0.455	62			
	D	0.879	4	1.87	2.62	3.87
	T	0.626	4	1.34	2.62	3.87
	DxT	0.274	16	0.58	1.93	2.54
	Error	0.469	38			
% of Fetal Activity in Carcass	Total	192.27	62			
	D	292.79	4	1.91	2.62	3.87
	T	365.86	4	2.39	2.62	3.87
	DxT	216.25	16	1.41	1.93	2.54
	Error	153.32	38			

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APPENDIX B Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
% of Fetal Activity in Liver	Total	3.41	62			
	D	9.85	4	3.99*	2.62	3.87
	T	7.45	4	3.02	2.62	3.87
	DxT	3.02	16	1.22	1.93	2.54
	Error	2.47	38			
% Dose in Maternal Liver	Total	15.27	69			
	D	37.82	4	4.46*	2.60	3.78
	T	73.43	4	8.65	2.60	3.78
	DxT	14.16	16	1.67	1.90	2.48
	Error	8.49	45			
% Dose in Maternal Kidney	Total	0.176	67			
	D	0.184	4	0.96	2.61	3.79
	T	0.264	4	1.38	2.61	3.79
	DxT	0.107	16	0.56	1.91	2.49
	Error	0.192	43			
% Dose in Maternal Brain	Total	0.0092	68			
	D	0.0049	4	3.98*	2.60	3.78
	T	0.0169	4	1.37	2.60	3.78
	DxT	0.0069	16	0.56	1.93	2.48
	Error	0.0123	44			

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APPENDIX B Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on ^{35}S-Distribution in the Mother and Fetal Rat (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
% Dose in Maternal Blood	Total	3.70	69			
	D	12.12	4	7.58*	2.60	3.78
	T	23.88	4	14.93*	2.60	3.78
	DxT	2.46	16	1.54	1.90	2.48
	Error	7.60	45			
% Dose in Uterus	Total	0.314	63			
	D	0.392	4	1.87*	2.62	3.87
	T	0.968	4	4.62*	2.62	3.87
	DxT	0.388	16	1.85	1.93	2.54
	Error	0.210	39			
% Dose in Muscle	Total	47.02	69			
	D	25.48	4	0.57*	2.62	3.87
	T	164.35	4	3.68*	2.62	3.87
	DxT	29.81	16	0.67	1.93	2.54
	Error	44.63	39			

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APPENDIX C Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
F_1 Generation						
Litter Size	Total	10.38	39			
	Diet	12.75	4	1.26	2.65	3.93
	Error	10.11	35			
Weaning Wt.	L	217.00	39			
	D	251.25	4	1.18**	2.65	3.93
	L/D	213.09	35	13.56**	1.62	1.98
	A/L	15.71	80			
	Total	81.68	119			
Weight gain	L	1126.82	39			
	D	1067.50	4	0.94**	2.65	3.93
	L/D	1133.60	35	6.03**	1.62	1.98
	A/L	187.94	80			
	Total	495.64	119			
Total Food Intake	L	4547.3	39			
	D	16742.0	4	5.31**	2.65	3.93
	L/D	3153.57	35	1.91*	1.62	1.98
	A/L	1649.34	80			
	Total	2599.08	119			

* Statistically significant at $P < 0.05$

** Statistically significant at $P < 0.01$

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APPENDIX C Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
F_1 Generation (continued)						
Protein Efficiency	L	0.266	39			
	D	0.315	4	1.212**	2.65	3.93
	L/D	0.260	35	7.027	1.62	1.98
	A/L	0.037	80			
	Total	0.112	119			
% Dose in Gut	L	25.99	39			
	D	39.78	4	1.63**	2.65	3.93
	L/D	24.40	35	3.86	1.62	1.98
	A/L	6.32	80			
	Total	12.76	119			
% Dose in Urine	L	557.78	39			
	D	121.75	4	0.20**	2.65	3.93
	L/D	607.62	35	64.37	1.62	1.98
	A/L	9.44	80			
	Total	189.14	119			
% Dose in Blood	L	0.456	39			
	D	0.780	4	1.81**	2.65	3.93
	L/D	0.431	35	10.26	1.62	1.98
	A/L	0.042	80			
	Total	0.178	119			

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APPENDIX C Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
F_1 Generation (continued)						
% Dose in Brain	L	0.039	39			
	D	0.067	4	1.86**	2.65	3.93
	L/D	0.036	35	9.00	1.62	1.98
	A/L	0.004	80			
	Total	0.015	119			
% Dose in Liver	L	13.80	39			
	D	11.52	4	0.82**	2.65	3.93
	L/D	14.06	35	15.62	1.62	1.98
	A/L	0.90	80			
	Total	5.13	119			
% Dose in Kidney	L	0.44	39			
	D	0.57	4	1.36**	2.65	3.93
	L/D	0.42	35	10.50	1.62	1.98
	A/L	0.04	80			
	Total	0.17	119			
% Dose in Muscle	L	60.97	39			
	D	46.75	4	0.75**	2.65	3.93
	L/D	62.60	35	5.86	1.62	1.98
	A/L	10.68	80			
	Total	27.16	119			

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APPENDIX C Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
F_2 Generation						
Litter Size	Total	5.63	24			
	Diet	3.10	4	0.51	2.87	4.43
	Error	6.13	20			
Weaning Weight	L	106.11	24			
	D	134.44	4	1.34**	2.87	4.43
	L/D	100.45	20	10.13	1.80	2.29
	A/L	9.92	50			
	Total	41.12	74			
Weight gain	L	1334.47	24			
	D	2012.37	4	1.68**	2.87	4.43
	L/D	1198.89	20	7.53	1.80	2.29
	A/L	159.33	50			
	Total	540.46	74			
Total Food Intake	L	4318.93	24			
	D	4798.43	4	1.14**	2.87	4.43
	L/D	4223.03	20	2.82	1.80	2.29
	A/L	1496.85	50			
	Total	2412.12	74			

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APPENDIX C Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
F_2 Generation (continued)						
PER	L	0.13	24			
	D	0.24	4	2.18**	2.87	4.43
	L/D	0.11	20	3.93**	1.80	2.29
	A/L	0.028	50			
	Total	0.062	74			
% Dose in Gut	L	18.21	24			
	D	59.38	4	5.96**	2.87	4.43
	L/D	9.97	20	1.47*	1.80	2.29
	A/L	6.77	50			
	Total	10.48	74			
% Dose in Urine	L	284.58	24			
	D	890.37	4	5.45**	2.87	4.43
	L/D	163.42	20	1.80	1.80	2.29
	A/L	90.79	50			
	Total	153.64	74			
% Dose in Blood	L	0.98	24			
	D	1.15	4	1.22**	2.87	4.43
	L/D	0.94	20	11.75**	1.80	2.29
	A/L	0.08	50			
	Total	0.37	74			

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APPENDIX C Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
F_2 Generation (continued)						
% Dose in Brain	L	0.063	24			
	D	0.173	4	4.22**	2.87	4.43
	L/D	0.041	20	4.00*	1.80	2.29
	A/L	0.014	50			
	Total	0.030	74			
% Dose in Liver	L	11.17	24			
	D	25.75	4	3.12*	2.87	4.43
	L/D	8.25	20	1.92	1.80	2.29
	A/L	4.31	50			
	Total	6.54	74			
% Dose in Kidney	L	0.383	24			
	D	1.253	4	5.96**	2.87	4.43
	L/D	0.209	20	3.32*	1.80	2.29
	A/L	0.063	50			
	Total	.167	74			
% Dose in Muscle	L	105.33	24			
	D	316.85	4	5.03**	2.87	4.43
	L/D	63.02	20	5.95*	1.80	2.29
	A/L	10.59	50			
	Total	41.32	74			

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APPENDIX C Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
F_3 Generation						
Litter Size	Total	7.95	24			
	Diet	10.66	4	1.44	2.87	4.43
	Error	7.41	20			
Weaning Wt.	L	137.09	24			
	D	264.40	4	2.37**	2.87	4.43
	L/D	111.63	20	14.59	1.80	2.29
	A/L	7.65	50			
	Total	49.63	74			
Weight gain	L	472.00	24			
	D	826.1	4	2.05*	2.87	4.43
	L/D	401.2	20	2.06	1.80	2.29
	A/L	195.3	50			
	Total	240.36	74			
Total Food Intake	L	2056.28	24			
	D	3813.00	4	2.24**	2.87	4.43
	L/D	1704.88	20	4.81	1.80	2.29
	A/L	354.23	50			
	Total	906.24	74			

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APPENDIX C Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
F_3 Generation (continued)						
PER	L	0.031	24			
	D	0.038	4	1.27**	2.87	4.43
	L/D	0.030	20	2.50**	1.80	2.29
	A/L	0.012	50			
	Total	0.017	74			
% Dose in Gut	L	54.00	24			
	D	54.00	4	6.51**	2.87	4.43
	L/D	8.30	20	251.52**	1.80	2.29
	A/L	0.033	50			
	Total	5.19	74			
% Dose in Urine	L	111.99	24			
	D	111.8	4	0.99**	2.87	4.43
	L/D	112.0	20	3.78**	1.80	2.29
	A/L	29.66	50			
	Total	56.36	74			
% Dose in Blood	L	0.34	24			
	D	1.31	4	8.73**	2.87	4.43
	L/D	0.15	20	1.25	1.80	2.29
	A/L	0.12	50			
	Total	0.19	74			

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APPENDIX C Statistical Analysis Results - Effect of Maternal Gestational Dietary Methionine Level on Performance and ^{35}S-Distribution in the Offspring (continued)

Criterion	Source of Variation	Mean Square	DF	F observed	F 0.05	F 0.01
F_3 Generation (continued)						
% Dose in Brain	L	.018	24			
	D	.070	4	10.00**	2.87	4.43
	L/D	.007	20	3.500	1.80	2.29
	A/L	.002	50			
	Total	0.008	74			
% Dose in Liver	L	7.40	24			
	D	23.2	4	5.45**	2.87	4.43
	L/D	4.26	20	6.17**	1.80	2.29
	A/L	0.69	50			
	Total	2.87	74			
% Dose in Kidney	L	.135	24			
	D	.560	4	11.20**	2.87	4.43
	L/D	.050	20	2.38*	1.80	2.29
	A/L	.021	50			
	Total	.058	74			
% Dose in Muscle	L	28.87	24			
	D	67.15	4	3.17*	2.87	4.43
	L/D	21.20	20	5.42**	1.80	2.29
	A/L	3.91	50			
	Total	12.14	74			

APPENDIX D Amino Acid Composition of Basal Rat Diet

Amino Acid	% of Diet
Asp	1.79
Thr	0.43
Ser	0.53
Glu	3.60
Pro	0.64
Gly	0.57
Ala	0.51
Val	0.64
Cys	-
Met	0.16
Ileu	0.70
Leu	1.12
Tyr	0.50
Phe	0.70
Lys	1.18
His	0.45
Arg	1.15