THE RELATIONSHIP OF DIET TO THE INCIDENCE OF CLINICAL SIGNS OF FOLATE DEFICIENCY DURING PREGNANCY IN PRIVATE AND CLINIC PATIENTS

by

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A thesis submitted to the Faculty of Graduate Studies
and Research in partial fulfilment of the requirements
for the degree of Master of Science

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McGill University,
Montreal.

August 1967

Suggested abbreviated title:

DIET AND FOLATE DEFICIENCY DURING PREGNANCY

M.Sc. Nutrition

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ABSTRACT

The Relationship of Diet to the Incidence of Clinical Signs of Folate Deficiency During Pregnancy in Private and Clinic Patients

Seven-day dietary records were used to investigate dietary habits of private and clinic pregnant women. Statistically significant differences between groups were found in the consumption of Calories and all nutrients except carbohydrate. Intakes of iron by clinic subjects in late pregnancy were below those recommended in the Canadian Dietary Standard. Diets of private patients provided greater quantities of both folate and B_{12} than those of clinic patients. The better quality of diets of private patients was attributed to a higher socio-economic status. Diets of "normal" and "folate-deficient" subjects were not significantly different. No high correlation was found between any dietary components and clinical parameters of folate nutrition. The incidence of folate deficiency was the same in both groups, suggesting that in addition to diet, other factors are involved in the development of the deficiency during pregnancy.

ACKNOWLEDGMENTS

The author wishes to express her sincere gratitude to Prof. Helen R. Neilson who acted as research director and whose invaluable guidance throughout this study was very much appreciated.

The author is indebted to Dr. L. Lowenstein and the staff of the Hematology Department of the Royal Victoria Hospital, Montreal, for supplying clinical data and other relevant information.

Special thanks are extended to Dr. L. E. Lloyd, Chairman of the Animal Science Department of Macdonald College, McGill University, whose advice and constructive criticism was most valued; to Mrs. M. Lister, medical technologist in charge of the Hematology Laboratory, Royal Victoria Hospital, who provided helpful assistance in dealing with the hematological data; and to Mrs. J. Vauthier for the accurate transcription of this thesis.

The author acknowledges receipt of financial support from the National Institute of Health (U.S.) during the first year of this study and from Quebec Agriculture Research Council during the second year.

Finally, the author wishes to say how grateful she is to all the subjects of this investigation and to private obstetricians for willing cooperation.

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I. GENERAL INTRODUCTION

The high incidence of clinical signs of folate deficiency in pregnancy, ranging from a very mild deficit evidenced by low serum folate levels to severe megaloblastic anaemias, has motivated the extensive studies on folate nutrition undertaken in the last several years.

Lowenstein et al. (1966a) reported an incidence of 48% of low blood folate levels and 25% of early megaloblastosis in an anaemic (iron-deficient) pregnant population attending the ante-partum clinic of the Royal Victoria Hospital in Montreal. Subsequently, the same researchers investigated in detail the hematologic and nutritional status of a large group of normal women from a comparable population (Lowenstein et al., 1966b). The hematologic study revealed the incidence of low blood folate levels to be approximately 50% in the patients unsupplemented with folic acid or vitamin B_{12} and megaloblastic bone-marrow changes occurred in 26% of the cases. The fact that megaloblastosis and low blood folates appeared as frequently in non-anaemic as in anaemic pregnant women suggested that an insufficient intake, excessive requirements and/or altered metabolism of folate was common in pregnancy. The dietary study of the non-anaemic pregnant population, performed by Moscovitch (1965) showed a suboptimal intake of total Calories, iron and folate, although the latter did not correlate significantly with the development of megaloblastic bone-marrow changes or with low blood folate values.

In order to confirm and extend these findings, it was decided by Lowenstein and co-workers to investigate a group of pregnant women under the care of private physicians. Based on the assumption that the two populations were from different socio-economic classes, the hypothesis that the incidence of folate deficiency signs would be lower, due to a better plane of nutrition, had to be tested. The author undertook the study of the dietary intake of these private patients; a sample of the clinic population was also surveyed. The results of the above mentioned investigation and of the concomitant hematologic findings are presented in this thesis.

II. LITERATURE REVIEW

A. FOLIC ACID NUTRITION IN PREGNANCY

1. Nature of Folic Acid and Naturally Occurring Forms of the Vitamin

It is now established that folic acid, previously identified as norite eluate factor (Snell, 1940), vitamin Bc (Pfiffner et al., 1945), L. casei factor from liver (Stokstad, 1943) and L. casei factor from fermentation residue (Hutchings et al., 1944), is a combination of glutamic acid, para-amino-benzoic acid and a pteridine nucleus. Folic acid is said not to occur in nature (Rabinowitz and Himes, 1960) although the subject remains controversial (Baker et al., 1964; Butterworth et al., 1963). It was first obtained synthetically by Angier et al. (1945).

The term "folate" is usually applied to folic acid (pteroyl-glutamic acid) and all its derivatives. Members of the folate group include pteroyl-triglutamic acid, pteroyl-heptaglutamic acid and the reduced forms: citrovorum factor (5-formyl tetrahydro-folic acid), heat labile 10-formyl tetrahydro-folic acid (Albrech and Broquist, 1956), 5-methyl tetrahydro-folic acid, the major folate compound of mammalian liver (Larrabee et al., 1961) and a few others.

Since the discovery of the vitamin, rapid progress has been made in the elucidation of the structure and distribution of the naturally occurring folates. Pteroyl-triglutamic acid was isolated from a variety of materials of animal and bacterial origin (Jukes and Stokstad, 1948) and pteroyl-heptaglutamic acid from yeast (Pfiffner et al.,

1945). Citrovorum factor occurs in natural materials in both free (monoglutamate) and combined (polyglutamate) forms. However, tetrahydrofolates, because of their susceptibility to oxidation, are found in relatively small amounts in nature.

2. Metabolic Functions of Folate

The folic acid group of vitamins are essential for many animal species including man. Their main function is to provide a powerful stimulus to blood cell formation and maturation through the enzymatic reactions involving folic acid.

The folic acid coenzymes participate in a variety of one-carbon transfer reactions, most of which have been elucidated. It is generally accepted that the tetrahydro-folates are the active coenzymes. Some of the better known reactions in which the folate coenzymes function are listed below:

- Interconversion of glycine and serine (Huennekens and Osborn, 1959; Peters and Greenberg, 1957).

- Conversion of homocysteine to methionine (Doctor et al., 1957; Nakao and Greenberg, 1958).

- Phenylalanine oxidation to tyrosine (Kaufman, 1959).

- Synthesis of purines (Hartman and Buchanan, 1959) and pyrimidines (Greenberg and Humphreys, 1958).

- Nucleic acid synthesis (Niewig et al., 1954).

3. Metabolic Interrelationships of Folate

a. Vitamin B_{12}

The ability of patients with vitamin B_{12} deficiency to derive

hematologic benefit from large doses of folic acid (Jandl and Lear, 1956) and vice versa (Zalusky et al., 1962) provided evidence that folate and vitamin B_{12} were interrelated in blood formation. The exact site of this interaction, which has given rise to various speculative theories (Noronha and Silverman, 1961; Herbert and Zalusky, 1962; Cooperman and Luhby, 1963; Gellene et al., 1964), is not as yet elucidated.

Observations of Dickerman et al. (1964) and Foster (1966) provided strong evidence that in most biological systems, both folate and vitamin B_{12} were involved in methionine biosynthesis. Furthermore, the possible need for B_{12} for adequate storage of folate was indicated by Cox et al. (1958) who found that the excretion of folic acid in the urine following a test dose was lower in pernicious anaemia patients than in normal patients. Concomitently, Dawborn et al. (1958) observed low liver levels of folic acid and citrovorum factor in vitamin B_{12} deficient sheep.

In view of the close relationship existing between folic acid and vitamin B_{12} metabolism, a relationship also exhibited in signs of deficiency of either vitamin, some aspects of the metabolism of vitamin B_{12} will be reviewed briefly.

Vitamin B_{12}, in either native or crystalline form, is poorly absorbed from the gastrointestinal tract of even clinically healthy subjects (Siegel et al., 1961; Sullivan and Herbert, 1965). However, Heyssel et al. (1966) estimated that normal adults eating a diet containing 5 to 15 μg. of the vitamin (Chung et al., 1961) absorbed at least 5 μg. daily and that there may be an age-related decrease in the ability to absorb vitamin B_{12}. An increased absorption was

observed by Siegel et al. (1961) when the vitamin was offered after feeding rather than on an empty stomach. Glass et al. (1954) showed that the percentage of utilization of B_{12} followed a regression curve, decreasing with increasing dosage levels, especially when the latter were beyond normal physiological range.

Since a minimal daily intake of 0.6 to 1.2 g. of vitamin B_{12} seemed adequate to maintain health and normal hemopoiesis in normal subjects operating with low body stores, Boziam et al. (1963) proposed 3 to 5 g. as the dietary requirement for a population. However, any additional needs imposed by pregnancy are not known.

Considerable amounts of vitamin B_{12} activity are destroyed by cooking, ranging from 23.7 to 89.6% (Banerjee and Chatterjea, 1963). Consequently, estimates of intake based on analysis of food prior to cooking should be interpreted with care.

Vitamin B_{12} deficiency, manifested by subnormal serum B_{12} concentrations (less than 120 g. per ml.), was shown by a number of workers to develop in strict vegetarians (Wokes et al., 1955; Mehta et al., 1964; Hines, 1966), and is due to the absence of animal protein in the diet, the major source of vitamin B_{12} (Herbert, 1963b). Serum vitamin B_{12} levels of pregnant women were shown to be significantly lower than those of non-pregnant normal women (Lowenstein et al., 1960), suggesting some effect of pregnancy upon the metabolism of this vitamin.

b. Iron

Vitale et al. (1966), studying the effects of iron deficiency on folate metabolism in rats, observed concurrent biochemical and

morphological changes due to folate deficiency, which he attributed to the decreased activity of the iron-dependent enzyme formimino-transferase. Similarly, Velez et al. (1966) reported that iron deficiency produced a secondary folate deficiency. Chanarin (1966) suggested that iron deficiency might, of itself, adversely affect folate status and be of some importance in the pathogenesis of folate deficiency anaemia in pregnancy. However, Lowenstein (1966) maintained that the incidence of macrogranulocytis was not significantly affected by the administration of iron; the author based his view upon observation of the same incidence of folate deficiency in iron-deficient and in iron supplemented patients.

c. Ascorbic acid

Ascorbic acid is functionally related to folic acid, not in actually stimulating the transformation of folic acid to citrovorum factor, but merely in protecting the citrovorum factor from destruction (Doctor, 1958). Herbert (1959) recognized the possibility of inadequate utilization of folic acid due to ascorbic acid deficiency.

d. Other vitamins

Riboflavin may be involved in folate metabolism. Foy and co-workers (1964, 1966) observed a decrease in serum folate and B_{12} but no megaloblasts in riboflavin deficient baboons; the addition of riboflavin to the diet produced a decrease in serum B_{12} and a rise in serum folate values. Havely and Guggenheim (1957) observed that rats deficient in vitamin A, pyridoxine and riboflavin, were less able to retain folic acid and citrovorum factor in their livers than normal rats; moreover, the conversion of folic acid to citrovorum factor seemed to be impaired

in deficiency of thiamine, pyridoxine and riboflavin. These findings are in contrast to work by Scott and Griffith (1958) who observed no interaction in growing rats between folic acid and any of the following vitamins - thiamine, riboflavin, pyridoxin and pantothenate.

4. Dietary Folate and Assays for Folic Acid Activity in Foods

a. Nature of food folate

The folic acid found in foods of vegetable origin appears to be in one or other of the conjugated forms (Toepfer et al., 1951; Stokstad, 1954), while much of the folate in foods of animal origin, especially liver, is in the form of the reduced monoglutamate with a methyl group in the N^5 position (Herbert and Zalusky, 1962). The former points out to the need of liberating folic acid from the conjugates during the digestion process.

b. Folate content of human dietaries

Most estimates of dietary folate activity are based on the tables published by Toepfer et al. (1951). However, it is likely that data for folic acid content of foodstuffs is too high because the microbiological assay organisms used in determining these values were shown to respond to a number of non-folate materials in food extracts. These compounds with folic acid-like activity in bacteriological assays may be inactive in human nutrition (Crosby, 1960), and conversely, as postulated by Luhby and Cooperman (1963), foods contain complex derivatives (conjugates) which are not useful for the micro-organisms but might be active for man. Butterworth et al. (1963) obtained, with S. faecalis, a growth response in folic acid-free medium to thymine, hypoxanthine, theobromine,

guanidine, guanosine, uric acid and others, in addition to at least ten derivatives of pteroylglutamic acid. The growth of L. casei was stimulated over a somewhat wider range of folic acid derivatives, including both methylated and triglutamate forms that did not support growth of S. faecalis.

L. casei is the bacterium which behaves more human-like regarding folate. It is clear, however, that only a human assay can provide a definite answer as to the available folate in foods for man, expressed in pteroylglutamic acid equivalence. Eventually a ratio (activity for L. casei/activity for man) might be defined. A large series of published investigations indicate that the folic acid activity of foods may be determined with S. faecalis following treatment with a conjugase to give values which correspond very closely in most cases with those obtained by biological assay with chicks. The chick biological assay is at this time the most reliable, although costly and time-consuming, method for determining folate activity. However, Flynn (1964) reported that the results of L. casei assays agreed well with the chick assay but that S. faecalis values did not. In a subsequent collaborative study on folic acid assay methods, Flynn (1965) found that the highest values for folic acid activity in a few food items were obtained with L. casei in extracts containing potassium ascorbate, due to the protective effect of the latter on the activity of 5-methyl tetrahydrofolic acid, which S. faecalis cannot use.

Conventional rats were used by Asenjo (1948) as the bio-assay organisms for folic acid activity; a sulfa drug was administered to prevent the intestinal synthesis of the vitamin. Daft and McDaniel (1963) stressed that for the bio-assay using rats to be valid, coprophagy must

be prevented. It was observed, however, that in germ-free rats, folic acid deficiency could be produced much faster than in conventional rats (McDaniel and Daft, 1959).

Folic acid assays by conventional methods using S. faecalis as the test organism showed that ordinary American diets provided daily 52 ± 14 μg. of free folate activity (Butterworth et al., 1963). Assays performed after conjugase treatment of the same diets with chicken pancreas yielded 184 ± 67 μg. of total folate per day. However, it is not known whether these determinations were made on the cooked or uncooked diet. Additional information on the daily total folate content of American dietaries was provided by Chung et al. (1961). Their average calculated values (from food tables) were, for high cost, low cost and "poor" diets, 120 μg., 117 μg., and 30.8 μg., respectively. The analyzed values, obtained from assays using S. faecalis on the cooked food were 193 μg., 157 μg., and 47 μg. respectively, for the three types of diets mentioned previously. Santini et al. (1964) found somewhat comparable values (65 μg. of free and 140 μg. of total folic acid) in cooked Puerto-Rican diets. Moscovitch (1965) reported a median total folate intake of 87 μg. in a dietary study of pregnant women attending an antenatal clinic in Montreal; the estimate was calculated from available tables on folate activity of food and referred to the uncooked diets.

The various reports on folate content of dietaries offer very little basis for comparison. Some estimates were calculated from available food composition tables, while others were derived from direct microbiological assays of diets. Moreover, in the latter case, different organisms and methods were used, free and/or total folate were

determined, and last but not least, some reports were on the basis of cooked, while others applied to uncooked diets. This latter decrepancy may be of major importance. In fact, Schweigert et al. (1946) reported cooking losses of folate ranging from 50 to 90%. Moscovitch (1965) observed a wider range in the loss of free folate but of a smaller order (i.e., 10-65%). Most of the cooking loss was shown to be due to water extraction. Herbert (1963a) produced folate deficiency in normal subjects by extensively boiling their food, reducing the folate content of normal dietaries to less than 5 μg. per day. However, Banerjee and Chatterjea (1964) found that dry cooking reduced the folic acid content of certain foods but also observed a rise in folate activity after boiling. The authors attributed this increase to the possible destruction of an inhibitor by boiling food - certain folate inhibitors are present in natural materials (Swenseid et al., 1947) - or to a better extraction of the vitamin. Nevertheless, the latter observations are rather isolated. The great majority of workers agree on the importance of cooking and processing losses of folate, although few data are available at this time on folate activity of individual cooked foods. Only recently did Ghitis (1966) publish a report of this type, where the extent of folate losses incurred in milk by heating and canning was studied. The scarcity of information on folate losses due to cooking may be attributed, at least partly, to the numerous complexities involved in the microbiological assays for folate activity.

5. Dietary Folate Requirement

The minimum daily adult requirement for folic acid was equated with that amount of pteroylglutamic acid required to maintain normality

of serum folate level, i.e., the earliest parameter of developing folate deficiency. Herbert's work (1962b) placed the minimal daily requirement for folic acid in the range of 50 μg. This value is in agreement with that of Sheehy and co-workers (1960) who estimated, through studies of individuals with tropical sprue, that man's minimum requirement was less than 100 μg.

The determination of dietary requirements for folic acid is complicated by a lack of information on the availability of folates. Most studies on folate requirements were done with pure folic acid. The amount of folates absorbed from foods and utilizable by man remain to be estimated. Crosby (1960) suggested that humans may have a specific requirement for pteroylglutamic acid in view of the discrepancy observed between the amount of folate activity in diets and the amount of pure folic acid required for the treatment of tropical sprue. Nevertheless, many workers believe that the normal animal (including man) utilizes equally free folic acid and the naturally occurring conjugates (Herbert, 1963b; Santini et al., 1964).

There is sufficient evidence to indicate that folate is synthesized by intestinal bacteria in man (Dzhelieva and Trufanov, 1964; Klipstein and Samloff, 1966). However, the relative importance of this source of folate in the nutrition of the host has not yet been elucidated.

The same obscurity applies to our knowledge of folate requirements for pregnancy. An increased need of this vitamin, as is the case for other nutrients, may be expected in the last part of pregnancy, when foetal growth is most rapid. This higher requirement may have some bearing on the widespread incidence of folic acid deficiency during

pregnancy. However, many factors could be responsible for the apparent deficiency and not until the metabolism of folates in pregnancy is fully understood will the specific requirements for gestation be delineated.

6. Folate Deficiency

a. Nature and incidence

Clinical signs of folate deficiency are far from uncommon during pregnancy, both in tropical (Das Gupta, 1954; Berry, 1955) and in non-tropical areas (Lillie et al., 1954; Giles and Shuttleworth, 1958; Chanarin et al., 1959; Herbert, 1962a; Amyot et al., 1965; Lowenstein et al., 1966a; Lowenstein et al., 1966b). The deficiency may cover a very wide spectrum, ranging from a very mild deficit evidenced by low serum folate levels accompanied by moderate morphological changes in hemopoietic and epithelial cells to severe megaloblastic anaemia with abnormally enlarged erythroblastic cells of the bone-marrow. The mechanisms by which folate deficiency signs appear are not completely clear. However, the development of megaloblastic anaemia as a result of the deficiency can be easily explained in terms of lack of tetrahydrofolate decreasing the supply of thymidilate required by the replicating cell (Friedkin, 1963).

Vitamin B_{12} deficiency may also produce megaloblastic anaemia but far less frequently than folate deficiency. Vitamin B_{12} tissue stores may remain normal for many years whereas folate stores will remain normal for little more than a month (Herbert, 1963b).

b. Possible causes

In addition to inadequate ingestion of folic acid, a deficiency state can be the consequence of impaired absorption or decreased

utilization of the vitamin.

The metabolism of folic acid may be altered significantly during pregnancy. While some workers found folate absorption to be normal in megaloblastic anaemia patients (Girdwood and Delamore, 1961), others reported that it was decreased as a result of pregnancy (Chanarin et al., 1959). Giles (1966) put forward the hypothesis that a mild degree of intestinal malabsorption might exist in a proportion of the general population - this section of the population then would be predisposed to develop severe folic acid deficiency and megaloblastic anaemia during pregnancy.

An abnormal renal clearance of folic acid was proposed as a possible explanation for the deficiency during pregnancy (Hytten and Leitch, 1964) on the basis of observations made by Chanarin et al. (1959) and by Girdwood and Delamore (1961). However, Hansen (1966) interpreted this increased folic acid elimination as a normal phenomenon in pregnancy.

The discrepancy between maternal and foetal levels of serum folate led Singh and Shinton (1965) to suggest a unidirectional transfer of folic acid across the placenta, resulting in a probable interference with the utilization of folic acid for maternal hemopoiesis. This would explain, at least partially, the rise in maternal serum folate observed a few weeks after delivery.

Grossowicz et al. (1960) found that foetal blood contained eight times more citrovorum factor than its maternal counterpart. However, total folic acid in foetal blood was only about twice that of the maternal blood. These findings suggested that conjugated folates may be mobilized from maternal red cells finding their way through the placenta

to the foetus where they are found mainly in metabolically active forms. Large foetal demand could consequently be responsible for a relative folic acid deficiency in the mother.

Badenoch et al. (1955) postulated that the cause of megaloblastic aenemia of pregnancy may be a resistance to the action of hemopoietic factors rather than a deficiency state. This view was supported by Gatenby (1956) who observed that a fair proportion of patients with a normal diet developed megaloblastic aenemia, while many patients with a grossly inadequate diet failed to develop the deficiency disease.

However, the great majority of workers attribute the folate deficiency in pregnancy to the failure to meet the increased requirements which may be further elevated by anorexia and vomiting. Although folic acid metabolism during pregnancy is not fully elucidated, it is probable from the observations of Giles and Shuttleworth (1958), Ungley (1952), Lowenstein et al. (1966b) and Metz (1966) that a deficient diet, because of inadequate intake of folate or its destruction by extensive cooking, will precipitate the appearance of clinical signs of folate deficiency.

c. Assessment

i) General.- Various methods for detecting folic acid deficiency in non-pregnant subjects have been devised. These methods, however, frequently give conflicting results in pregnancy. "It is still open to question whether the diagnosis of folic acid deficiency in pregnancy should be based on the range of normal variation in non-pregnants, or should be based on the values obtained from healthy pregnant and puerperal women" (Hansen, 1966).

ii) FIGLU excretion.- Many investigators accept the measurement of urinary excretion of formimino-glutamic acid (FIGLU) after a loading

dose of histidine as a sensitive index of folate deficiency (Luhby et al., 1958; Knowles et al., 1960; Kohn et al., 1961). A deficiency of folic acid due either to malabsorption or to dietary insufficiency results in an increase of formimino-glutamic acid - tetrahydro-folic acid not being available to effect further breakdown of FIGLU to glutamic acid. The resulting accumulated FIGLU then is excreted in urine (Broquist and Luhby, 1959). However, it is felt by many that FIGLU excretion cannot be relied upon as a test for folate deficiency in pregnancy since the metabolism of histidine seems to be altered, leading to decreased FIGLU excretion in the course of normal pregnancy (Hansen, 1966; Chanarin, 1966).

Furthermore, instances of folate deficiency without FIGLU excretion (Zalusky and Herbert, 1962) and of vitamin B_{12} deficiency accompanied by FIGLU excretion (Silverman and Pitney, 1958; Stocksland et al., 1966; Vitale and Hegsted, 1967) were reported. Vitale and Hegsted (1967) attributed the latter effect to a decreased activity of the enzyme formimino-transferase in vitamin B_{12} deficiency.

iii) Erythrocyte and serum folate levels.- Microbiological assays for folic acid activity in serum and red blood cells have been extensively used in the assessment of folate deficiency. The technique adopted by Baker et al. (1959) from that described by Usdin et al. (1956) and by Jukes (1955) has been reported to provide a reliable index and a convenient way of recognizing the deficiency in man. However, most researchers have observed that about half of pregnant women had subnormal serum folate values in their last trimester compared with non-pregnant women of the same age. Nevertheless, Cooper and Lowenstein (1961) and Harper (1965) found that all patients with serum folate values below 3.0 mμg.

per ml. almost certainly had folate deficiency. The normal range of serum folate has been established as being between 7 and 23 mμg./ml. (Herbert et al., 1960; Izak et al., 1961; Grossowicz et al., 1962; Water and Mollin, 1962).

Cooper and Lowenstein (1961) suggested that the assay for folic acid activity in red blood cells might be somewhat superior to the serum assay as a measure of folic acid deficiency in that "it permits a sharper distinction of the lower limit of the normal range than does the serum method." This could be attributed to the well-known fact that the major part of folate activity in the blood resides in the cells. The two methods may be complementary in the assessment of folate deficiency. Cooper and Lowenstein (1966) reported that low erythrocyte folate concentration (less than 150 mμg./ml.) in patients with serum folate below 4.1 mμg./ml. indicated that megaloblastic or macrogranulocytic changes would be found in the bone-marrow. This confirmed the findings of Izak et al. (1961).

B. DIETARY STUDIES OF HUMAN PREGNANCY

1. Appraisal of Dietary Intake

a. Dietary survey techniques and their reliability

A number of techniques have been developed to obtain data on the dietary intake of individuals. The majority of these are modifications of the two basic dietary survey methods - (1) the recall method and (2) the record or dietary diary. Qualitative and quantitative evaluations of the various techniques are presented in many reports (Burke, 1947; Bransby et al., 1948a; Leitch and Aitken, 1950; Young et al., 1952a;

Adelson, 1960; Trulson and McCann, 1960; Young and Trulson, 1960; Cellier and Hankin, 1963). The adequacy of a method depends primarily on the purpose of the investigation, the nutrient or nutrients to be assessed and the precision required. Consideration must also be given to the number of subjects involved in the study and the number of personnel available to conduct the survey.

A precise evaluation of the composition of diets eaten by experimental subjects is feasible only under laboratory conditions, with a restricted number of individuals, and is difficult even then. However, many estimates of dietary intake have been derived from the dietary record which consists of a detailed, quantitative listing of all foods consumed by an individual over a given period of time. This technique is the most widely used for research purposes, where a reasonably accurate assessment of the diet is needed.

Many authorities feel that a dietary record covering a period of seven consecutive days or 20 consecutive meals is the shortest length feasible from the standpoint of accuracy. This period covers a week-end when eating patterns are often modified (Cellier and Hankin, 1963). However, Walker (1965) felt that although eating patterns may change during week-ends, less accurate recording is also frequent. Therefore, he used five-day records, covering Monday through Friday. Any period of record keeping longer than a week was shown to decrease the subject's accuracy in reporting subsequent food intake. Adelson (1960) considered a second week unnecessary, since averages for one week gave as satisfactory information about current diets as two consecutive weeks.

When assessing the dietary intake of a large number of subjects and characterizing a group by its mean intake, a one-day record was

found most efficient (Chalmers et al., 1952). The same authors showed, however, for individuals and for groups of 10 and 25 subjects, an increase in precision when the number of days in the dietary record was increased. Since there are difficulties in obtaining diet sheets for seven days - Burke (1947) found that the longest period she could get the pregnant women in her study to co-operate was three days - Cellier and Hankin (1963) investigated the adequacy and reliability of three- and four-day records as compared to seven-day records. While the three-day records were clearly inadequate, the four-day diet sheets retained 90% of the precision of the seven-day diet sheets for each of the major nutrients. It was emphasized, however, that the bias introduced when the four-day records were adopted was negligible only when the group was large enough, that is, in the order of 100 subjects.

The recall method and the dietary history, established by Burke (1947) consist of interviewing the individuals in regard to their food habits with the help of a more or less extensive list of foods. The subject is asked to recall his food intake on the previous one, three, four or seven days or even to estimate an average consumption of foods on a monthly basis, this being performed with the amount-and-frequency questionnaire. It has been shown that such "frequency studies" can supply useful information although they cannot give more than a very rough approximation of the average food intake. A study of pregnant women in Jerusalem (Abramson et al., 1963) showed that the amounts of foods consumed by these women correlated very well with the frequency with which these foods were eaten. A similar method used in England to study diet and heart disease showed that food frequency scores from the menu records correlated highly with the weights of the food consumed

(Marr et al., 1961). The amount of accuracy and the representative value of such a technique depends largely on the co-operation of the subject, his ability to remember and estimate portions of foods and on the skill of the interviewer. Young (1959) believes in the value of good dietary interviews when taken by a skilled interviewer, provided sufficient time is allowed and adequate circumstances are chosen for the interview. Nevertheless, Young et al. (1952b), in a comparative study of dietary survey methods, found almost unanimously for 10 nutrients and for all population groups studied, that the dietary recall or history method did not give the same estimate of intake for an individual as the seven-day record. Similarly, Blake and Durnin (1963) found that the nutrient intake values obtained, in this case from elderly people, by a 24-hour recall and a seven-day diary frequently showed much discrepancy; however, mean intakes of protein, calcium, iron and energy agreed fairly well. Thomson (1958) observed that the recall procedure resulted in underestimation of the food intake and concluded that for reasonable accuracy, a dietary survey of pregnant women should rely upon direct measurement of the food consumed.

Yudkin and Roddy (1966) found a short questionnaire method highly satisfactory for the assessment of sugar intake only. However, they recognized that for estimating the intake of all dietary components, the dietary record was indubitably more precise.

b. Conversion of food intake to nutrient intake

i) Food composition tables.- The constant development of food tables is based on the concept that a single representative value for each food is serviceable to more users than several statistical expressions of the data (Watt, 1962). By representative is meant the value

that most closely approaches the content of a nutrient in one food on a year-round basis and in a given country.

Among nutrition workers, there are two schools of thought in regard to food tables. One tends to regard the figures in themselves as having the accuracy of atomic weight determinations; the other dismisses them as valueless on the grounds that the chemical composition of a foodstuff may vary so much that no figure can be a reliable guide to its composition. The truth probably lies somewhere between these two extremes. The usefulness and limitations of food tables were discussed by Whiting and Leverton (1960), Mayer (1960), Harris (1962) and McNaughton (1963).

There is a high degree of variability in the composition of samples of the same food due to the influence of genetic inheritance, the soil, the stage of maturity and to factors which destroy or inactivate nutrients (Asenjo, 1962). Hopkins and co-workers (1966), however, recently reported that the variations in the nutritive value of crops associated with growth on different soils are of no practical significance in the American dietary.

Dietary intake values may be determined by laboratory analysis or calculated from food composition tables. It is not feasible in dietary surveys to resort to laboratory analyses of aliquot samples of the diet. An assessment of the possible discrepancy between analyzed and calculated values is therefore important for the interpretation of the data obtained by the use of food composition tables. Bransby et al. (1948a,b) found good agreement between calculated and analyzed values for Calories, fat, carbohydrate and calcium but less agreement for protein and iron. They expressed the view that differences were in many instances so large as to throw doubt on the usefulness of individual results obtained by calculation.

Whiting and Leverton (1960) studied the discrepancy between protein and fat content of diets when calculated from tables and when analyzed chemically. Calculated protein values were within 10% of the analyzed values in 54% of the cases, but the fat determinations were within this range in only 25% of the cases. In a similar experiment, Eagles et al. (1966) reported that in 84% of the diets, the calculated values for protein agreed with the analyzed values within 10%; in the case of total fat, only 57% of the calculated values agreed with the analyzed values; both fat and protein were overestimated by reference to food tables. Parente and co-workers (1965), however, found that calculated values for protein tended to be underestimated. Walberg and Adams (1965) and Manalo and Jones (1966) obtained a close approximation for all mineral elements except phosphorus and magnesium. Unfortunately, it appears from a search of the literature that no such comparative data are available for vitamins.

There is partial disagreement in published reports as to the differences that exist between various food composition tables. Anderson et al. (1962) compared the nutrient intake of 100 diets when calculated from two different food tables. Statistically significant differences were found for four diet components, namely calories, fat, niacin and iron, out of 11 components studied. However, Eagles et al. (1966) found no significant differences in the tables in common use when 10 nutritionists calculated three out-patients' diets.

ii) The nutritionist.- The validity of dietary intake measurement is difficult to assess. Nutritive values calculated from tables of food composition depend upon the nutritionist's interpretation of the descriptive items and approximate measurements reported in the diet records.

The closeness of results obtained from different nutritionists may then depend upon similarities in their background or training. This source of variation may be eliminated if all the computation is performed by the same nutritionist. It can otherwise be estimated by comparing the results obtained by several nutritionists calculating the same dietary records. Eppright et al. (1952) compared weights of "standard" portions as independantly estimated by three nutritionists. They found close agreement among nutritionists for certain staple items but a wide range of differences for composite dishes. Steele and Tucker (1952) reported statistically significant differences in two (iron and niacin) out of 10 nutrients, in two series of trials in which six nutritionists calculated seven-day dietary records. Similarly, Eagles et al. (1966) in a recent study found significant variation among nutritionists in calculating three out-patients' diets.

2. Interpretation of Dietary Intake Data

a. Dietary requirements and allowances for pregnancy

Knowledge of the optimum requirements of the mother and foetus during pregnancy is still far from complete, especially for the lesser known vitamins. The 'recommended allowances' for pregnancy depend more upon observations of the diets ordinarily taken by healthy pregnant women with a good reproductive performance than upon intimate knowledge of the physiological effects of the nutrients involved. However, at present in this country, food habits of groups of pregnant women which result in intakes of nutrients similar to those recommended in the Canadian Dietary Standard (1964), are believed to be satisfactory.

An understanding of the basis of the dietary standards formulated

in various countries by official bodies or nutritionists in the last 25 years is essential in order to adequately interpret dietary survey data. A dietary standard is "a statement of amounts of Calories and various nutrients proposed as daily intakes" (Dietary Standard for Canada, 1964). Dietary standards generally include 'recommended allowances' or recommended quantities of nutrients to maintain the health of the majority of individuals of a population. These recommended intakes are usually designed to afford a margin of safety over and above the minimum physiologic requirements. Therefore, they are not intended for use in the assessment of nutritional status of individuals, but of large groups of people (Dietary Standard for Canada, 1964).

However, Krehl and Hodges (1965) proposed an arbitrary but recommendable approach: "In interpreting nutrient intakes, it is safe to suggest that those which are less than the Recommended Allowances may not be adequate at least for the continuous maintenance of good health."

b. Food habits during pregnancy

Many factors - geographical, social, economic and cultural - combine to determine human dietary habits, which vary widely between individuals and between groups. Any changes, quantitative or qualitative, which may take place during pregnancy will be superimposed on pre-existing food habits. Taggart (1961) studied these changes in food habits in a pregnant population group. Half of the subjects reported a definite increase in appetite, generally during the first trimester. Increased thirst was even more common. Appetite seemed to return to normal during late pregnancy. Nausea and vomiting affected 50% of the patients in early pregnancy, accompanied by poor appetite and reduced food intake.

Cravings or aversions were reported by approximately two thirds of the women, whereas Hankin and Burden (1964) observed the occurrence of cravings in half of the pregnant women surveyed. These changes apparently bore no relationship to the patient's knowledge of, or opinions about nutrition. Marr et al. (1955) observed social class differences in the consumption of orange juice, vitamin A and D concentrates, as well as in the consumption of milk during pregnancy. Similarly, Thomson (1959) observed that the mean nutritive values of pregnant women's diets diminished with falling social status. However, the variability between individuals in all classes was large and there was considerable overlap between classes. The author also reported that a high proportion of subjects, even in the higher social class, were taking nutrients in amounts less than those recommended by the British Medical Association (1950). Another important finding in that study was that Thomson obtained apparently reliable data from 93% of the subjects in the higher social class (husbands in non-manual occupations), from 75% of the subjects in the middle class (husbands in skilled manual occupations) and from only 61% of the subjects in the lower social class (husbands in semi-skilled and unskilled occupations). Similarly, in a study of pregnant women in Israel, Guggenheim et al. (1964) found that the diets eaten by the subjects belonging to the highest social class were significantly superior with respect to consumption of animal protein, calcium, vitamin A, riboflavin and ascorbic acid. They attributed the difference between groups to social class rather than to country of origin. To complement these findings, Stevens and Ohlson (1967) observed in a group of medically indigent women, that these subjects used a limited variety of foods and that, in general, quality was achieved through quantity.

Darby et al. (1953) related their data to the trimester of pregnancy in which the dietary information was obtained. The intakes determined for the first two trimesters were slightly higher than for the third trimester. Cellier and Hankin (1963) observed no difference for carbohydrate and total Calories. Protein intake increased throughout pregnancy but fat intake was higher in the second than in the third trimester.

Thomson and Billewicz (1961) found a positive correlation between food intake, height and weight gain during pregnancy. They observed higher average Calorie intake in tall women, even after corrections were made for differences in body weight. The Calorie intake of overweight and underweight women were similar, which led the authors to suggest that the relatively obese women were less active than the non-obese.

c. Clinical significance of the diet

It seems clear from all these surveys that patients differ widely in the amounts and nutritive values of the diets they consume during pregnancy. The controversial question is whether these variations have any clinical significance. While Burke (1943) found striking correlations between their dietary and clinical findings in a study of "middle-class" American women, McGanity et al. (1954) found none in a study of women of "low to moderate income" in another area of the United States. Thomson (1957) expressed the view that "none of the common disabilities of pregnancy are deficiency diseases of classical type; some of these seem to be expressions of inefficient physiologic adaptation to pregnancy, due in some measure only to chronic and non-specific inadequacy or imbalance of the diet." Although it is the belief of many workers that within a

wide range of dietetic conditions, the human foetus will grow normally, if necessary at the expense of maternal tissue, many reports have appeared to support the view that a low level of intake of the basic foodstuffs during pregnancy, with presumably some degree of vitamin deficiency, is associated with an increased incidence of congenital malformations. However, in attempting to relate the clinical events of pregnancy to the mother's diet, the past dietary pattern has to be considered as well as that at the time of pregnancy. The nutritional status of a pregnant woman is a function of her diet before pregnancy to the same degree as during pregnancy. As pointed out by Miller and Woollam (1960), there is need for further investigation of maternal nutrition during pregnancy, with particular emphasis on the possible importance of minor degrees of vitamin deficiencies in the first three months of pregnancy.

d. Statistical aspects of dietary surveys

For the statistical analysis of data to be valid, systematic error and bias must be avoided in all steps of the investigation. Woolf (1954) and Trémolières (1963) discussed the kinds of error which can be associated with the statistical analysis of dietary surveys. Error may be introduced because of bias in the selection of the population sample, in the sampling periods, in the estimation of foodstuffs eaten and in the estimation of nutrients.

In surveys, especially dietary surveys, it is difficult to obtain a statistically valid sample. Adelson (1960) expressed the view that a representative sample is more easily obtained if the recall technique is used because the subjects are required to cooperate only for the period of the interview, whereas the participants filling a dietary diary or

record have to perform duties for at least a few days length of time at meals. As pointed out by many workers, accurate weighing and measuring of food, which must for practical reasons be undertaken by the subjects themselves, calls for care, patience and a certain skill. Therefore, any sample which includes careless and unintelligent subjects, as well as conscientious and co-operative subjects will yield a number of inaccurate and incomplete records to be compared with highly accurate diet sheets.

The sampling periods are of great importance for the subsequent interpretation of the data since the dietary intake during the period sampled is assumed to be representative of the usual eating pattern of an individual. It is a recognized fact that the amount of different foods consumed varies from day to day and from week to week (Yudkin, 1951). It is then possible for a person to have an intake of any of the dietary components which is apparently adequate in one week and inadequate in another. Data based on dietary intake of a few days or even a week should consequently be interpreted with caution. However, Thomson (1958) found that the weekly variation in the diet of a given subject is less than the variation between subjects, while the diet of an individual appeared to retain its main nutritional characteristic in two widely separated weeks.

When a survey is more complex in structure, the kind of errors to be considered are correspondingly increased. Thus, if nutritional status is to be correlated with social class or economic resources, there will be additional errors of sampling, of possible wrong assessment of income or attribution to social class (Woolf, 1954).

The nutritional status of a group is usually judged by comparing its estimated average intake of a nutrient with a standard requirement. Superimposed on the possibility of excessively high or low standards, is the fact that groups with a deficient average intake may still contain members getting more than the standard; conversely, groups with averages above the standard might contain some members with a deficient intake. The greater the spread about the mean, the less representative will be the average intake values. If the results of a dietary survey are to be justly interpreted, figures for averages must consequently be supplemented by information on the spread about the mean; the orthodox measure of relative spread is the coefficient of variation (Woolf, 1954).

In spite of the inherent variabilities of the material and subjects, the great majority of workers believe that dietary intake data do provide useful guides for interpreting nutrient intake within reasonable limits. The application of statistical methods to such data, although it is a controversial subject, is justified by many researchers. Accordingly, an accurate knowledge of the actual intake is not synonymous with the evaluation of nutritional status (Thomson, 1957). The conclusion of adequate supply or deficiency is justified only when intakes are extremely high or low.

III. OBJECT OF RESEARCH

The primary objectives of the investigation were:

a) to study the relationship between diet and the appearance of clinical signs of folic acid deficiency during pregnancy

b) to compare in this respect two pregnant population groups of Montreal - clinic patients and private patients

c) to further compare these two groups with the population of clinic patients investigated by Moscovitch (1965).

Consequently, the research involved (1) a study of the food habits of the subjects with particular emphasis on their intake of folic acid and other nutrients related to its functions, and (2) a comparison of the dietary intake of the normal pregnant women with that of the group which exhibited clinical signs of folic acid deficiency.

IV. EXPERIMENTAL PROCEDURE

A. ASSESSING THE NUTRIENT INTAKE

1. Collection of Dietary Intake Data

a. Sampling procedure

The women attending the public antenatal clinic of the Royal Victoria Hospital constituted the "clinic population" to be surveyed. Only those coming to the clinic before the 29th week of their pregnancy and considered normal when clinically examined were included in the project. Although all the women who met these requirements were automatically enlisted in the hematological study, only those who volunteered to contribute to the dietary survey formed the clinic group studied by the author. The patients were randomly assigned to three groups: E, F and G. Members of group E, considered a control group for the hematological studies, received daily a polyvitamin-mineral tablet containing 75 mg. of elemental iron; group F received the same standard supplement plus 200 micrograms of folic acid daily and Group G, the standard supplement except that it contained 10 mg. of pyridoxine instead of 3 mg. as in groups E and F, and contained no folic acid. The composition of these supplements is shown in Appendix Table I. The patients who were prescribed special diets by the physician were excluded from the study; their food habits had to be changed and would not have been representative of their usual eating patterns. Those who only needed dietary advice because of inadequate food habits were so instructed, but only after they had first provided the author with a

dietary sheet. During the course of the study, no attempt was made to regulate diets or amounts of weight gained during pregnancy.

The "private" patients were pregnant women under the care of the obstetricians participating in this cooperative study. They were referred to the author after they had agreed to contribute to the survey. They received the same supplement as group E: a polyvitamin-mineral tablet daily containing iron but no folic acid.

The distinction made between "clinic" and "private" patients was relative and arbitrary. Although it was assumed that subjects attending the public clinic were from a lower economic class than those under the care of private physicians, this criterion was not infallible. The choice of attending an out-patient clinic or seeing a private obstetrician is sometimes a function of the importance various people attach to medical care.

A control group which was initially planned to be a stratified sample of healthy non-pregnant women, because of technical difficulties, was later restricted to a small group of hospital workers - i.e., nurses, student nurses and laboratory technicians.

b. The nutritionist

In order to reduce the error introduced when different nutritionists are involved (Eagles et al., 1966), the author carried out all steps of the dietary study. These included interviews of patients, estimation of dietary intakes and calculation of nutrient intakes.

c. Timing of interviews

An attempt was made to interview the clinic patients included in the survey on their first visit to the clinic, although this was not always

possible. Some patients ignored or forgot the request that was made to see the nutritionist before they left. In a few cases, the interview had to be postponed until the next visit because of lack of time on the part of the patient. In addition, since the study started in early September, 1965, and the nutritionist commenced the dietary study in October of the same year, some patients could be seen only at a more advanced stage of their pregnancy. Medical examinations were scheduled every month for the pregnant women attending the clinic and from the 32nd week of pregnancy, every fortnight. Some women missed their appointments, thus delaying their dietary interviews until a month later. An attempt was made by the nutritionist to see each patient every time she came to the clinic. The interviews were held in the antenatal clinic of the Royal Victoria Hospital every Monday, Tuesday and Thursday from one to five p.m., and during the period October, 1965 to December, 1966.

Generally the private patients were interviewed in the hospital, in an office adjacent to the hematology laboratory. As soon as one subject was referred to the hematology clinic, an appointment was made for her to see the nutritionist, at a time suitable to both. It was often possible for the patient to save a trip by having her appointment coincide with her visit to the obstetrician. This was possible if his office was in the hospital - otherwise, the interview was arranged to coincide with her scheduled appointment for hematological tests. The nutritionist interviewed a few patients in their homes. This was done when it was inconvenient or impossible for them to come to the hospital soon enough because of distance or employment outside the home. The investigation of private patients covered a period of 16 months: from October, 1965 until February, 1967.

The control subjects were interviewed in the hospital, at a time when they could visit the nutritionist without interfering with their work. There was no definite timing for the interview, which in all cases lasted from 15 to 30 minutes, depending on the ability of the individuals to understand the tasks we asked them to perform.

d. Survey technique

The seven-day dietary diary was the only investigational technique used to obtain reliable quantitative data on food intake. It was accepted as inevitable that some subjects would not cooperate adequately. However, according to many workers, seven days is the shortest feasible length of time from the standpoint of accuracy. Much time and thought were given to explaining to the patients the purpose of the study and the value of their cooperation. Motivation had to be created so that the subjects would respond favorably and produce reliable records. The size of the sample would have increased if less detailed and demanding methods had been used and if the quality of the information had not been obtained at the expense of quantity.

On the first contact with the nutritionist, the patient was informed of the study, its purpose and of the tasks she was expected to perform. She was then allowed to decide whether to accept to cooperate in the study or to withdraw. No pressure was imposed on her. The willing subject was subsequently carefully instructed about the record keeping procedures.

Each patient was provided with a "kit" containing an eight-ounce scale, a set of measuring cups and spoons and a booklet to be filled in according to instructions given on the front page. This booklet contained seven one-day recording sheets in addition to the "instruction

sheet" and lastly, a "sample sheet" to illustrate the way in which various foods should be recorded (see Appendix, Forms i to iii). The record forms, in either French or English, were designed to avoid any suggestion as to the types or amounts of food that should be eaten, as was pointed out by Young (1959). The patients were asked to record, qualitatively and quantitatively, their food intake for seven consecutive days. Fish, meat and cheese had to be weighed because measuring these foods is impractical and inadequate. Edible and inedible waste was weighed and a suitable deduction made. All the other items were recorded in fluid ounces or units with the exception of salt, pepper, dried herbs and water. Foods were weighed or measured after cooking. Information pertaining to the cooking method and the brand name also had to be recorded. For composite dishes, recipe cards were provided originally but were soon discarded, for the reason that too many forms to be filled confused or frightened the subjects. Ingredients, amounts and number of servings obtained from recipes subsequently were entered at the back of the diet sheet. Meals or snacks consumed outside the home were to be recorded in estimated amounts. The importance of recording accessory items such as cooking fat, salad dressings, gravies, sugar, other sweets and all beverages, was stressed.

After the instruction was completed and all the patients' questions answered, a general information sheet was completed by the nutritionist (see Appendix, Form iv). This form, in many ways similar to that used by Moscovitch (1965), contained the usual information such as address, nationality, age, number of children, occupation, weight, height and expected date of delivery. Specific information pertinent to the dietary background was obtained from the questions on food likes and

dislikes, the proportion of meals eaten at home or outside in one week and the occurrence of nausea and vomiting during the course of pregnancy. Space was saved under "comments" for noting any idiosyncrasies of the patient with regard to her food habits and any other significant factors. This section was also reserved for the interviewer's evaluation of the probable reliability of the record, the cooperation of the subject and of her ability to understand the record-keeping procedure.

On the subsequent visit, which generally took place between two to four weeks after completion of the dietary record, the dietary sheets were checked by the nutritionist with the help of the patient. Whenever possible, omissions or errors were detected and additional information was obtained if required. In the case of lack of details concerning amounts, the size of an "average" portion was inserted, or preferably the size of the serving of that specific food consumed on another day. Only to that extent could memory be relied upon, especially when the diary had been completed long before the return interview. Records which exhibited an obvious lack of information, accuracy or care were discarded.

The women who kept a first record adequately were asked to keep a second one, at least three months later, in order to compare intakes at different stages of pregnancy. The same dietary study technique was used. The second interview took much less time, however, the patient being already familiar with the recording process. If the first record was weak in certain respects, the second instruction stressed these special points.

The same basic technique, that is, the seven-day dietary diary, was used for the control subjects. However, slight modifications had to be introduced because most of the members of this group ate at least

one meal outside the home every day. Consequently, the majority of the records did not consist of weighed or measured food intake but of estimated portion sizes of different foods eaten. The "general information sheet" (Appendix, Form v) included questions on marital status, living conditions and eating places, in addition to the basic information contained in the pregnant women's "general information sheet." The completed records were checked in the previously described manner. The subjects were not requested to keep a second record.

2. Calculating Caloric and Nutrient Intakes from Seven-Day Dietary Records

a. Preparation of a food list

In addition to folate and vitamin B_{12}, of major interest in this study, all nutrients for which recommended intakes have been estimated, excluding vitamin D, were considered in order to give more scope to the survey. The "skeleton" of the food table was obtained from the Home and Garden Bulletin No. 72 of the U.S.D.A. (1964). The 500 foods listed are the ones commonly used and most of them are in ready-to-eat form, raw or cooked. The weight in grams is shown for an approximate measure of each food given in cups, ounces, pounds, a piece of a certain size or some other unit easily recorded by an individual at home. Values for Calories and nutrients were extracted from the table, for each food listed. Additional items had to be added to cover the wide variety of foods consumed by the subjects under the study. The accessory tables referred to in order to complete the food list were those in common use (Church and Church, 1963; Watt and Merrill, 1963; and Nutrition Division, Department of Health and Welfare, Ottawa, 1960). The entire list contained 764 food items.

Values for folic acid content of foods were obtained primarily from the monograph of Toepfer et al. (1951) and from a few partial tables (Aitken and Duncan, 1960; Hardinge and Crooks, 1961; Kiernat et al., 1964). No important work has been published on the folate content of foods since Moscovitch (1965) prepared a table of folate activity of food items from the existing literature for the study of dietary intake of pregnant women. The summary of nutrient content of meats published by Kiernat et al. (1964) includes more data than the one published previously (Schweigert and Payne, 1956), but figures for total folic acid content are for raw foods and are taken from existing tables. Consequently, as is the case for vitamin B_{12} content of dietaries, "total" folate values of the subjects' diets could be compared with the figures obtained by Moscovitch (1965). The figures that were extracted from the above mentioned tables of folic acid content were those obtained from microbiological assays using Lactobacillus casei as the test organism: these bacteria are the ones which give the best estimate of the"total" folate content (Herbert, 1961). When available, figures for "total" folate after treatment of the samples with chicken pancreas deconjugating enzyme were used.

Two major tables (Kiernat et al., 1964; Lichtenstein et al., 1961) were referred to in completing the food list with vitamin B_{12} content of foods. Figures from the Lactobacillus Leichmannii assay were used.

Although the figures representing the folic acid and vitamin B_{12} content of foods are primarily for raw foods, no correction was attempted for the loss or gain in weight of individual foods during cooking, as previously reported by Moscovitch (1965). It is felt that this correction gave an estimate of the "total" folate which is much too high and which

is hardly representative of the amount of the vitamin actually ingested. In the present study, the "apparent" folate and vitamin B_{12} content of diets was estimated. For example, if three ounces of cooked meat appeared in a record, the "total" folate and vitamin B_{12} figures were those listed for three ounces of raw meat, and in certain instances where data were available, of cooked meat. The tables for folic acid and vitamin B_{12} activity in foods are far from complete; many items have not been assayed and data for cooked foods are almost non-existant. In spite of these shortcomings, since figures for the main dietary sources of the vitamins were available, the estimation of the apparent total folate and vitamin B_{12} of dietaries was considered satisfactory for the comparison of the two population groups under study.

The Consumer and Food Economics Research Division of the United States Department of Agriculture (1964) described a set of punched cards carrying the nutritive values per specified food unit as published in the Home and Garden Bulletin No. 72 (1964). The six-digit identification numbers for all the foods were used as described and the items added to the food list were assigned identification numbers following the same procedure. One IBM card was punched for each food item (see Appendix, Fig. 1).

b. Coding of food intake records

An identification number (9 digits) was assigned to each subject. The subject line number (3 digits), the age group (1 digit), the trimester of pregnancy (1 digit), the reliability (for discussion on assessment of reliability, see Chapter V, B, 1) of the information (1 digit) and the number of previous pregnancies (1 digit) constituted the

identification number devised for further sorting and statistical analyses. The identification code for the "control" subjects carried seven digits: line number (3 digits), age group (1 digit), living conditions (1 digit), reliability (1 digit) and status (1 digit).

The diet list of each individual was then converted to a code suitable for the computation of the data on the IBM 1620 at Macdonald College, McGill University. Each food item was recorded as its identification code. The amount listed in the dietary record was converted to a multiple of the unit appearing in the food list. A field of four digits with two decimals was allocated for this factor. For example - the nutritive value of milk appearing in the table is for one cup (8 fluid ounces). If the subject has recorded half a cup of milk, the identification number for milk being 001111, the coded item becomes 001111 0.50. The intake of all foods over the seven days was coded in a similar manner, as a continuous list, without treating days separately. The average daily intake of each nutrient was desired and no attempt was made to compare the intake of different days. The complete list was subsequently punched on cards.

c. Computation of the data

An IBM program was set up to compute the total Calorie and nutrient intake over the seven days and the average daily intake of Calories and nutrients for each patient. The food list deck was first stored in the memory of the computer after which the individual consumption cards were read into it according to the instructions given in the program (see Appendix, Chart i). A fetching operation, followed by a series of multiplications and additions and finally a division of the total intake by 7 were the major instructions given in the program.

d. Statistical analyses of dietary intake data

As recommended by Woolf (1954), the mean values for the intake of Calories and nutrients were supplemented with standard deviations and coefficients of variation. Dietary intake comparisons were made between private and public patients, between pregnant women and "control" subjects, between groups E, F and G within the clinic population and between trimesters of pregnancy within the two groups of pregnant women. The significance of the differences observed was tested using the unpaired and paired "t" test (Cochran and Cox, 1961). The chi-square test was used to evaluate the incidence of folate deficiency signs in both groups of pregnant women. Correlations between the intakes of various nutrients, and the serum and erythrocyte levels of folic acid and vitamin B_{12} were studied.

B. HEMATOLOGICAL TESTS

In addition to the routine blood tests, the Hematology and Vitamin Research Laboratories of the Royal Victoria Hospital performed the following tests: serum and erythrocyte folates, serum vitamin B_{12} and, in a few cases, FIGLU excretion. Bone marrow aspirates were examined by hematologists for morphological changes characteristic of megaloblastic anaemia. Serum was assayed for folate and vitamin B_{12} at 38 weeks of pregnancy, 7 days post-partum and 6 weeks post-partum. Erythrocytes were assayed for folate at the same intervals of time. Only the 38th week of pregnancy data were used in the study being reported. The procedure followed for these determinations were previously described (Cooper and Lowenstein, 1961, 1964; Mollin and Ross, 1952).

V. RESULTS AND DISCUSSION

A. SIZE AND CHARACTERISTICS OF THE GROUPS STUDIED

1. Private Pregnant Women

Fifty-four private patients were interviewed by the nutritionist. Fifty kept one or more dietary records for seven days. Two patients had to be deleted from the project for lack of cooperation, one for illness and one because of language difficulties. The initial goal was to interview 100 pregnant women referred to the Hematology Department of the Royal Victoria Hospital by private obstetricians. However, due to a slow rate of admission to the study, the final number of subjects was half what it was first expected to be. It was realized that such a small sample made statistical inference uncertain and reduced the possibility of performing tests of significance on various comparisons. Nevertheless, for 50 cooperative subjects, among which 15 produced two dietary records, dietary intake characteristics and general trends in food habits could be detected with reasonable assurance.

The age distribution of the private patients is presented in Figure 1. Ages ranged from 19 to 40 years, with a mean of 27. The majority of the subjects were between 20 and 30 years of age. The average weight of these patients was 124 pounds and their average height was 64 inches. Patients reported their weight as of before pregnancy; therefore slight bias might have been introduced. The average height value corresponded within 0.5 in. with that reported by Thomson (1959) for 101 pregnant women of the higher social class studied.

Figure II, illustrating the distribution of patients according to number of previous pregnancies, shows that 52% of the subjects were primiparous, while the highest number of pregnancies reported in this group was six.

The English speaking group constituted 86% of the total and the French only 4%. Although 10% of the subjects were from different ethnic origins, they were fluent in either French or English. Fourteen women had employment outside the home, on a full- or part-time basis.

2. Clinic Pregnant Women

The pregnant women attending the antenatal clinic were much less cooperative than the private patients with respect to the dietary study. This was evidenced by the small proportion of patients who agreed to keep a dietary record for seven days. In fact, from a total of well over 150 interviews, only 46 subjects were included in the study. Because the population attending a public clinic in Montreal comprises a large number of immigrants, many women could not participate due to language difficulties.

When a large number of observations is desired from a clinic population group, it is probably preferable to use survey techniques which do not require too much work from the subjects. Moscovitch (1965), for instance, in a study of a similar group, obtained more data from dietary histories, three- and four-day dietary diaries than from seven-day dietary records. In the present survey, however, the technique applied to the two distinct groups being investigated had to be identical to minimize the difference attributable to variations in the experimental procedure. The private group (50 patients) and the clinic group (46 subjects)

FIG. I. Age distribution of private patients

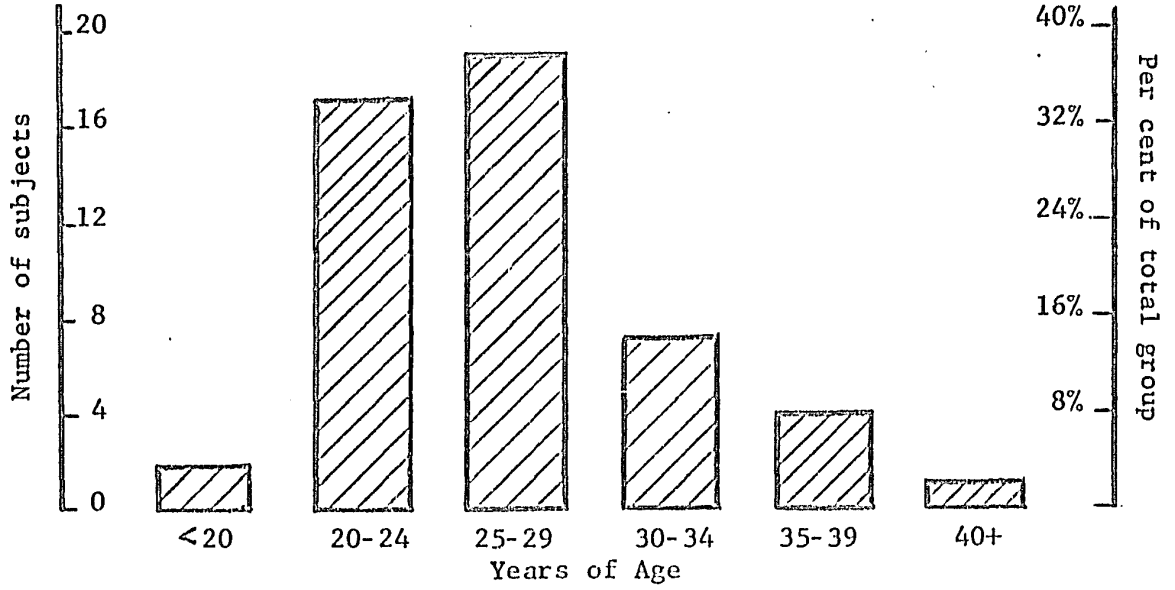
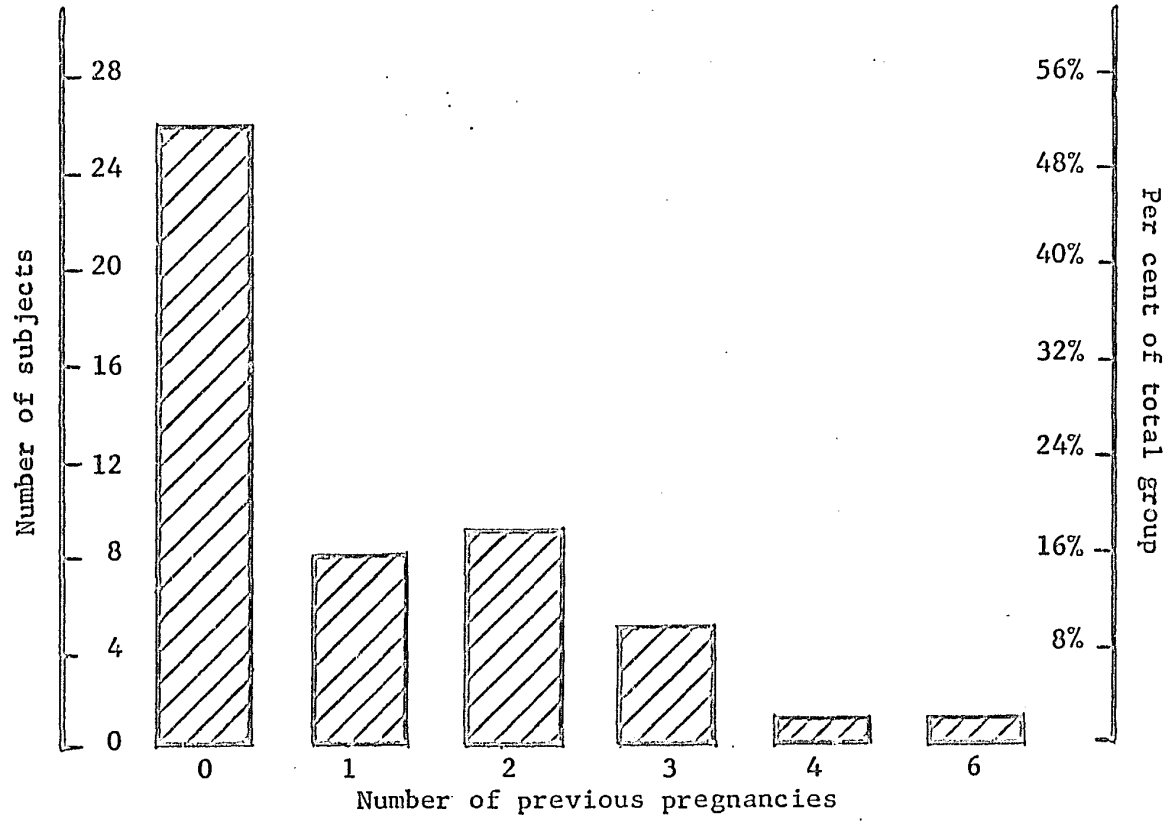


FIG. II. Number of previous pregnancies of private patients



could be compared on the basis of parity in the sample sizes and in the survey technique employed.

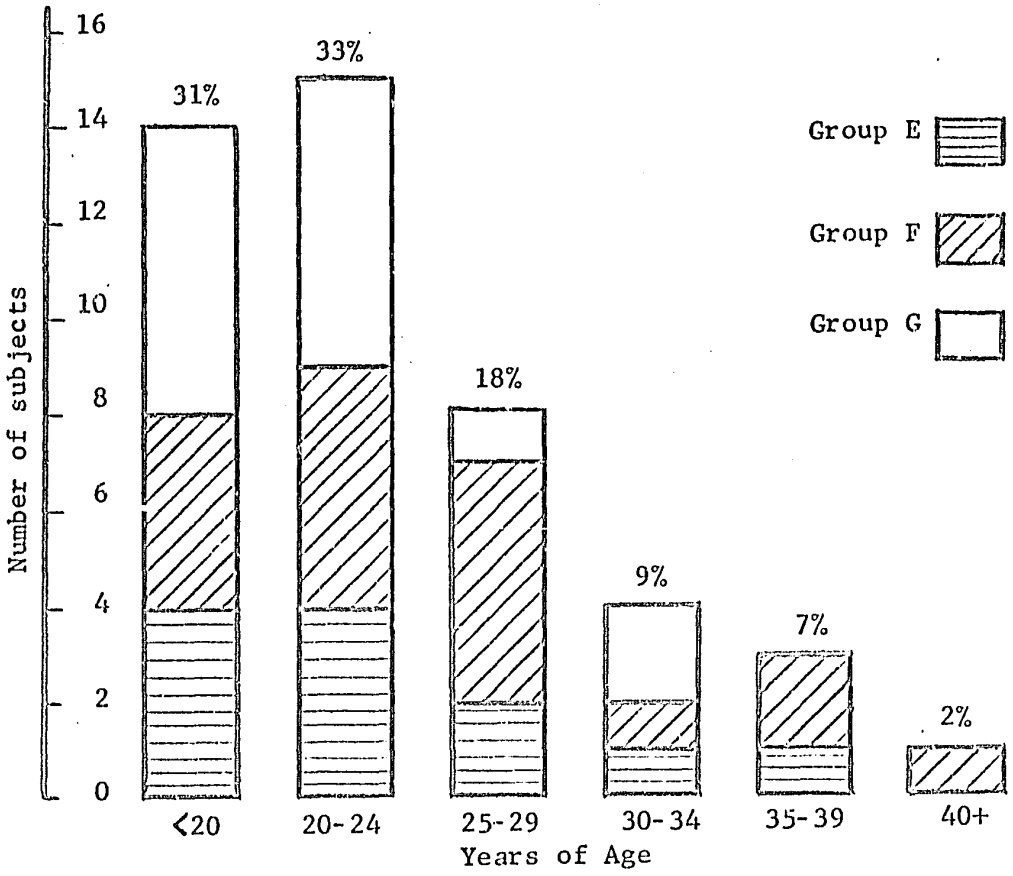
The age distribution was roughly similar in the three clinic groups (see Figure III). For a general average of 24 years of age, most subjects were between 17 and 25; the first two age groups formed 64% of the total number of subjects. The clinic group was considerably younger on the average than the private group for which the mean age was 27. This could have been due to the fact that the clinic receives a high proportion of unmarried pregnant women, as can be seen in Table 1. If the five subjects for which no information was available concerning marital status are excluded, 22% of the clinic patients were single.

Average weights and heights of the clinic patients (Table 2) were comparable to those of the private patients. The distribution of clinic patients according to number of previous pregnancies (Figure IV) showed that 21 subjects, or 47% were primiparous. This compared to 52% in the private group.

Fifty-two per cent of the clinic patients were English speaking and 39% French speaking. Since the majority of them were Canadians, the dietary intake data were not biased by the inclusion of many subjects from a foreign cultural background and basically different food habits.

Only eight clinic patients or 17% were working outside the home, as compared to 28% of the private subjects. If the assumption holds that clinic patients usually belong to a lower socio-economic class than private patients, it could be said, on the basis of this observation, that women who work do not generally do so for financial reasons.

FIG. III. Age distribution of clinic patients[1]



[1]For one patient in Group F, there was no available information.

TABLE 1. Marital status of clinic patients

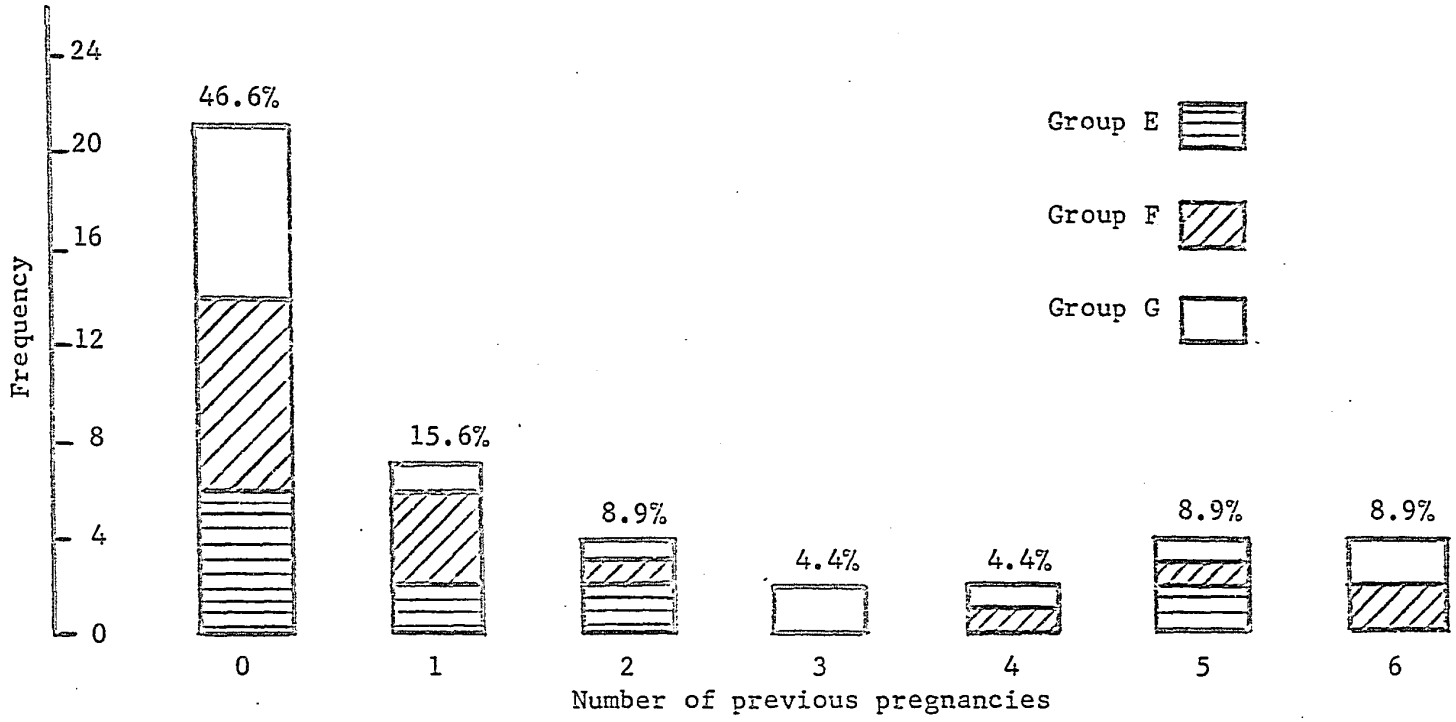
	Married	Single	Unknown
Group E	7	3	2
Group F	11	6	1
Group G	14	0	2
Total	32	9	5

TABLE 2. Average weight and height of clinic patients[1]

	Weight (lbs.)	Height (in.)
Group E	123.8	63.2
Group F	122.7	64.4
Group G	115.3	63.0
Total average	121.0	63.7

[1]No information was available for one patient in Group G.

FIG. IV. Number of previous pregnancies of clinic patients[1]



[1]No information was available for one patient in Group G and one patient in Group F had 14 previous pregnancies.

3. Non-pregnant Control Subjects

This group, contrary to expectations, was unfortunately too small to be of any comparative value and was subsequently excluded from the clinical investigation. However, since nine subjects provided a dietary journal, their nutrient intake was assessed, although most likely it was not representative of a stratified sample of non-pregnant women.

Ages ranged from 19 to 32 with an average of 23 years. Their average weight and height were 127 pounds and 64 inches, respectively. Two were married and all of them were working at the Royal Victoria Hospital - five as nurses or student-nurses and four as laboratory technicians. They were all eating a minimum of one meal outside the home every day. Consequently, records included a higher proportion of estimated values than those of the two pregnant groups.

B. NUMBER, DISTRIBUTION AND RELIABILITY OF THE DIETARY RECORDS

1. Private Patients

Fifteen patients (30%) were able to keep two records, giving a total of 65 records. Fourteen diaries were filled in the first, 33 in the second and 18 in the last trimester of pregnancy. It was first intended to obtain a minimum of two records from each patient to allow comparisons of nutrient intakes by individuals at different stages of pregnancy. However, it soon became apparent that this was rather difficult to accomplish. Some patients claimed lack of time because of children or work. A few patients moved out of the city, were prescribed special diets or joined the study shortly before it had to be terminated.

Finally, in a few cases, patients simply admitted that they were not willing to keep a second dietary diary.

Yudkin (1951) showed a high variation in intakes of various nutrients when a seven-day record was kept by the same individual at the beginning of each month throughout the year. Differences observed between intakes in trimesters of pregnancy may then be the mere reflection of this variation of intake, or of the seasonal variation in eating patterns, superimposed on the true variation due to pregnancy. For this reason, comparison of intakes between trimesters could be of doubtful relevance. Consequently, in this study more importance was attached to the average daily dietary intake derived from one or two records, without reference to the stage of gestation, although the latter was considered for evaluation of diets by comparison with Dietary Standards which recommend different levels of intake for the latter part of pregnancy. When two records were submitted by one patient, the average of the two, undoubtedly more representative of an individual's dietary pattern, was used in the computation of group averages. Of the 15 patients who submitted two dietary sheets, four kept these in the first and third, one in the first and second and ten in the second and third trimesters of pregnancy.

Records were rated for their reliability. The first category ("1") included diet sheets which provided as much detail on amounts, description and cooking methods of food as can possibly be expected from subjects on self-chosen diets. Most records falling in this category (45 or 69%) were from women who were not employed outside the home and consequently for whom it was feasible to measure and weigh their food at every meal. Records which often included approximate measures of the

food eaten or a large amount of exotic foods without the necessary information on ingredients, number of servings, etc., were rated "2". The 14 diaries (22%) comprised in this group came primarily from working women and from patients belonging to a foreign ethnic group. Records were rated "3" when they exhibited a lack of information or doubtful reliability, although the nutrient intakes could still be estimated with reasonable assurance. Omissions were frequent or a decline in the quality and quantity of recording could be observed towards the end of the seven-day period. Whether this decline was due to a progressive lack of interest or to mere coincidence could not be established. Six records, or only 9% of the total, were so rated. Rating dietary sheets in such a manner may be subjected to criticism on the grounds that subjectiveness may play an important role and that the reliability groups may well overlap. However, it was felt that even an imperfect assessment of the reliability of the information was necessary if comparisons between groups considered different according to certain criteria were to be made.

2. Clinic Patients

The number and distribution of records for groups E, F and G, and for stage of pregnancy, are given in Table 3. Twelve records were provided by members of group E, 21 by members of group F and 18 by members of group G. Eight records were filled in the first, 25 in the second and 18 in the last trimester of pregnancy. Only five clinic patients kept two records of their food intake. This is likely a direct result of the reduced cooperation obtained from clinic patients compared to that of private patients. This discrepancy was also evident

from the assessment of the reliability of records shown in Table 4. Of a total number of 51, 28% of the records were rated "1" as compared to 69% of the records from the private group; 37% were rated "2" while 22% of the private patients' records were so rated. Category "3" included 35% of the clinic records but only 9% of the private records.

3. Control Subjects

One record was provided by each subject included in the study. Of the nine records, one was rated "1", five "2" and three "3". This was so because, as mentioned previously, these women did not eat regularly at home and provided rather imprecise records.

C. DIETARY FINDINGS

1. Pregnant Women - Private vs Clinic Patients

a. Comparison of food habits

Thirty per cent of the private patients and 50% of the clinic patients reported the occurrence of nausea and vomiting, usually at the beginning of their pregnancy. This was rather consistent with an incidence of 50% observed by Taggart (1961). However, a careful examination of dietary records revealed that food habits differed considerably in the two groups. Private patients seemed to have more regular eating patterns than clinic patients who often skipped meals, especially breakfast. In addition, a wider variety of foods appeared in the diets of private patients. Concomitantly, Stevens and Ohlson (1967) observed that medically indigent pregnant women used a limited variety of foods and achieved quality through quantity. The consumption of organ meat and other protein foods, of dark green vegetables and of

TABLE 3. Number and distribution of dietary records obtained from clinic patients

		Stage of pregnancy		
		1st trim.	2nd trim.	3rd trim.
Group E	Number of records	0	9	3
	Subtotal = 12			
Group F	Number of records	4	9	8
	Subtotal = 21			
Group G	Number of records	4	7	7
	Subtotal = 18			
	Grand total = 51	8	25	18

TABLE 4. Reliability of dietary records obtained from clinic patients

Group	Reliability		
	"1"	"2"	"3"
E	3	3	6
F	7	7	7
G	4	9	5
Total	14 (28%)	19 (37%)	18 (35%)

fresh fruit was more frequent in the private than in the clinic group despite the fact that organ meats and dark green vegetables were almost equally unpopular in both groups of pregnant women. These observations are in accordance with those of Hankin and Burden (1964) who found that most frequently disliked foods included liver and other organ meats, turnips and spinach. It could also be noticed that sweets and starchy foods constituted a relatively more important part of the diets of clinic patients.

b. Comparison of average daily Calorie and nutrient intakes

Average daily Calorie and nutrient intakes of private patients are summarized in Table 5. As recommended by Woolf (1954), ranges, standard deviations and coefficients of variation have been included to provide information on the variability associated with intakes and hence to better characterize the data. Ranges were wide for most nutrients, giving rise to high standard deviations and coefficients of variation.

Thomson (1959) investigated the diet of pregnant women in relation to social class. Her reported average dietary intakes of subjects belonging to the highest social stratum were higher than those of the private group in this study with respect to Calories, fat, carbohydrate, calcium and vitamin A but lower for protein, riboflavin, niacin and ascorbic acid.

Average daily dietary intakes of clinic patients are shown in Table 6. Although there was no reason to suspect differences in the nutritive value of diets consumed by members of groups E, F and G since clinic patients had been randomly assigned to experimental groups, intakes were calculated separately for respective groups. The observed

TABLE 5. Average daily Calorie and nutrient intakes of private patients (50 subjects)

		Mean	Range	St. deviation	Coeff. variation %
Calories		2100	1328-3390	484	23
Protein	(gm.)	87	59-125	18	20
Fat	(gm.)	97	35-195	27	28
Carbohydrate	(gm.)	228	107-384	61	27
Calcium	(mg.)	1069	385-1898	407	38
Iron	(mg.)	13.0	7.9-21.9	3.3	25
Vitamin A	(I.U.)	9135	2543-22722	5383	56
Thiamine	(mg.)	1.2	0.5-2.3	0.3	30
Riboflavin	(mg.)	2.2	0.9-4.2	0.8	36
Niacin	(mg.)	15.3	8.0-27.7	4.1	27
Ascorbic acid	(mg.)	127	31-281	58	46
Folate	(μg.)	86.5	36.9-157.1	26.1	30
Vitamin B_{12}	(μg.)	9.8	2.2-29.0	6.5	69

TABLE 6. Average daily Calorie and nutrient intakes of clinic groups E, F and G

		Group E (12)	Group F (18)	Group G (16)	Observed "t" values[1] E vs F	E vs G	E vs G
Calories		1729±841	1635±479	1925±512	0.35ns	0.72ns	1.70ns
Protein	(gm.)	61±20	65±16	69±16	0.59ns	1.14ns	0.74ns[1]
Fat	(gm.)	88±53	72±20	84±22	1.00ns	0.25ns	1.67ns
Carbohydrate	(gm.)	184±86	191±69	234±80	0.24ns	1.57ns	1.67ns
Calcium	(mg.)	666±378	792±384	790±276	0.89ns	0.96ns	0.02ns
Iron	(mg.)	9.8±2.8	9.9±2.9	11.1±2.7	0.95ns	1.24ns	1.25ns
Vitamin A	(I.U.)	6001±4637	5165±2945	4792±3219	0.74ns	0.77ns	0.35ns
Thiamine	(mg.)	0.9±0.3	1.0±0.3	1.1±0.3	0.91ns[1]	1.72ns[1]	1.00ns[1]
Riboflavin	(mg.)	1.4±0.7	1.5±0.5	1.6±0.5	0.45ns	0.87ns	0.59ns[1]
Niacin	(mg.)	12.3±3.6	12.1±3.4	12.9±3.2	0.15ns	0.46ns	0.73ns
Ascorbic acid	(mg.)	80±53	86±44	73±33	0.32ns	0.35ns	1.00ns
Folate	(μg.)	66.7±29.2	65.6±30.5	65.9±24.0	0.09ns	0.08ns	0.03ns
Vitamin B_{12}	(μg.)	4.0±3.9	4.4±2.4	4.3±2.7	0.32ns	0.23ns	0.11ns

[1]"t" test for equal variance and unequal number of observations (unmarked values were for unequal variances and unequal number of observations).

differences between groups were tested for significance using the unpaired "t" test for unequal number of observations and unequal or equal variance (Cochran and Cox, 1957). The differences[1] were not statistically significant (P<.05), indicating homogeneity of the clinic population surveyed. Therefore, average Calorie and nutrient intakes for the clinic group as a whole were used for comparison between private and clinic patients.

Intake values for the complete clinic group, appearing in Table 7, were considerably lower than those reported in previous studies of comparable populations of pregnant women (Thomson, 1958; Stevens and Ohlson, 1967), with the exception of niacin and ascorbic acid. In addition, intakes reported by Stevens and Ohlson (1967) were higher than those of private patients in the present study for Calories and for all nutrients except vitamin A, thiamine and ascorbic acid.

Statistically significant (P<.01 or <.05) differences were found between dietary intakes of private and clinic pregnant women (Table 8), where, with one exception, the intakes of the former were higher. Only mean carbohydrate intakes, although higher in the private group, were not significantly different. The differences observed in Calorie and nutrient intakes could not be attributed to differences in body size or age since average heights, weights and ages were comparable in the two groups. However, the fact that 69% of the private patients but only 28% of the

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[1]In this thesis, whenever differences are tested for significance, the level of significance, following the observed "t" value, is indicated as follows:

non significant	= ns
significant (P<.05)	= *
highly significant (P<.01)	= **

TABLE 7. Average daily Calorie and nutrient intakes of clinic patients

		Mean	Range	St. deviation	Coeff. variation %
Calories		1760	879-3696	603	34
Protein	(gm.)	65	34-106	17	26
Fat	(gm.)	81	34-227	33	41
Carbohydrate	(gm.)	204	91-398	79	39
Calcium	(mg.)	759	252-1815	346	46
Iron	(mg.)	10.3	5.1-17.1	2.8	27
Vitamin A	(I.U.)	5254	1771-18604	3495	67
Thiamine	(mg.)	1.0	0.5-1.7	0.3	31
Riboflavin	(mg.)	1.5	0.8-2.9	0.6	40
Niacin	(mg.)	12.4	4.8-19.2	3.3	27
Ascorbic acid	(mg.)	80	13-183	43	53
Folate	(μg.)	66.0	21.4-160.9	27.4	42
Vitamin B_{12}	(μg.)	4.3	1.1-15.9	2.8	66

TABLE 8. Comparison between average daily Calorie and nutrient intakes of private and clinic patients

		Private group (50)	Clinic group (46)	Observed "t" values
Calories		2100 ± 484	1760 ± 603	3.03 **
Protein	(gm.)	87 ± 18	65 ± 17	6.11 **
Fat	(gm.)	97 ± 27	81 ± 33	2.59 *
Carbohydrate	(gm.)	228 ± 61	204 ± 79	1.66 ns
Calcium	(mg.)	1069 ± 407	759 ± 346	4.03 **
Iron	(mg.)	13.0 ± 3.3	10.3 ± 2.8	4.33 **
Vitamin A	(I.U.)	9135 ± 5383	5254 ± 3495	4.22 **
Thiamine	(mg.)	1.2 ± 0.3	1.0 ± 0.3	3.33 **[1]
Riboflavin	(mg.)	2.2 ± 0.8	1.5 ± 0.6	4.90 **
Niacin	(mg.)	15.3 ± 4.1	12.4 ± 3.3	3.84 **
Ascorbic acid	(mg.)	127 ± 58	80 ± 43	4.54 **
Folate	(μg.)	86.5 ± 26.1	66.0 ± 27.4	3.75 **
Vitamin B_{12}	(μg.)	9.8 ± 6.5	4.3 ± 2.8	5.46 **

[1] "t" test for equal variances and unequal number of observations (unmarked values were for unequal variances and unequal number of observations).

clinic patients submitted highly reliable records may have had some bearing on the marked discrepancy obtained between groups. For that reason, average intakes of Calories, protein, fat and carbohydrate were computed within each group according to the reliability score. From Table 9, it appears that these values tended to decrease slightly with falling reliability, this being even more evident for the clinic group. It is therefore possible that for a larger proportion of clinic than of private patients, inaccurate or incomplete recording may have resulted in lower estimates of intake. However, the possibility that the assessment of reliability was affected by the quantity of recorded foods is not excluded. Consequently, these means may be real or may be the result of the assumptions which had to be made in scoring the dietary records for reliability and in analyzing more doubtful data. The same question was raised by Thomson (1958) who observed that doubtful subjects were, on the average, socially, intellectually and clinically inferior to reliable subjects and that their diets also tended to be somewhat inferior.

The daily caloric intakes in this survey ranged from less than the probable basal energy expenditure to almost 4,000 Calories. Only with the help of an accurate way of assessing the reliance that can be placed on such data would it be possible to accept these extremes as real or as resulting from under- or over-estimation. However, values summarized in Table 9 are consistently higher for the private group than for the clinic group, suggesting that despite the possible effect of unequal quality of the information obtained from both categories of subjects, private patients had a better diet, qualitatively and quantitatively, than clinic patients. This further supports the findings of

TABLE 9. Comparison between average dietary intakes of private and clinic patients grouped on the basis of reliability

	Reliability index	No. of patients	Calories	Protein (gm.)	Fat (gm.)	Carbohydrate (gm.)
Private patients	"1"	35	2107	86	97	234
	"2"	10	2183	94	103	230
	"3"	5	1887	87	92	184
Clinic patients	"1"	12	1928	71	93	213
	"2"	18	1817	67	77	224
	"3"	16	1570	59	74	174

Thomson (1959) that with falling social status, the mean nutritive value of pregnant women's diets diminished.

Moscovitch (1965) studied the diet of pregnant women attending an antenatal public clinic in order to relate dietary and clinical findings of folate and vitamin B_{12} nutrition. On the basis of similarity of objectives and parity of the clinic groups investigated, results of this previous survey and of the present survey were compared. Moscovitch used various dietary survey techniques. However, only data obtained from seven-day dietary records were compared with the findings of the present study. Table 10 provides a comparison of average daily intakes of Calories and of seven nutrients for clinic groups belonging to each survey. In general, Moscovitch obtained higher values, but the differences were not significant ($P<.05$) for Calories, carbohydrate and iron intakes. Protein, folate and vitamin B_{12} consumption were significantly ($P<.05$ or $<.01$) higher in the earlier study. Only ascorbic acid and fat intakes were higher in the present clinic group, although not significantly ($P<.05$) so. Discrepancies in folate and vitamin B_{12} consumption by the two groups of clinic subjects were undoubtedly intensified by the fact that Moscovitch estimated folate and vitamin B_{12} content on the basis of uncooked diets, whereas "apparent" folate and B_{12} intakes (see Experimental Procedure, section 2, a) were measured in this study. Excluding folate and vitamin B_{12}, differences observed in the dietary intakes of these comparable groups of pregnant women can only be explained conjecturally. They may reflect real differences in food intake, incomplete recording by the present clinic group or a different estimation by the nutritionist of intakes when only average servings were reported. The last variable could be the most important one since Eagles

TABLE 10. Comparison between average dietary intakes of two clinic population groups, estimated from seven-day dietary records

		Present study No. of subjects:46	Moscovitch's study (1965)[1] No. of subjects:26	Observed "t" values
Calories		1760 ± 603	1874 ± 459	0.90ns
Protein	(gm.)	65 ± 17	75 ± 18	2.36*
Fat	(gm.)	81 ± 33	77 ± 20	0.64ns
Carbohydrate	(gm.)	204 ± 79	223 ± 67	1.09ns
Iron	(mg.)	10.3 ± 2.8	11.4 ± 3.6	1.38ns
Ascorbic acid	(mg.)	80 ± 43	75 ± 37	0.52ns
Folate	(μg.)	66.0 ± 27.4	97.0 ± 57.6	2.58*
Vitamin B_{12}	(μg.)	4.3 ± 2.8	12.7 ± 15.3	2.78**

[1]Standard deviations were calculated by the author from reported standard error of means.

et al. (1966) observed that the variation encountered among nutritionists in calculating the same diets was sometimes large enough to reach significance.

A further comparison was made between intakes of private patients in this study and of clinic patients studied by Moscovitch (1965). Table 11 shows that Calorie, carbohydrate and iron intakes were higher in the private than in the clinic group, although the differences were not significant ($P<.05$). However, the diets of private patients supplied significantly ($P<.01$) more protein, fat and ascorbic acid. Folate and vitamin B_{12} intakes were slightly higher, but not significantly so ($P<.05$) in the clinic group. It can consequently be inferred with reasonable assurance that private patients have on the average, better diets than pregnant women attending a public clinic since differences were apparent when comparing intakes of the private group with those obtained in two separate studies of clinic pregnant women.

c. Within-group variability: comparison of coefficients of variation

Within-group variability is best assessed by the coefficient of variation, which is the ratio of the standard deviation over the mean. It can be seen from the first part of Table 12 that the private group was more homogeneous than the clinic group, in which coefficients of variation were higher than in the private group for Calories and for all nutrients except niacin and vitamin B_{12}. Coefficients of variation were especially large, in both groups, for calcium, vitamin A, ascorbic acid and vitamin B_{12} intakes, indicating a tendency of the data to be skewed.

Coefficients of variation obtained in previous dietary surveys are presented in the second part of Table 12. The same trends were

TABLE 11. Comparison between average dietary intakes of private patients and of clinic patients from a previous study

		Private patients No. of subjects:50	Clinic patients (Moscovitch, 1965) No. of subjects:26	Observed "t" values
Calories		2100 ± 484	1874 ± 459	2.0 ns
Protein	(gm.)	87 ± 18	75 ± 18	2.79**[1]
Fat	(gm.)	97 ± 27	77 ± 20	3.70**
Carbohydrate	(gm.)	228 ± 61	223 ± 67	0.32ns
Iron	(mg.)	13.0 ± 3.3	11.4 ± 3.6	1.88ns
Ascorbic acid	(mg.)	127 ± 58	75 ± 37	4.73**
Folate	(μg.)	86.5 ± 26.1	97 ± 57.6	0.64ns
Vitamin B_{12}	(μg.)	9.8 ± 6.5	12.7 ± 15.3	0.93ns

[1] "t" test for equal variances and unequal number of observations (unmarked values were for unequal variances and unequal number of observations).

TABLE 12. Comparison of coefficients of variation of Calorie and nutrient intakes

	Present study		Previous studies			
	Clinic Group	Private Group	Stevens and Ohlson (1967)[1]	Moscovitch (1965)	Guggenheim et al. (1964)[1]	Cellier and Hankin (1963)
Calories	34	23	24	26	30	21
Protein	26	20	26	27	29	22
Fat	41	28	27	30	-	25
Carbohydrate	39	27	28	29	-	26
Calcium	46	38	43	-	44	-
Iron	27	25	26	33	31	22
Vitamin A	62	56	62	-	14	-
Thiamine	31	30	27	-	44	-
Riboflavin	40	36	39	-	50	-
Niacin	27	27	28	-	41	-
Ascorbic acid	53	46	41	60	6	48
Folate	42	30	-	52	-	-
Vitamin B_{12}	66	69	-	91	-	-

[1]Coefficients of variation were calculated from the data by the author.

observed with respect to variability, although Guggenheim et al. (1964) obtained much smaller variation in the consumption of vitamin A and ascorbic acid. However, the survey involved pregnant women from Israel and it is likely that different food habits would be reflected by a somewhat different pattern of variability. Nevertheless, whenever data were available for comparison, coefficients of variation for calcium, vitamin A, ascorbic acid and vitamin B_{12} intakes were in fairly good agreement in all other studies for which values appear in Table 12. Accordingly, Chappel (1955) observed, in long-term individual surveys, that intakes of vitamin A and C were generally more variable than those of other nutrients. Especially for these two vitamins, a seasonal variation in intakes (Harris and Oliver, 1943) may be responsible for the high coefficients of variation obtained.

The within-group variability observed in the present study being comparable to that of previous studies of homogeneous population groups, it was felt that no benefit would be obtained by regrouping the data according to certain criteria such as age and body weight of subjects and season. Furthermore, groups were not large enough to allow such comparisons.

d. Correlation between components of diets

Very few comprehensive investigations of the correlation between nutrient contents of dietaries have been reported previously, apart from correlation between intake of energy and single nutrients, such as protein. A search of the literature revealed that data on correlation between nutrients were available in three dietary surveys: the McCance Survey (McCance et al., 1938), measuring the diets of pregnant women; the Carnegie Survey of family diets (Rowett Research Institute, 1955)

and the Aberdeen Survey (Thomson, 1959) including 489 primigravidae. All three surveys were done in various parts of the United Kingdom.

Tables 13, 14 and 15 illustrate linear correlation and regression coefficients between the components of diets of private patients, clinic patients and women of the Aberdeen survey (Thomson, 1959) respectively. The three tables follow the same pattern. In each cell, the top figure is the correlation coefficient between the "pair" of nutrients. The middle figure is the regression coefficient of the variable in a given row on the variable in a given column. The bottom figure is the standard error of the estimate. The McCance and Carnegie Surveys (ref. cit.) were not used for comparison because the former did not include such data for any vitamin and the latter excluded riboflavin and niacin.

It is apparent from Tables 13 and 14 that almost all dietary components were highly correlated - i.e., in both private and clinic patients. However, the correlation was usually higher in the clinic than in the private group. Coefficients of correlation shown in Table 15, pertaining to the Aberdeen Survey (ref. cit.), were in better agreement with those of the actual clinic patients than of the private patients. The Aberdeen Survey included a high proportion of subjects from a low economic class. It is therefore possible that, because of reduced variety of foods consumed, dietary components are more highly correlated in lower income groups.

Regression coefficients obtained in both studies were of the same order. The discrepancy which can be observed between all figures involving calcium is only apparent and is due to the use of milligrams as the unit in the present survey and of grams in the previous survey. However, despite the high proportion of significant coefficients, regression

TABLE 13. Linear correlation (r) and regression (the diets of pri

	Calories	Protein	Fat	Carboh.	Calcium	Iron
Protein (r)	0.69					
(b)	0.025					
(S.E.)	12.8					
Fat	0.90	0.56				
	0.05	0.85				
	11.8	22.4				
Carbohydrate	0.90	0.52	0.66			
	0.114	1.80	1.49			
	26.1	52.0	45.9			
Calcium	0.42	0.64	0.26	0.42		
	0.36	14.65	3.93	2.79		
	368.4	311.4	392.5	369.2		
Iron	0.74	0.75	0.59	0.64	0.19	
	0.005	0.137	0.071	0.034	0.002	
	2.21	2.17	2.63	2.52	3.21	
Vitamin A	0.29	0.55	0.18	0.20	0.32	0.50
	3.19	164.9	36.45	17.89	4.25	829.9
	5156.8	4506.3	5291.5	5270.9	5098.2	4650.1
Thiamine	0.54	0.57	0.46	0.46	0.26	0.69
	0.0004	0.011	0.006	0.003	0.0002	0.07
	0.29	0.28	0.31	0.31	0.33	0.25
Riboflavin	0.50	0.81	0.35	0.42	0.86	0.46
	0.0008	0.036	0.010	0.005	0.002	0.11
	0.69	0.47	0.75	0.73	0.41	0.71
Niacin	0.57	0.68	0.50	0.42	-0.02	0.85
	0.005	0.158	0.077	0.028	-0.0002	1.08
	3.39	3.03	3.58	3.75	4.14	2.16
Ascorbate	0.29	0.40	0.15	0.29	0.13	0.49
	0.035	1.28	0.312	0.279	0.019	8.63
	55.3	53.1	57.2	55.3	57.3	50.5

ı (r) and regression (b) coefficients between components of the diets of private patients[1]

ı.	Calcium	Iron	Vitamin A	Thiamine	Ribof.	Niacin	Ascorbic a.	Folate
	0.19							
	0.002							
	3.21							
	0.32	0.50						
	4.25	829.9						
	5098.2	4650.1						
	0.26	0.69	0.51					
	0.0002	0.073	0.00003					
	0.33	0.25	0.30					
	0.86	0.46	0.67	0.51				
	0.002	0.113	0.0001	1.17				
	0.41	0.71	0.59	0.69				
	-0.02	0.85	0.53	0.70	0.33			
	-0.0002	1.08	0.0004	8.38	1.71			
	4.14	2.16	3.52	3.0	3.9			
	0.13	0.49	0.50	0.57	0.32	0.48		
	0.019	8.63	0.005	96.13	23.54	6.68		
	57.3	50.5	50.02	47.3	54.7	50.8		

Table 13 (cont'd)

	Calories	Protein	Fat	Carboh.	Calcium	Iron
Folate	0.38	0.64	0.14	0.39	0.33	0.71
	0.021	0.942	0.132	0.167	0.021	5.70
	24.1	19.9	25.8	24.0	24.6	18.2
Vitamin B_{12}	0.27	0.66	0.20	0.12	0.49	0.44
	0.004	0.240	0.049	0.013	0.008	0.883
	6.30	4.9	6.4	6.49	5.7	5.9

[1]Correlation coefficients of .28 and over were significant ($P<.05$).

ble 13 (cont'd)

Calcium	Iron	Vitamin A	Thiamine	Ribof.	Niacin	Ascorbic a.	Folate
0.33	0.71	0.58	0.53	0.54	0.59	0.53	
0.021	5.70	0.003	39.82	17.64	3.69	0.237	
4.6	18.2	21.2	22.2	21.9	21.2	22.2	
0.49	0.44	0.86	0.45	0.82	0.42	0.38	0.57
0.008	0.883	0.001	8.60	6.72	0.662	0.043	0.143
5.7	5.9	3.4	5.8	3.7	5.9	6.1	5.4

TABLE 14. Linear correlation (r) and regression (b) c
the diets of clinic

	Calories	Protein	Fat	Carboh.	Calcium	Iron
Protein (r)	0.86					
(b)	0.02					
(S.E.)	8.54					
Fat	0.91	0.72				
	0.05	1.41				
	13.56	22.8				
Carbohydrate	0.93	0.80	0.71			
	0.12	3.77	1.71			
	28.35	47.4	55.5			
Calcium	0.70	0.83	0.57	0.67		
	0.40	17.27	6.01	2.97		
	248.18	193.67	286.3	256.7		
Iron	0.80	0.79	0.62	0.82	0.59	
	0.004	0.13	0.05	0.03	0.005	
	1.67	1.71	2.19	1.58	2.26	
Vitamin A	0.49	0.50	0.42	0.48	0.58	0.58
	2.87	104.2	16.61	21.3	5.84	727.4
	3038	3031	3175	3064	2846	2846
Thiamine	0.72	0.67	0.56	0.74	0.52	0.81
	0.0004	0.012	0.005	0.003	0.0004	0.09
	0.21	0.22	0.26	0.21	0.26	0.18
Riboflavin	0.76	0.85	0.62	0.74	0.93	0.70
	0.0007	0.03	0.01	0.005	0.001	0.14
	0.36	0.29	0.44	0.37	0.20	0.40
Niacin	0.72	0.71	0.64	0.67	0.43	0.83
	0.004	0.14	0.06	0.03	0.004	0.98
	2.31	2.35	2.56	2.46	3.00	1.87
Ascorbate	0.46	0.49	0.37	0.45	0.55	0.55
	0.03	1.26	0.48	0.24	0.07	8.48
	37.8	37.06	39.59	38.05	35.71	35.48

) and regression (b) coefficients between components of the diets of clinic patients[1]

Calcium	Iron	Vitamin A	Thiamine	Ribof.	Niacin	Ascorbic a.	Folate
0.59							
0.005							
2.26							
0.58	0.58						
5.84	727.4						
2846	2846						
0.52	0.81	0.53					
0.0004	0.09	0.00005					
0.26	0.18	0.26					
0.93	0.70	0.73	0.64				
0.001	0.14	0.0001	1.19				
0.20	0.40	0.38	0.43				
0.43	0.83	0.57	0.74	0.61			
0.004	0.98	0.005	8.12	3.61			
3.00	1.87	2.73	2.25	2.64			
0.55	0.55	0.51	0.63	0.59	0.43		
0.07	8.48	0.006	89.44	45.48	5.50		
35.71	35.48	36.64	33.06	34.31	38.5		

TABLE 14 (cont'd

	Calories	Protein	Fat	Carboh.	Calcium	Iron
Folate	0.61	0.73	0.43	0.65	0.67	0.73
	0.03	1.2	0.36	0.22	0.05	7.19
	21.6	18.7	24.72	20.9	20.39	18.68
Vitamin B_{12}	0.36	0.42	0.24	0.39	0.49	0.45
	0.002	0.07	0.02	0.01	0.004	0.45
	2.63	2.55	2.73	2.60	2.46	2.51

Correlation coefficients of .30 and over were significant ($P<.05$).

TABLE 14 (cont'd

Calcium	Iron	Vitamin A	Thiamine	Ribof.	Niacin	Ascorbic a.	Folate
0.67	0.73	0.62	0.57	0.67	0.59	0.58	
0.05	7.19	0.005	52.20	33.14	4.88	0.37	
20.39	18.68	21.45	22.45	20.26	22.11	22.36	
0.49	0.45	0.72	0.38	0.70	0.42	0.39	0.40
0.004	0.45	0.0006	3.56	3.54	0.36	0.03	0.04
2.46	2.51	1.96	2.60	2.01	2.55	2.59	2.57

TABLE 15. Linear correlation (r) and regression (l
the diets of pregnant w

	Calories	Protein	Fat	Carboh.	Calcium	Vitami
Protein (r)	0.86					
(b)	0.027					
(S.E.)	8.3					
Fat	0.89	0.80				
	0.043	0.52				
	11.3	9.8				
Carbohydrate	0.92	0.71	0.65			
	0.133	0.156	1.94			
	29.4	11.5	56.5			
Calcium	0.69	0.76	0.63	0.59		
	0.0004	0.016	0.008	0.003		
	0.241	0.215	0.259	0.269		
Vitamin A	0.32	0.34	0.33	0.22	0.44	
	2.4	79.8	51.3	11.6	51.90	
	3671	3697	3654	3775	3474	
Thiamine	0.78	0.79	0.71	0.68	0.55	0.34
	0.0004	0.013	0.007	0.002	0.43	5059
	0.150	0.159	0.181	0.188	0.216	3645
Riboflavin	0.72	0.91	0.68	0.57	0.84	0.48
	0.0007	0.028	0.014	0.004	1.25	3747
	0.347	0.211	0.368	0.407	0.272	3393

id regression (b) coefficients between components of
s of pregnant women (Thomson, 1959)[1]

alcium	Vitamin A	Thiamine	Ribof.	Niacin
).44				
1.90				
3474				
).55	0.34			
).43	5059			
).216	3645			
).84	0.48	0.71		
:.25	3747	1.36		
).272	3393	0.35		

Table 15 (cont'd)

	Calories	Protein	Fat	Carboh.	Calcium	Vitamin
Niacin	0.64	0.71	0.62	0.52	0.34	0.45
	0.004	0.12	0.07	0.02	2.87	619
	2.12	1.95	2.18	2.37	2.60	3464
Ascorbate	0.35	0.33	0.27	0.34	0.39	0.35
	0.02	0.64	0.34	0.14	37.2	0.003
	29.5	29.7	30.3	29.6	28.9	29.4

All correlation coefficients were significant ($P<.01$).

ble 15 (cont'd)

Calcium	Vitamin A	Thiamine	Ribof.	Niacin
0.34	0.45	0.71	0.65	
2.87	619	7.63	3.61	
2.60	3464	1.95	2.08	
0.39	0.35	0.36	0.37	0.32
37.2	0.003	44.3	23.7	3.63
28.9	29.4	29.3	29.1	29.8

coefficients could not be used in estimating an individual's intake of a given nutrient if the other member of the "pair" is known because of extremely large values for the standard errors of estimate. Increasing the number of observations commensurately reduces the size of error. This is the reason for lower standard errors of estimate in the Aberdeen Survey, which included 489 pregnant women, compared to 50 private and 46 clinic patients included in the present study.

High correlation values between dietary components suggest that in interpreting dietary survey data, intakes of Calories or of any nutrient should not be considered independently. Differences in the intake of a given dietary component would imply proportional differences in other components with which it is highly correlated. The caloric value of diets being highly and significantly ($P<.05$) correlated with all other nutrients, it follows that a reduction in caloric intakes will automatically be accompanied by a reduction in intakes of most nutrients. This is evidenced in Table 16, in which are presented average daily nutrient intakes at various levels of caloric intakes. Subjects consuming less than 2000 Calories daily had nutrient intakes appreciably lower than when the caloric value of diets was over 2000 or 2500 Calories; this was especially true in clinic patients.

Similarly, since dietary folate was highly correlated with protein and iron content of diets, low intakes of one should result in low values for the others. A high correlation (over 0.7) was also observed between vitamin B_{12} and vitamin A, and between vitamin B_{12} and riboflavin intakes. However, vitamin B_{12} and protein were more highly correlated in the private group than in the clinic group. It is likely that more animal protein was consumed by private than clinic subjects, since

TABLE 16. Average daily nutrient intakes at various levels of Calorie intakes

Calorie intakes	No. of subjects	Protein gm.	Fat gm.	Carb. gm.	Calcium mg.	Iron mg.	Vit.A I.U.	Thiam. mg.	Ribof. mg.	Niac. mg.	Asc.a. mg.	Folate µg.	Vit.B_{12} µg.
						Private Patients							
2500	10	108	117	307	1220	17.1	13960	1.4	2.8	20.2	151	102.3	13.9
2000-2500	19	91	102	238	1213	13.5	10169	1.3	2.5	15.4	145	91.1	11.2
2000	21	75	76	181	866	10.5	6442	0.9	1.7	12.9	99	74.8	6.6
						Clinic Patients							
2500	6	93	133	348	1180	14.5	7118	1.4	2.2	17.0	120	100.3	5.1
2000-2500	8	97	102	268	975	12.5	8341	1.2	1.9	14.9	87	84.1	6.9
2000	32	57	65	161	625	9.0	4132	0.8	1.2	11.0	71	55.1	3.5

vitamin B_{12} is found mainly in animal protein foods.

e. Proportion of total Calories derived from protein, fat and carbohydrate

For the private and clinic groups, mean percentages of total Calories derived from protein, fat and carbohydrate are shown in the upper part of Table 17. The contribution of protein to Calories was higher in dietaries of private than of clinic patients. Conversely, the diets of clinic subjects supplied relatively more Calories in the form of carbohydrate than did diets of private patients.

The contribution of protein towards Calories seemed markedly higher in both population groups than reported previously by Dole (1959) from the statistics of food consumption in 32 nations, and by Thomson (1959) for a large population of pregnant women. It was, in addition, higher than the average Canadian diet (Nutrition Division, 1959), in which protein supplies approximately 12% of total Calories. However, if intakes are broken down within each group according to caloric values of diets (Table 17, lower part), it becomes apparent that percentages of Calories supplied by protein tended to increase with decreasing caloric intake. Therefore, for diets supplying 2500 Calories and over daily, the protein contribution towards Calories was comparable, at least for the clinic group, with the average value of Canadian diets, which supply 3000 Calories per day. Nevertheless, consistently higher percentages of protein Calories could be observed in the private group, at all levels of total Calorie intakes. Moreover, carbohydrate supplied more Calories than fat in the clinic group whereas the proportion of total Calories derived from fat and from carbohydrate in diets of private subjects was roughly similar.

TABLE 17. Mean percentages of Calories derived from protein, fat and carbohydrate in the diet of private and clinic patients

	No. of subjects		Per cent of total Calories derived from		
			Protein	Fat	Carbohydrate
Private group	50		17.1	41.9	41.0
Clinic group	46		15.3	41.1	43.6
Private group					
Daily caloric intake					
2500 and over	10	(20%)	15.6	42.5	41.9
2000 - 2500	19	(38%)	16.9	42.5	40.6
under 2000	21	(42%)	17.9	40.9	41.2
Clinic group					
Daily caloric intake					
2500 and over	6	(13%)	12.8	41.2	46.0
2000 - 2500	8	(17%)	13.7	40.6	45.7
under 2000	32	(70%)	16.1	41.2	42.7

f. Average daily Calorie and nutrient intakes in each trimester of pregnancy

Dietary records were grouped according to the trimester of pregnancy during which they had been kept and intakes averaged for each trimester, within each of the two groups. Table 18 illustrates dietary intakes of private patients in each trimester and in Table 19 similar data are found for clinic patients. Differences between trimesters, with respect to Calorie and nutrient content of diets of private patients, were not statistically significant ($P<.05$). However, intakes of Calories and most nutrients in the first and second trimesters were slightly higher than in the last trimester of pregnancy and in this respect the results agreed with the findings of Darby et al. (1953). Cellier and Hankin (1963) reported that protein intakes significantly increased throughout pregnancy and that fat intake was significantly higher in the second than in the third trimester. It is possible, on the basis of previous observations, that if three dietary sheets had been submitted by each subject of the present study, individual comparisons between intakes at various stages of pregnancy would have given different results. Nevertheless, because of individual variation in food consumption from week to week (Yudkin, 1951), comparisons between intakes of individuals or groups in trimesters may not be very divergent. The slight reduction observed in food intake of private patients by the end of pregnancy could very well be due to subjects' self-regulation of eating patterns in order to avoid excessive gain of weight or else to an increased appetite during the first part of pregnancy, as was proposed by Taggart (1961).

A somewhat different trend was observed in clinic patients. Intakes of Calories and of a few other nutrients tended to be elevated in

TABLE 18. Average daily Calorie and nutrient intakes of private patients; comparison between trimesters of pregnancy

		First Trimester (14)	Second Trimester (33)	Third Trimester (18)	Observed "t" values		
					1st vs 2nd	2nd vs 3rd	1st vs 3rd
Calories	(gm.)	2180 ± 709	2087 ± 417	2057 ± 475	0.46 ns	0.22 ns	0.56 ns
Protein	(gm.)	90 ± 21	86 ± 18	84 ± 20	0.63 ns	0.36 ns	0.82 ns
Fat	(gm.)	99 ± 42	98 ± 21	96 ± 26	0.09 ns	0.28 ns	0.23 ns
Carbohydrate	(gm.)	240 ± 79	225 ± 57	226 ± 65	0.65 ns	0.05 ns	0.54 ns
Calcium	(mg.)	1056 ± 427	1084 ± 457	998 ± 383	0.01 ns	0.71 ns	0.40 ns
Iron	(mg.)	13.4 ± 4.0	12.7 ± 3.1	12.8 ± 3.9	0.59 ns	0.10 ns	0.44 ns
Vitamin A	(I.U.)	10383 ± 5046	8615 ± 5404	8714 ± 5568	1.38 ns	0.06 ns	1.15 ns
Thiamine	(mg.)	1.2 ± 0.4	1.1 ± 0.3	1.1 ± 0.3	0.88 ns	-	0.25 ns
Riboflavin	(mg.)	2.3 ± 0.7	2.2 ± 0.9	2.1 ± 0.7	0.42 ns	0.45 ns	0.83 ns[1]
Niacin	(mg.)	16.7 ± 5.4	15.1 ± 3.5	14.9 ± 4.3	1.66 ns	0.17 ns	1.06 ns
Ascorbic acid	(mg.)	143 ± 57	119 ± 59	140 ± 85	1.31 ns	0.93 ns	0.12 ns
Folate	(μg.)	91.3 ± 33.1	83.7 ± 24.8	83.6 ± 25.5	0.78 ns	0.01 ns	0.72 ns
Vitamin B_{12}	(μg.)	10.2 ± 5.8	9.8 ± 7.1	7.9 ± 5.6	0.20 ns	1.06 ns	1.13 ns

[1]"t" test for equal variances and unequal number of observations (unmarked values were for unequal variances and unequal number of observations).

TABLE 19. Average daily Calorie and nutrient intakes of clinic patients; comparison between trimesters of pregnancy

		First Trimester (8)	Second Trimester (25)	Third Trimester (18)	Observed "t" values		
					1st vs 2nd	2nd vs 3rd	1st vs 3rd
Calories		1708 ± 510	1704 ± 538	1781 ± 724	0.02 ns	0.44 ns	0.29 ns
Protein	(gm.)	68 ± 15	63 ± 16	64 ± 20	0.81 ns	0.18 ns	0.57 ns
Fat	(gm.)	78 ± 20	76 ± 27	85 ± 43	0.22 ns	0.79 ns	0.57 ns
Carbohydrate	(gm.)	194 ± 77	201 ± 77	201 ± 83	0.22 ns[1]	-	0.10 ns
Calcium	(mg.)	630 ± 315	511 ± 427	830 ± 406	0.85 ns	2.49*	1.36 ns
Iron	(mg.)	12.2 ± 3.1	9.9 ± 2.4	10.0 ± 3.4	1.21 ns	0.11 ns	1.63 ns
Vitamin A	(I.U.)	4531 ± 2581	5024 ± 3369	5700 ± 3873	0.43 ns	0.60 ns	0.91 ns
Thiamine	(mg.)	1.0 ± 0.3	1.0 ± 0.3	0.9 ± 0.3	-	1.11 ns[1]	0.79 ns[1]
Riboflavin	(mg.)	1.4 ± 0.5	1.4 ± 0.5	1.6 ± 0.6	-	1.18 ns	0.91 ns
Niacin	(mg.)	13.5 ± 2.0	11.8 ± 3.2	12.3 ± 3.6	1.79 ns	0.47 ns	1.09 ns
Ascorbic acid	(mg.)	69 ± 55	83 ± 44	74 ± 40	0.66 ns	0.70 ns	0.23 ns
Folate	(µg.)	64.5 ± 16.1	65.8 ± 27.1	64.4 ± 34.2	0.17 ns	0.14 ns	0.10 ns
Vitamin B_{12}	(µg.)	4.5 ± 2.4	3.8 ± 2.8	4.2 ± 2.7	0.70 ns	0.48 ns	0.29 ns

[1]"t" test for equal variances and unequal number of observations (unmarked values were for unequal variances and unequal number of observations).

the third trimester, in relation to earlier pregnancy (Table 19). However, differences were statistically significant ($P<.05$) only for calcium. Higher intakes of calcium by clinic patients in late pregnancy were probably caused by increased consumption of milk.

It can be said that in both groups of pregnant women, there was no evidence of any marked increase of appetite or food intake during the last part of pregnancy, as might be inferred from accepted standards of energy requirements during pregnancy (see Section D, 1).

2. Non-pregnant Control Subjects

Average daily Calorie and nutrient intakes of nine control subjects are summarized in Table 20. Protein supplied 14.4%, fat, 39.7% and carbohydrate, 45.9% of total Calories. Carbohydrate intakes were higher in this group than in the private group; however, for Calories and all other nutrients, the values were lower. In addition, mean intakes of calcium, thiamine, ascorbic acid and folate were less than observed in the clinic group. The control group was unfortunately much too small to allow any statistical comparisons or inferences concerning usual dietary intakes of non-pregnant healthy young females. Very irregular eating habits were detectable from dietary records submitted by members of this particular group. However, the pattern of within-group variability was roughly similar to that of the pregnant groups, with vitamin A, ascorbic acid and vitamin B_{12} consumption showing the highest coefficients of variation.

A comparison with the Canadian Recommended Dietary Allowances for women between 18 and 35 years of age, involved in light activity, revealed that intakes were adequate, according to this Standard, for all

TABLE 20. Average daily Calorie and nutrient intakes of control subjects and comparison with the Canadian recommended Dietary Allowances

		Mean	Standard deviation	Coefficient of variation %	Mean intake as percentage of Canadian Dietary Allowances
Calories		1995	394	20	83
Protein	(gm.)	72	18	25	185
Fat	(gm.)	88	20	23	-
Carbohydrate	(gm.)	232	51	22	-
Calcium	(mg.)	755	253	34	151
Iron	(mg.)	11.2	2.9	26	112
Vitamin A	(I.U.)	6248	3979	64	169
Thiamine	(mg.)	0.9	0.2	22	129
Riboflavin	(mg.)	1.6	0.5	32	133
Niacin	(mg.)	13.9	4.9	35	199
Ascorbic acid	(mg.)	71	26	37	237
Folate	(μg.)	60.5	19.9	33	-
Vitamin B_{12}	(μg.)	6.2	5.0	80	-

nutrients but did not meet the proposed intakes for Calories (Table 20). However, Calorie requirements, as presented in most dietary standards, are for a certain level of physical activity and it seems that they are set too high for the amount of activity of some individuals. Consequently, since nutrient intakes met the Canadian requirements, control subjects likely consumed sufficient amounts of Calories.

D. ASSESSMENT OF DIETARY ADEQUACY OF THE PREGNANT GROUPS STUDIED

1. Comparison of Calorie and Nutrient Intakes with the Canadian and American Recommended Dietary Allowances for Pregnancy

The Canadian and American Dietary Standards differ widely in their Recommended Allowances for Calories and for a few nutrients. This discrepancy comes from a different definition of dietary allowances. Whereas the recommended intakes of the Canadian Standard (1964) are intended to "give reasonable assurance of health for the majority of Canadians without encouraging excess," the dietary allowances of the American Standard (1964) "will maintain good nutrition in essentially all healthy persons in the United States under current conditions of living." Consequently, the margin of sufficiency over and above average physiological requirements, intended to cover individual physical and biochemical variation, is much more generous in the latter than in the former Dietary Standard with the exception of recommended daily Calorie intakes, which have been recently adjusted downwards in view of the progressive decrease in activity of most people living in the United States.

The Canadian and American Recommended Dietary Allowances for various stages of pregnancy are listed in Table 21. The levels of intake

TABLE 21. Canadian and American Recommended Dietary Allowances for various stages of pregnancy

		1st trimester		2nd trimester		3rd trimester	
		Can. Standard	Am. Standard	Can. Standard	Am. Standard	Can. Standard	Am. Standard
Calories		2400	2100	2400	2300	2900	2300
Protein	(gm.)	39	58	39	78	49	78
Calcium	(mg.)	.5	.8	.5	1.3	.7	1.3
Iron	(mg.)	10	15	10	20	.13	20
Vitamin A	(I.U.)	3700	5000	3700	6000	4200	6000
Ascorbic Acid	(mg.)	30	70	30	100	40	100
Thiamine	(mg.)	17	.8	.7	1.0	.85	1.0
Riboflavin	(mg.)	1.2	1.3	1.2	1.6	1,45	1.6
Niacin	(mg.)	7	14	7	17	8.5	17

appearing under "Canadian Standard" are those recommended for women approximately 25 years of age, weighing 124 pounds and measuring 67.2 inches and involved in light activity. Only allowances for the third trimester of pregnancy are higher than for non-pregnant females. The American Allowances are for women 25 years of age, weighing 128 pounds and measuring 64 inches. They are intended for persons normally active in the climate of the country. Additional intakes are recommended for pregnant women by the second trimester, accounting for the wide differences between the Canadian and American Dietary Allowances during this stage of pregnancy.

Average daily Calorie and nutrient intakes of private and clinic patients in relation to the Canadian and American Dietary Allowances are illustrated in Figures V, VI and VII for the first, second and third trimester of pregnancy, respectively. Intakes of both groups during the first trimester were above the Canadian Standard for all nutrients. Although Calories did not measure up to the Canadian Recommended Allowances, it is felt that the energy supply of diets was adequate in most cases since the activity of the subjects was rather limited. During the second trimester, values for iron were below the Canadian Standard in the clinic group. Dietary iron of the last trimester of pregnancy was slightly inferior to the Canadian requirements in the private group and was probably inadequate in the clinic group. This was also observed by Moscovitch (1965) for diets of clinic pregnant women. Iron intakes were often found insufficient during pregnancy. Therefore, supplementing the diet with iron is now a common practice. In addition, clinic patients' diets were suboptimal in thiamine during late pregnancy, according to the Canadian Standard.

FIG. V. Average daily Calorie and nutrient intakes of private and clinic patients in the first trimester of pregnancy in relation to Canadian and American recommended dietary allowances

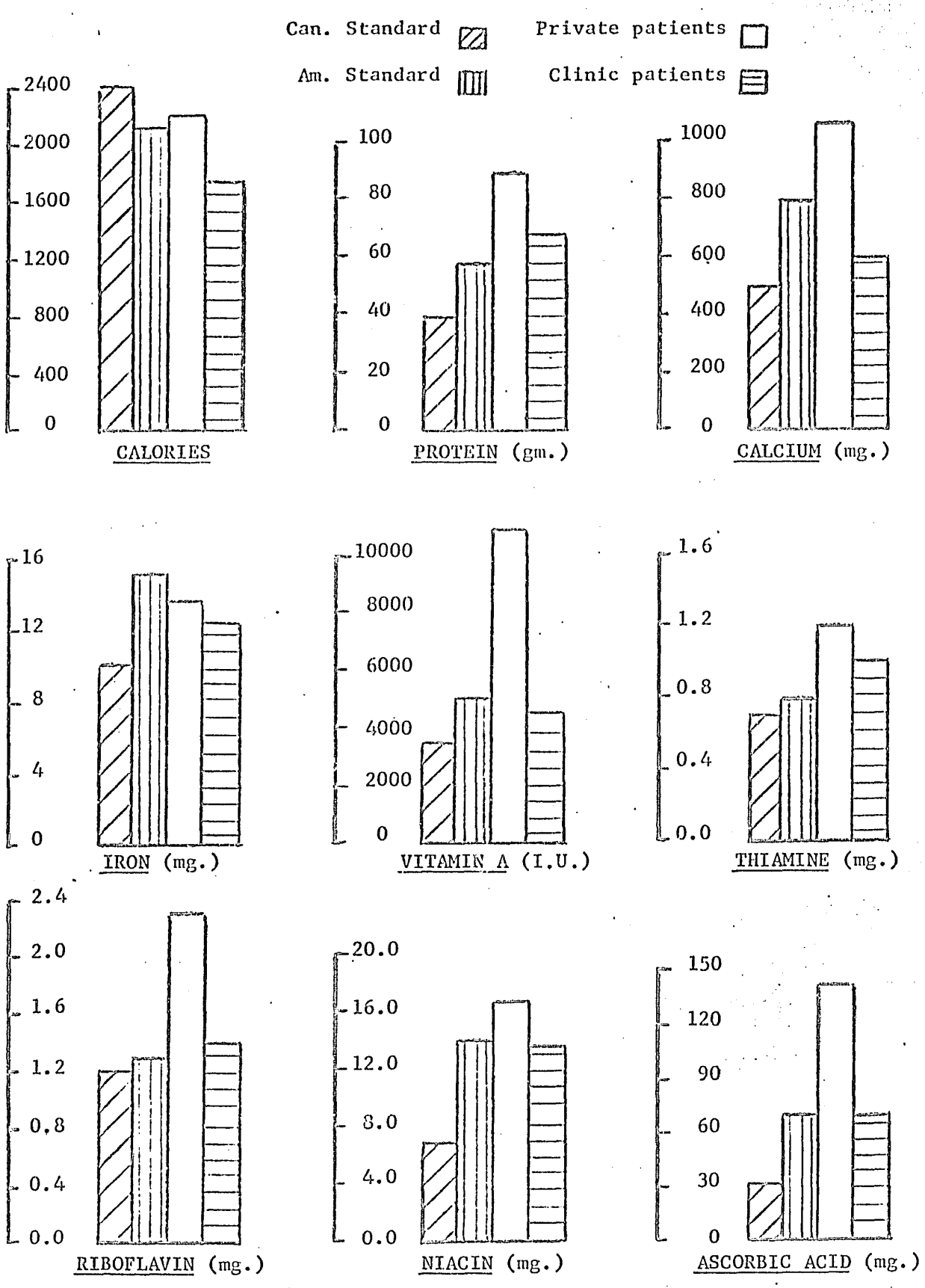


FIG. VI. Average daily Calorie and nutrient intakes of private and clinic patients in the second trimester of pregnancy in relation to Canadian and American Recommended Dietary Allowances

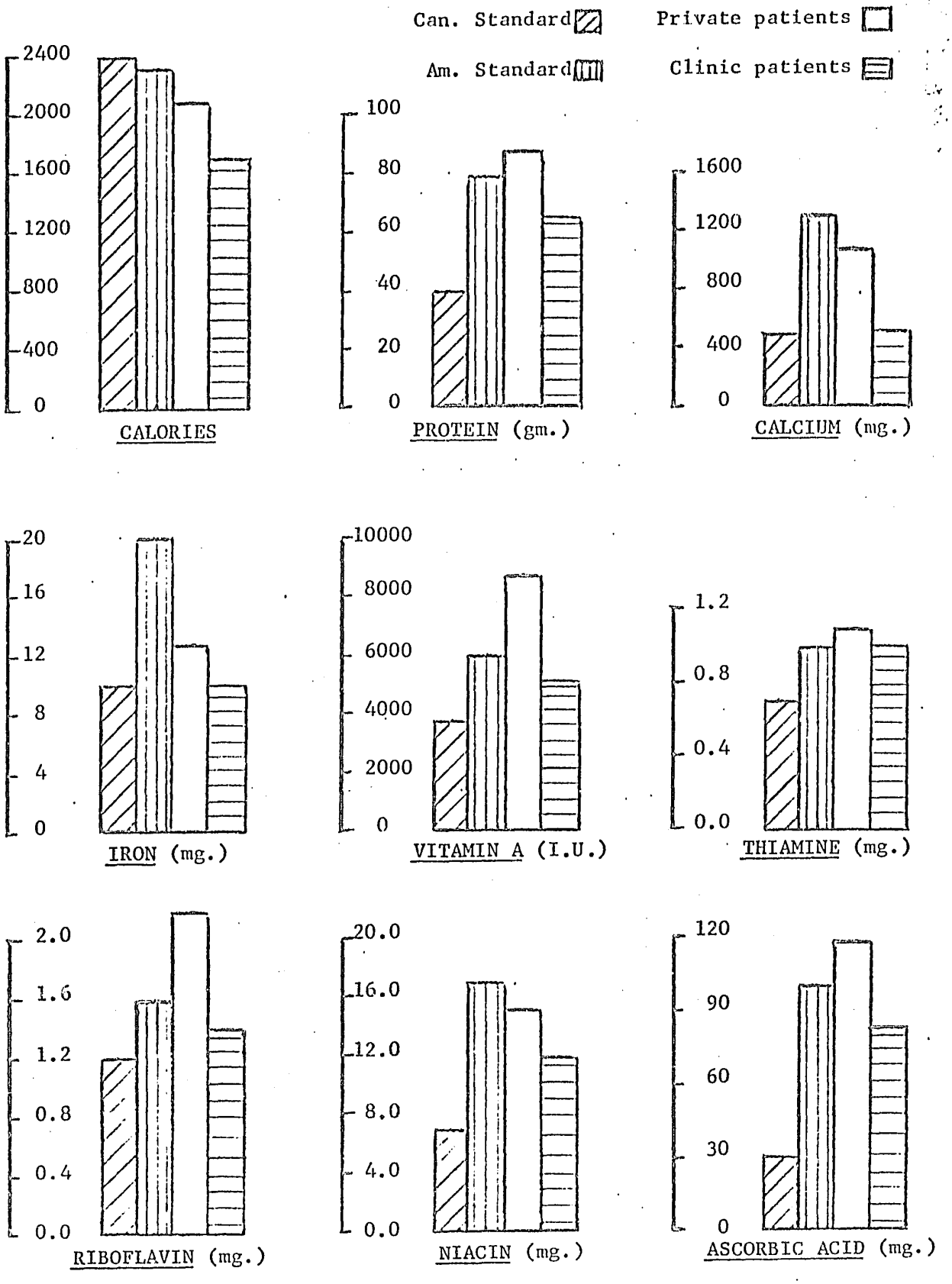
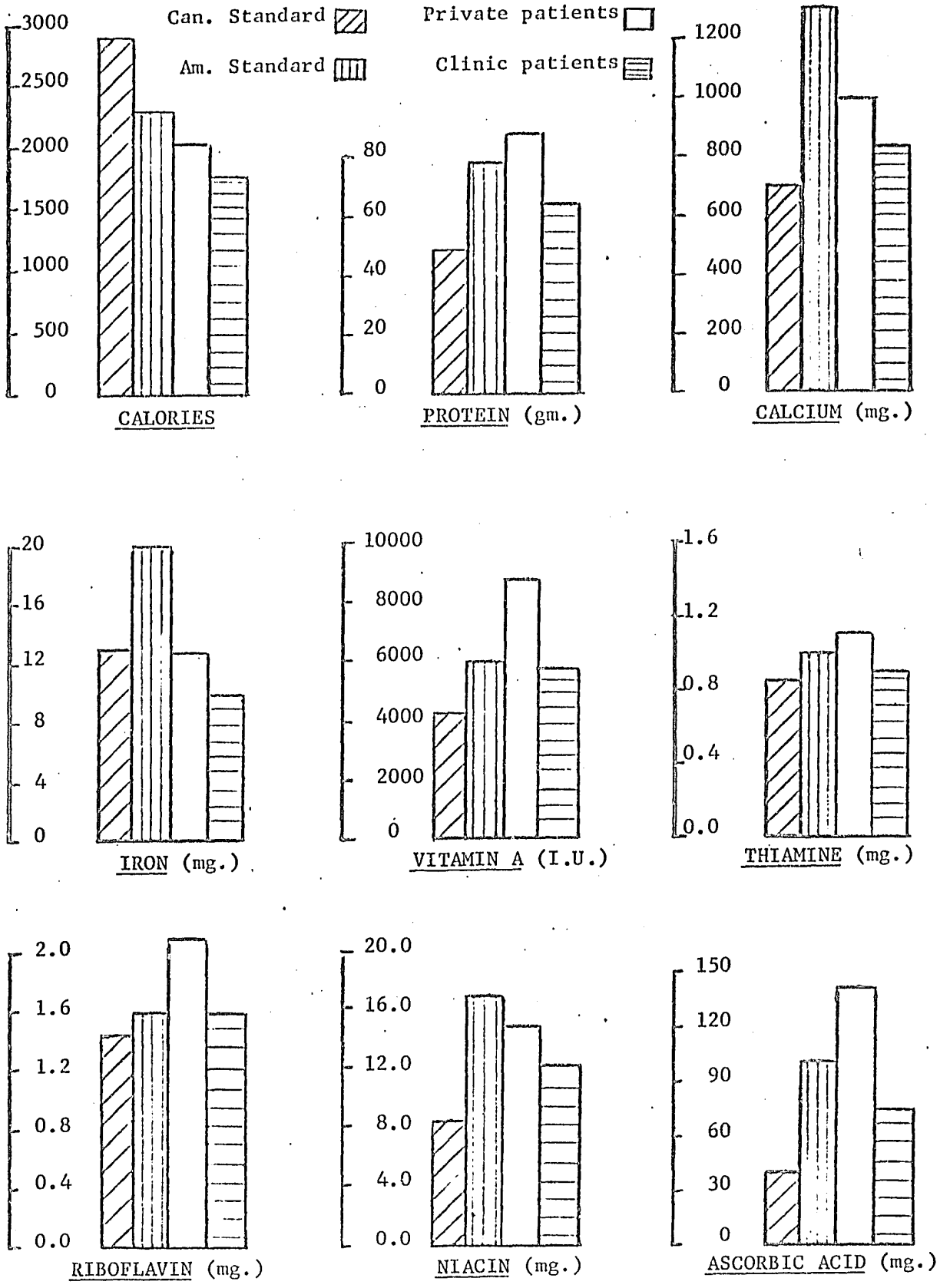


FIG. VII. Average daily Calorie and nutrient intakes of private and clinic patients during the last trimester of pregnancy in relation to Canadian and American Recommended Dietary Allowances



The American Standard being set much higher than the Canadian Standard in all trimesters of pregnancy, average intakes did not meet the American requirements for many nutrients. Intakes of clinic patients met the American allowances only for protein, thiamine and riboflavin in the first trimester, for thiamine in the second trimester and for riboflavin in the last trimester. Intakes of private patients were below the American Standard for iron in the first trimester, calcium, iron and niacin in the second trimester and protein, calcium, iron and niacin in the last trimester of pregnancy. These results are quite different from those of Stevens and Ohlson (1967) who found that the estimated mean values for nutrients in 129 medically indigent pregnant women met or exceeded the 1958 American Dietary Allowances for pregnancy, except for calcium, iron and ascorbic acid in the youngest and oldest groups of subjects.

Because of the wide spread of values around means in the two groups of pregnant women studied, percentages of groups whose intakes were below the Canadian Recommended Dietary Allowances have been determined and are listed in Table 22. A high proportion of clinic patients' diets did not meet the requirements and this is most evident for iron in all trimesters and for calcium and vitamin A in the first trimester. Most patients belonging to either group had energy intakes lower than those proposed in the Canadian Standard. The significance of the latter findings was discussed previously. Recommended dietary allowances for persons of the same age, sex and degree of activity provide at best an approximate estimate of individual requirements, which vary according to basal metabolism, muscular efficiency and other factors. In addition, requirements are in direct relation to the energy value of diets.

TABLE 22. Proportion of private and clinic patients with dietary intakes below the Canadian Recommended Dietary Allowances

Stage of pregnancy			Calories	Protein	Calcium	Iron	Vit. A	Thiam.	Ribof.	Niac.	Asc. a.
1st trim.	Clinic	(%)	88	0	50	50	50	13	38	0	25
	Private	(%)	64	0	7	21	7	7	0	0	0
2nd trim.	Clinic	(%)	88	8	32	60	24	16	44	4	16
	Private	(%)	76	0	6	15	12	0	0	0	0
3rd trim.	Clinic	(%)	94	22	39	83	39	44	44	11	17
	Private	(%)	100	0	17	61	17	22	22	66	11

Consequently, it would be wrong to conclude that percentages shown in Table 22 indicate the proportion of truly "deficient" diets. However, it can be said that few diets of clinic patients were manifestly adequate in terms of recommended intakes for "optimum" nutrition.

2. Folate and Vitamin B_{12} Consumption

The exact requirements for folate and vitamin B_{12} have not been defined as yet for normal adults. Therefore, the adequacy of intakes of these vitamins by pregnant women, for whom requirements are increased, can only be assessed through comparisons with intakes of healthy pregnant women consuming diets adequate with respect to all other nutrients.

It was previously shown (see Section D, 1) that the private patients had much better diets than the clinic patients, according to the Canadian or American Recommended Dietary Allowances. Consequently, it can be inferred that their consumption of folate and vitamin B_{12} was on the average adequate unless extremely high requirements for these two vitamins have to be met during pregnancy. Mean and median folate and vitamin B_{12} intakes of private and clinic patients are shown in Table 23. It can be seen in this table that diets of clinic patients supplied less folate and vitamin B_{12} than those of private patients. A lower consumption of organ meats and dark green vegetables by clinic patients may be reflected in these lower values. Medians were at the left of means, except for folate intakes of clinic patients. However, because of the high coefficients of variation associated with averages of both vitamins, a distribution table may be more representative of intakes. Table 24 shows that only 6% of the private patients but 32% of the clinic patients had folate intakes lower than 50 μg., a value recommended

TABLE 23. Mean and median folate and vitamin B_{12} intakes of private and clinic patients

			Private patients	Clinic patients
Folate	(μg.)	Mean	86.5 ± 26.1	66.0 ± 7.4
		Median	84.4	66.0
Vitamin B_{12}	(μg.)	Mean	9.8 ± 6.5	4.3 ± 2.8
		Median	7.3	3.4

TABLE 24. Distribution of folate and vitamin B_{12} daily intakes of private and clinic patients

	Private patients		Clinic patients	
	No. of subjects	% of group	No. of subjects	% of group
Folate intakes				
100 μg. and over	15	30	4	9
50 - 100 μg.	32	64	27	59
under 50 μg.	3	6	15	32
Vitamin B_{12} intakes				
10 μg. and over	19	38	2	4
5 - 10 μg.	16	32	10	22
under 5 μg.	15	30	34	74

by Herbert (1962b) as the minimal daily intake for non-pregnant adults. Three to five μg. of vitamin B_{12} daily was proposed by Bozian et al. (1963) as the dietary requirement for a normal population. Seventy per cent of the private group had vitamin B_{12} intakes above five μg. daily whereas 74% of the clinic group consumed less that five μg. per day.

In view of these findings, it is possibly safe to suggest that those subjects whose diets were estimated to contain less than 50 μg. of folate daily were more prone to develop a deficiency. This would be so particularly if the hypothesis of decreased absorption (Chanarin et al., 1959; Giles, 1966) or decreased utilization of folate during pregnancy (Singh and Shinton, 1965) is true. With respect to vitamin B_{12}, it is very unlikely that patients consuming less than five μg. of the vitamin daily will become deficient, even if vitamin B_{12} is poorly absorbed (Siegel et al., 1961; Sullivan and Herbert, 1965) or much higher requirements have to be met during pregnancy. This is so, since body stores of the vitamin were shown to remain normal for many years even when the diet was poor in B_{12} (Herbert, 1963b).

The average values for folate intake of both private and clinic patients were lower than reported in previous studies. Chung (1961) obtained a daily average of 193 μg. of folate in high cost diets, when microbiological assays were performed using S. faecalis as the test organism. When the folate content of the same diets was calculated from a food composition table (Toepfer et al., 1951), the values were definitely lower (120 μg.), but remained higher than folate intakes calculated in the present study. However, private and clinic patients' diets contained substantially more folate than the "poor" diets calculated by Chung (1961), which contained 30.8 μg. of the vitamin daily.

In addition, it was interesting to note that in Chung's work, mean calculated values were consistently below the analyzed values for folate in cooked diets. On the basis of these observations, it may be suggested that the discrepancy occurring between calculated and analyzed results for folate content of dietaries is due primarily to lack of data on folate content of many food items and only to a lesser extent, to absence of information on the losses of the vitamin during cooking. Otherwise, calculated values would have been consistently greater than the analyzed values.

Similarly, the average vitamin B_{12} intakes of the two pregnant groups in the present study were lower than the average content of high cost (31.6 μg.) and low cost diets (16.0 μg.) analyzed by Chung (1961), although they fell within the range of these analyzed values. However, Jolliffe and Peterman (1956) observed that a good diet may furnish as little as six μg. of vitamin B_{12} daily. On this basis, it would appear that private patients' diets could be considered adequate whereas diets of clinic patients were slightly suboptimal with respect to vitamin B_{12}.

E. CLINICAL FINDINGS

1. Group Averages for Serum Folate, Erythrocyte Folate and Serum Vitamin B_{12} Levels

The hematological results are not presented and discussed at length in this thesis. Only the data pertinent to the study of the relationship between diet and folate deficiency were used. The hematological aspects of this joint clinical and nutritional study are expected to be published later.

Serum folate, erythrocyte folate and serum vitamin B_{12} values are

summarized in Table 25. Lowenstein and co-workers (1966b) expressed the view that medians may better characterize groups than means because of the skewedness of the data and also because, with the method used, serum folate values below 2.5 mμg./ml. could not be quantitated. To obtain serum folate averages, an arbitrary value of 2.0 mμg. was given to folate levels falling below 2.5 mμg./ml., otherwise bias would have been introduced if all these had been excluded from calculations. The number of observations varied from group to group and within any given group for the various parameters studied. This is due to the fact that data for the 38th week of pregnancy were sometimes missing or, in a few cases, the growth of micro-organisms used in assays was inhibited by detergents or by antibiotics. It should also be pointed out that the clinical findings herein presented, except when otherwise mentioned, do not pertain only to patients from whom dietary information was obtained. Conversely, for some patients who supplied diet sheets, clinical data were not available. However, it is felt that the sample groups of private and clinic patients surveyed by the author were representative of the private and clinic populations, so that a parallel could be drawn between dietary and clinical findings of respective groups.

It can be seen in Table 25 that, with the exception of clinic group F who received supplemental folic acid, median folate levels were only slightly above 3.0 mμg./ml., a value considered by Cooper and Lowenstein (1961) and by Harper (1965) to be indicative of mild folate deficiency. Mean erythrocyte folate levels were slightly lower in clinic groups E and G than in the private group. All groups were comparable with respect to serum vitamin B_{12} values. It is evident from this table that clinic group F had higher serum folate and erythrocyte folate means

TABLE 25. Hematological findings in the private and clinic groups at 38th week of pregnancy

	Private patients	Clinic patients		
		Group E	Group F	Group G
Serum folate levels (mμg./ml.)				
Mean	4.4	4.1	7.0	4.2
St. dev.	2.7	2.1	4.6	3.2
Coeff. var. (%)	61.3	50.2	65.7	76.1
Median	3.6	3.4	5.7	3.6
Number of observations	39	41	44	44
Erythrocyte folate levels (mμg./ml.)				
Mean	226	216	326	213
St. dev.	119	119	113	124
Coeff. var. (%)	52.7	55.1	34.7	58.2
Median	188	168	282	189
Number of observations	38	41	44	44
Serum B_{12} levels (μμg./ml.)				
Mean	201	198	222	198
St. dev.	92	101	117	77
Coeff. var. (%)	45.8	51.0	52.7	38.9
Median	200	162	200	200
Number of observations	38	38	38	43

and medians. However, it seems the folate supplementation did not appreciably affect the levels of serum vitamin B_{12} and that pyridoxine supplements did not seem to have any affect.

The statistical analysis of differences between groups with regard to serum folate, erythrocyte folate and serum B_{12} levels was performed using the "t" test; results appear in Table 26. It can be seen that the private group did not differ significantly ($P<.05$) from group E for average values of the parameters studied. Similarly, since group E and group G showed no significant ($P<.05$) differences, values of the latter group were not significantly ($P<.05$) different from those of the private group. However, differences observed between group F and all other groups with respect to folate levels were highly significant ($P<.01$); as could be expected from the results shown in Table 25. The effect of folate supplementation on the major clinical parameters of folate nutrition is therefore evident.

2. Incidence of Folate Deficiency Signs in Private and Clinic Patients

The proportion of patients in each group exhibiting signs of folate deficiency is shown in Table 27. A mild deficiency (serum folate below 3.0 mμg./ml.) appeared in 44% of the private patients, 41% of clinic group E, 16% of group F and 34% of group G. Low erythrocyte folate levels (below 150 mμg./ml.), indicative of a more advanced folate deficit, appeared in 21% of the private group, 37% of clinic group E, 20% of group F and 34% of group G. However, low erythrocyte folate levels were not necessarily associated with low serum folate values, although in the majority of subjects, these observations were concordant

TABLE 26. Observed "t" values for differences in average serum folate, erythrocyte folate and serum B12 levels of private and clinic patients

	Private group vs Clinic group E	Private group vs Clinic group F	Private group vs Clinic group G	Group E vs Group F	Group E vs Group G	Group F vs Group G
Serum folate	0.56 ns	3.21**	0.31 ns	4.08**	0.17 ns	3.33**
Erythrocyte folate	0.37 ns	3.89**	0.49 ns	4.37**	0.11 ns	4.48**
Serum B_{12}	0.14 ns	0.88 ns	0.16 ns	0.96 ns	-	1.08 ns

TABLE 27. Incidence of folate deficiency signs in private and clinic patients

	Private patients	Clinic patients		
		Group E	Group F	Group G
	%	%	%	%
Low serum folate levels (3.0 mμg./ml.)	44	41	16	34
Low erythrocyte folate levels (150 mμg./ml.)	21	37	20	34
Low serum B_{12} levels (100 μμg./ml.)	8	8	11	0
Megaloblastic bone-marrow	13 (16)	26 (24)	23 (26)	23 (22)

(r=0.71). Low serum B_{12} levels (below 100 μμg./ml.) were much less common in all groups. It is suggested that these low serum B_{12} values were not associated with a deficiency of vitamin B_{12} since serum vitamin B_{12} levels of pregnant women were shown to be in general significantly lower than those of non-pregnant normal women (Lowenstein et al., 1960). The incidence of megaloblastic transition of the bone-marrow seemed lower in the private group than in the clinic groups. However, the number of bone-marrow smears examined in the private group was smaller than that in other groups. Folate supplementation of group F did not appear to decrease the incidence of megaloblastosis although, as was mentioned previously, it certainly had an effect on serum folate and erythrocyte folate levels. As a result, the incidence of low serum and low erythrocyte folate levels was decreased in group F; this can be seen in Table 27.

The incidence of folate deficiency signs in groups not receiving folate supplements was studied by application of the chi-square test. The contingency table and chi-square value for each parameter of folate nutrition being considered are presented in Table 28. None of the chi-square values was statistically significant ($P<.05$). In view of these findings, the incidence of folate deficiency signs was taken as being comparable in private and clinic patients not supplemented with folic acid.

F. COMPARISON BETWEEN DIETARY AND CLINICAL FINDINGS

1. Comparison Between Dietary Intakes of "Normal" and "Folate-deficient" Patients

The incidence of folate deficiency signs was found comparable in

TABLE 28. Comparison of incidence of folate deficiency in private and clinic (groups E and G) patients; contingency tables and chi-square values

	Private patients No. of subjects	Clinic patients Group E No. of subjects	Clinic patients Group G No. of subjects
Low serum folate	17 (15.4)[1]	17 (16.2)	15 (17.4)
Normal serum folate	22 (23.6)	24 (24.8)	29 (26.6)
	χ^2 = 0.95 with 2 df, ns		
Low erythrocyte folate	8 (11.7)	15 (12.7)	15 (13.6)
Normal " "	30 (26.3)	26 (28.3)	29 (30.4)
	χ^2 = 2.50 with 2 df, ns		
Low serum B_{12}	3 (1.9)	3 (1.9)	0 (2.1)
Normal serum B_{12}	35 (36.1)	35 (36.1)	43 (40.9)
	χ^2 = 2.45 with 2 df, ns		
Megoloblastic bone marrow	2 (3.6)	7 (5.4)	5 (5)
Normoblastic " "	14 (12.4)	17 (18.6)	17 (17)
	χ^2 = 1.43 with 2 df, ns		

[1]Expected values are within parentheses.

the complete private and clinic groups not receiving folate supplements. However, restricted comparison between only private and clinic patients for whom some information on dietary intake and on folate clinical status at the 38th week of pregnancy was available, regardless of the vitamin supplements received, gave somewhat different results. Such information was available for 38 private and 36 clinic patients. It was found that 40% of the private group but only 33% of the clinic group were free from any symptom of folate deficiency. However, this difference was not statistically ($P<.05$) significant. In order to determine if the diets of these "normal" subjects were different from those of the individuals considered "folate-deficient" on the basis of serum folate, erythrocyte folate and for bone-marrow morphology, dietary intakes were compared within the private group and the clinic group. "Normal" private and clinic patients were not grouped in order to compare their intakes to those of "folate-deficient" private and clinic pregnant women because of the previously found differences between the private and clinic group with respect to diet. Dietary intakes of private patients regrouped according to their folate nutritional status are shown in Table 29. The diet of these two sub-groups was not significantly different, as evidenced by the observed "t" values, not reaching significance at the 5% level of probability. However, there was a trend towards higher consumption of iron, vitamin A, thiamine, ascorbic acid, folate and vitamin B_{12} in the "normal" group of private patients. The highest discrepancy observed was in ascorbic acid intakes. Statistically speaking, significant differences could not be demonstrated, probably due to the high variation in intakes among individuals. However, these results may be indicative of a lower intake of ascorbic acid, folate and vitamin B_{12}

TABLE 29. Comparison between dietary intakes of "normal" and "folate-deficient" private patients

		"Normal" Private patients (16)	"Folate-deficient" Private patients (22)	Observed "t" values
Calories		1995 ± 415	2202 ± 556	1.31 ns
Protein	(gm.)	84 ± 17	86 ± 18	0.35 ns
Fat	(gm.)	92 ± 24	104 ± 29	1.40 ns
Carbohydrate	(gm.)	217 ± 52	241 ± 70	1.22 ns
Calcium	(mg.)	950 ± 350	1027 ± 369	0.65 ns
Iron	(mg.)	13.5 ± 3.5	12.6 ± 3.3	0.80 ns
Vitamin A	(I.U.)	9318 ± 5770	7887 ± 4079	0.87 ns
Thiamine	(mg.)	1.2 ± 0.4	1.1 ± 0.2	0.91 ns
Riboflavin	(mg.)	2.1 ± 0.7	2.1 ± 0.6	-
Niacin	(mg.)	15.2 ± 4.7	15.2 ± 4.0	-
Ascorbic acid	(mg.)	145 ± 54	118 ± 54	1.53 ns[1]
Folate	(μg.)	89.5 ± 21.4	80.2 ± 19.9	1.37 ns
Vitamin B_{12}	(μg.)	9.7 ± 5.6	7.4 ± 4.5	1.35 ns

[1]"t" test for equal variances and unequal number of observations (unmarked values were for unequal variances and unequal number of observations).

by "folate deficient" subjects.

Table 30 summarizes dietary intakes of "normal" and "folate-deficient" clinic patients. Groups E, F and G were combined. "Folate-deficient" individuals had slightly lower intakes of all nutrients except fat and carbohydrate. However, differences were much smaller than between "normal" and "folate-deficient" private patients and all "t" values were below 1.00. In addition, it could be observed that "normal" clinic patients still had mean intakes inferior to "folate-deficient" private patients. It appears from Table 31 that the diet of "folate-deficient" private patients supplied significantly ($P<.05$ or $P<.01$) more Calories, protein, fat, riboflavin and vitamin B_{12} than did diets of "normal" clinic patients. However, calcium, iron, vitamin A, thiamine, niacin, ascorbic acid and folate intakes of both groups being compared were not significantly ($P<.05$) different. "Folate-deficient" private patients were consequently more comparable to "normal" clinic patients with respect to diet than were the two whole groups. Therefore, diets of "folate-deficient" subjects were slightly inferior to those of "normal" subjects within the private and clinic groups, but the lower quality of clinic patients' diets was apparent, even when a parallel was drawn between dietary intakes of "folate-deficient" private patients and "normal" clinic subjects.

2. Correlation Between Dietary Components and Clinical Parameters of Folate Nutrition

No definite or statistically significant differences could be found between diets of "normal" and "folate-deficient" pregnant women in this study. However, since the demarcation line between "normality"

TABLE 30. Comparison between dietary intakes of "normal" and "folate-deficient" clinic patients

		"Normal" Clinic patients (12)	"Folate-deficient" Clinic patients (24)	Observed "t" values
Calories		1655 ± 523	1781 ± 588	0.65 ns
Protein	(gm.)	66 ± 15	65 ± 18	0.19 ns
Fat	(gm.)	75 ± 23	79 ± 25	0.48 ns
Carbohydrate	(gm.)	189 ± 78	213 ± 85	0.85 ns
Calcium	(mg.)	808 ± 412	708 ± 317	0.74 ns
Iron	(mg.)	10.6 ± 3.3	10.4 ± 2.9	0.18 ns
Vitamin A	(I.U.)	6013 ± 5115	4878 ± 2999	0.71 ns
Thiamine	(mg.)	1.0 ± 0.3	0.9 ± 0.3	0.91 ns[1]
Riboflavin	(mg.)	1.6 ± 0.7	1.4 ± 0.5	0.95 ns
Niacin	(mg.)	13.3 ± 3.7	12.2 ± 4.0	0.82 ns
Ascorbic acid	(mg.)	86 ± 38	73 ± 46	0.90 ns
Folate	(μg.)	73.7 ± 37.9	62.9 ± 24.2	0.90 ns
Vitamin B_{12}	(μg.)	4.6 ± 3.7	4.1 ± 2.7	0.42 ns

[1]"t" test for equal variances and unequal number of observations (unmarked values were for unequal variances and unequal number of observations).

TABLE 31. Comparison between dietary intakes of "folate-deficient" private patients and "normal" clinic patients

		"Folate-deficient" private patients (22)	"Normal" Clinic patients (12)	Observed "t" values
Calories		2202 ± 556	1655 ± 523	3.50**
Protein	(gm.)	86 ± 18	66 ± 15	4.00**
Fat	(gm.)	104 ± 29	75 ± 23	3.76**
Carbohydrate	(gm.)	241 ± 70	189 ± 78	2.39*
Calcium	(mg.)	1029 ± 369	808 ± 412	1.90 ns
Iron	(mg.)	12.6 ± 3.3	10.6 ± 3.3	2.06 ns[1]
Vitamin A	(I.U.)	7887 ± 4079	6013 ± 5115	1.38 ns
Thiamine	(mg.)	1.1 ± 0.2	1.0 ± 0.3	1.30 ns
Riboflavin	(mg.)	2.1 ± 0.6	1.6 ± 0.7	2.08*
Niacin	(mg.)	15.2 ± 4.0	13.3 ± 3.7	1.67 ns
Ascorbic acid	(mg.)	118 ± 54	86 ± 38	0.23 ns
Folate	(μg.)	80.2 ± 19.9	73.7 ± 37.9	0.74 ns
Vitamin B_{12}	(μg.)	7.4 ± 4.5	4.6 ± 3.7	2.33*

[1]"t" test for equal variances and unequal number of observations (unmarked values were for unequal variances and unequal number of observations).

and "deficiency" with respect to folate nutrition during pregnancy is rather difficult to draw, an attempt was made to correlate directly the dietary intakes of certain nutrients with the levels of folic acid in serum or erythrocytes, or of vitamin B_{12} in serum. Private patients and clinic patients belonging to group E received the same daily vitamin and mineral supplements. Clinic group F was supplemented with folate and group G received supplements containing no folic acid but higher amounts of pyridoxine than those given to other groups. For that reason, the study of any relationship between dietary components and the clinical parameters of folate nutrition involved only subjects receiving the same supplements - private and clinic patients from group E. For 32 private patients and six clinic patients (group E), full information, dietary and clinical, was available. Since bone-marrow samples could be obtained only from a small number of patients, the correlation between nutrients of diets and bone-marrow morphology (megaloblastic or normoblastic) could not be studied.

Table 32 shows the correlation (r) and regression (b) coefficients which were found between dietary components which may be related to folate (i.e., protein, iron, riboflavin, ascorbic acid, folate, vitamin B_{12}), and serum folate, erythrocyte folate and serum B_{12} levels. As can be seen in this table, there was a low but significant correlation ($P<.05$) between dietary ascorbic acid and serum folate and between the same dietary component and erythrocyte folate values. It is believed that with a greater number of observations, dietary folate and vitamin B_{12} might both have been significantly correlated with serum and erythrocyte folate levels. Furthermore, it is interesting to note that the correlation between dietary folate and serum B_{12} was higher than

TABLE 32. Linear correlation (r) and regression (b) coefficients between dietary components and blood findings in private patients and in group E of clinic patients

Dietary components		Serum folate	Erythrocyte folate	Serum B_{12}
Protein	(r)	-.008	.009	-.07
	(b)	-.0009	.05	-.319
Iron	(r)	.10	.15	.03
	(b)	.073	4.97	.826
Riboflavin	(r)	.10	.08	.16
	(b)	.357	12.7	19.5
Ascorbic acid	(r)	.33*	.35*	-.05
	(b)	.013	.658	-.067
Folate	(r)	.25	.28	.20
	(b)	.025	1.35	.72
Vitamin B_{12}	(r)	.24	.25	.09
	(b)	.122	6.035	1.71

between dietary vitamin B_{12} and serum B_{12} values, although these coefficients were not statistically significant ($P<.05$).

The inability to show a high or significant relationship between diet and any of the clinical findings of folate nutrition could be attributed to a high variability of serum and erythrocyte folate levels and to the low sensitivity of the survey technique for estimating folate intake. In addition, folate levels in serum or erythrocytes may well reflect the intake of the vitamin and/or other nutrients in the few days prior to blood tests taken during the 38th week of pregnancy. Dietary records were sometimes obtained in the last trimester of pregnancy and often before, so that values obtained from these diet sheets may not be representative of the intake shortly before the blood tests were performed. However, Moscovitch (1965) found no correlation between dietary folate ingested prior to blood tests and serum folate levels.

The present findings lead to the belief that there is a possible relationship between certain dietary components, such as ascorbic acid, folic acid and vitamin B_{12}, and the folate clinical status during pregnancy. However, only further investigation will permit a clarification of the importance of this relationship.

VI. SUMMARY

The relationship of diet to the incidence of folate deficiency in a private and clinic population of pregnant women was studied. The investigation involved first the appraisal of dietary intake, secondly the assessment of dietary adequacy and lastly a comparison between dietary and clinical findings in the private and in the clinic group.

A seven-day dietary record was obtained from 50 pregnant women under the care of private obstetricians and from 46 pregnant women attending the antenatal clinic of the Royal Victoria Hospital, Montreal. The diets of private patients supplied significantly ($P<.01$) more Calories, protein, calcium, iron, vitamin A, thiamine, riboflavin, niacin, ascorbic acid, folate and vitamin B_{12} than did diets of clinic patients. The observed discrepancy in the Calorie and nutrient content of diets came from notable differences in the food habits of the private and clinic patients. Dietary intakes by the clinic patients were, in general, lower than reported by Moscovitch (1965) for a comparable clinic group. A comparison of dietary intakes at various stages of pregnancy revealed that in neither group was there a significant ($P<.05$) increase in Calorie and nutrient intake in late pregnancy. An exception was the intake of calcium in the clinic group.

Dietary allowances for pregnancy were used as guidelines in the assessment of dietary adequacy. According to the Canadian Dietary Standard (1964), the diets of pregnant women were adequate in all trimesters of pregnancy, although average iron intakes in the third trimester were slightly below the Canadian recommended allowance. The

diets of clinic patients were well below the Canadian Standard for iron in the last trimester and were borderline in the second trimester with respect to this nutrient. It is believed that caloric intakes were in general sufficient in view of the amount of activity of most subjects, although they did not meet the values recommended in the Canadian Dietary Standard.

Average daily folate and vitamin B_{12} intakes were respectively 86.5 μg. and 9.8 μg. in the private group, and 66.0 μg. and 4.3 μg. in the clinic group. These values were lower than reported in previous studies (Chung, 1961; Moscovitch, 1965). Six per cent of the private patients and 32% of the clinic patients had folate intakes below 50 μg. daily, the minimal requirement for non-pregnant subjects (Herbert, 1962). Thirty per cent of the private group and 74% of the clinic group consumed less than 5 μg. of vitamin B_{12} per day, a level considered adequate for a population (Bozian et al., 1963).

However, serum folate, erythrocyte folate and serum B_{12} levels were not significantly ($P<.05$) different between the private patients and the clinic patients not receiving folate supplements. In addition, the incidence of any clinical sign of folate deficiency was not significantly ($P<.05$) higher in the clinic group. Nevertheless, of the subjects for whom dietary and clinical data were available, 40% of the private group compared to 33% of the clinic group were free from any symptom of folate deficiency. Diets of "folate-deficient" patients were slightly inferior to those of "normal" patients within each group, but differences were not statistically significant ($P<.05$). In addition, "folate-deficient" private patients had diets which supplied significantly ($P<.05$ or $P<.01$) more Calories, protein, fat, carbohydrate, riboflavin and

vitamin B_{12} than diets of "normal" clinic patients.

Dietary components were not significantly ($P<.05$) correlated with any parameter of folate nutrition, with the exception of ascorbic acid which showed a low but significant ($P<.05$) correlation with both serum folate and erythrocyte folate levels.

VII. CONCLUSIONS

The following conclusions may be drawn on the basis of the findings in the present study:

1. Diets of private patients were of a better quality than those of clinic patients. The observed discrepancy in dietary intakes was attributed to a difference in socio-economic status of a private and a clinic population group.

2. Diet may not be the major determining factor in the development of folate deficiency during pregnancy. This conclusion was reached since intakes of "normal" and of "folate-deficient" patients in this study were not statistically different ($P < .05$), and since the incidence of clinical signs of folate deficiency was comparable in the private and clinic groups.

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APPENDIX

APPENDIX TABLE i. Composition of vitamin-mineral supplements given to clinic patients

	Vitamin-mineral supplements given to		
	Group E	Group F	Group G
Ferrous fumarate (elemental iron 75 mg.)	225 mg.	225 mg.	225 mg.
Vitamin A	5000 I.U.	5000 I.U.	5000 I.U.
Vitamin D	800 I.U.	800 I.U.	800 I.U.
Thiamine mononitrate	2.0 mg.	2.0 mg.	2.0 mg.
Riboflavin	2.0 mg.	2.0 mg.	2.0 mg.
Pyridoxine	3.0 mg.	3.0 mg.	10.0 mg.
Niacinamide	10.0 mg.	10.0 mg.	10.0 mg.
Ascorbic acid	75 mg.	75 mg.	75 mg.
Folic acid	-	0.2 mg.	-

APPENDIX FORM i

INSTRUCTIONS FOR YOUR FOOD RECORD FORMS

1. Record all the food you eat for seven days, starting on Wednesday.

2. Before you eat anything, be sure to fill in the column A: Description of food and the column B: Amount served. Use measuring cups and spoons.

3. For meat, fish and cheese, use a scale.

4. Do not forget also to record the fat used in cooking, one pat of butter = 1 level teaspoon.

5. Record in level, not rounded measure.

6. In the column A: Description of food, indicate, for example:
   - for juices: fresh (how many fruit used), canned or frozen
   - for bread: kind, whole wheat, white enriched, rye.

7. Always measure the food when cooked, right before eating.

8. After eating, measure the amounts of food left on the plate and record in the Column C: Amount left. For meat or fish, weigh the amount left, including bones.

9. You can calculate column D: Amount eaten by subtracting Column C from Column A.

10. For mixed dishes, give the recipe at the back of the day-sheet.

11. For meals eaten out, record estimate portions eaten.

12. Your cooperation in this study is most appreciated. Please try to be as accurate as possible. Thank you.

APPENDIX FORM ii.

ONE-DAY DIETARY RECORD

Name_____ Day of the Week_____ Date_____

(A) Description of Food	Amount (B) Served	Amount (C) Left	Amount (D) Eaten	(E) Cooking Method	(F) Brand

APPENDIX FORM iii.

"SAMPLE SHEET"

Name _____ Day of Week _____ Date_____

(A) Description of Food	Amount (B) Served	(C) Left	(D) Eaten	(E) Cooking Method	(F) Brand
Orange Juice, Frozen without sugar	½ cup	-	½ cup		Birdseye
White Toast	2 slices	-	2 slices		
Butter	2 tsp.	-	2 tsp.		
Orange Marmalade	1 tbsp.	-	1 tbsp.		Sherriff
Coffee					
Cream, light	1 oz.	-	1 oz.		
Sugar	1 tsp.	-	1 tsp.		
Sandwiches: white bread	2 sli.	-	2 sli.		
Tomato	1 small	-	1 small		
Mayonnaise	1 tsp.	-	1 tsp.		
Lettuce	1 leaf	-	1 leaf		
Chocolate	1 bar	-	1 bar		Aero
Ginger Ale (8 oz.)	1 bottle	-	1 bottle		Canada Dry
Beef Consomme	1 cup	-	1 cup		Campbell
Pork Chops, 2	4 oz.	1 oz.	3 oz.	oil Fried in 2 tbsp.	
Green Beans	½ cup	-	½ cup		Aylmer
Butter	1 tsp.	-	1 tsp.		
Mashed Potatoes + Milk & Butter	½ cup	-	½ cup		
Apple pie	1/6	-	1/6		Pillsbury
Milk, skim	1 cup	-	1 cup		

APPENDIX FORM iv.

GENERAL INFORMATION SHEET

Name:_____ Date _____

Address:_____ Nationality_____

Phone Number:_____ Language spoken most frequently_____

Occupation: Homemaker _____

Other _____ Religion_____

E.D.C._____

Week of pregnancy when first seen:_____

No children _____

Family: Boys:_____ Ages_____

Girls:_____ Ages_____

	No	Yes	No.
Previous pregnancies	____	____	____
Stillbirths	____	____	____
Deaths	____	____	____

	Frequency of /week	
Trim.	Nausea	Vomiting
1st		
2nd		
3rd		

Food Likes	Food Dislikes
Meats:	Meats:
Fish:	Fish:
Fruit:	Fruit:
Vegetable:	Vegetable:

Age:_____ Height_____

Frame: Small__Medium__Large__

Weight (before preg.)__ideal__

Cooking: herself____other____

No. of meals eaten out/week__

Date	Comments

APPENDIX FORM v.

GENERAL INFORMATION SHEET (Non-pregnant Women)

Name _____ Age_____ Food Likes Food Dislikes

Address _____ Phone No._____ Meats Meats

Occupation_____ Religion_____ Fish Fish

Language spoken most frequently_____ Fruit Fruit

Single _____ Married_____ Other_____ Vegetable Vegetable

Height_____ Weight_____ Ideal Wt._____ Other Other

Living conditions for the last year: Date Seen_____

1. Alone _____ Comments:
2. Family_____ No. of people_____
3. residence _____

Meals Eaten Most Frequently

1. At home _____

   Cooking: herself_____ Other_____
2. Restaurant _____
3. Institution _____

APPENDIX FIGURE i. CARD DESIGN FOR FOOD LIST

Description	Card Columns
Food identification number	1 - 6
Weight, gm. (1 decimal)	7 - 10
Calories (whole No.)	11 - 15
Protein, gm. (whole No.)	16 - 20
Fat, gm. (whole No.)	21 - 25
Carbohydrate, gm. (whole No.)	26 - 30
Calcium, mg. (whole No.)	31 - 35
Iron, mg. (1 decimal)	36 - 40
Vitamin A, I.U. (in tens)	41 - 45
Thiamine, mg. (2 decimals)	46 - 50
Riboflavin, mg. (2 decimals)	51 - 55
Niacin, mg. (1 decimal)	56 - 60
Ascorbic acid, mg. (whole No.)	61 - 65
Folate, g. (3 decimals)	66 - 72
Vitamin B_{12}, g. (3 decimals)	73 - 80

I.B.M. PROGRAM

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C DELISLE HELENE              PC12
      CALORIE AND NUTRIENT INTAKES OF PRIVATE AND CLINIC PATIENTS
      DIMENSION R(15) ,F(350) ,G(350) ,Y(16),PQ(14) ,QQ(14)
      DEFINE DISK(15,1600)
      L=1
    5 READ 1 ,R
      IF (R(1))2,3,2
    2 RECORD (L) R
      GO TO 5
    3 READ 10,X
      IF(X)500,501,500
  501 CALL EXIT
  500 LL=1
   20 READ 11,Y
      IF (Y(1)) 14,15,14
   14 LM=LL+7
      DO 18 JJ=LL,LM
      JX=2*(JJ-LL+1)
      F(JJ)=Y(JX-1)
   18 G(JJ)=Y(JX)
      LL=LL+8
      GO TO 20
   15 DO 80 N=1,14
   80 PQ(N)=0.0
      I=1
  105 II=F(1)/1000.0
      FETCH(II)R
      DO 81 N=1,14
   81 PQ(N)=PQ(N)+G(I)*R(N+1)
      IF (F(I+1)) 100,100,101
  101 IF (I-LM) 102,100,102
  102 I=I+1
      GO TO 105
  100 DO 108 N=1,14
      QQ(N)=PQ(N)/7.0+0.0005
  108 PQ(N)=PQ(N)+0.0005
      PRINT 110,X,PQ,QQ
      GO TO 3
    1 FORMAT(F6.0,F4.0,11F5.0,F7.0,F8.0)
   10 FORMAT(F 10.0)
   11 FORMAT(8(F6.0,F4.2))
  110 FORMAT(8HOPATIENT,F11.0/8H TOTAL  ,7F16.3/ 8X,7F16.3//8H MEAN   ,
     1                                   7F16.3/ 8X,7F16.3///)
      END
```