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STUDIES IN METABOLISM DURING PREGNANCY

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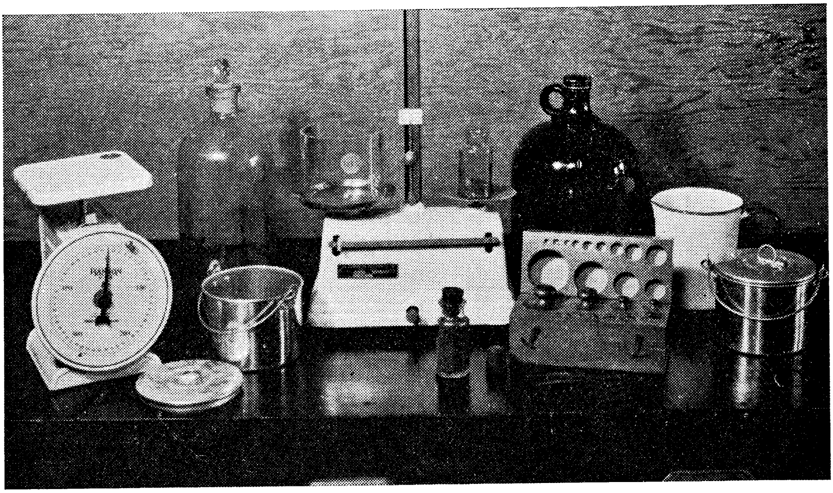


Fig. I.—Equipment used in the homes of the subjects during a balance experiment.

STUDIES IN METABOLISM DURING PREGNANCY

INTRODUCTION

The chief aim in beginning the present series of studies in metabolism during pregnancy was to learn something of the tendencies shown by women of the southern United States in the retention of certain minerals, particularly calcium and phosphorus. The results of the first few balances suggested a need for information concerning the fate in metabolism of other inorganic constituents of the diet, and for the most complete possible definition of the character of the diets chosen by these women. Accordingly, the scope of the project was extended to include a determination of the intake and outgo of nitrogen and of the imporatnt minerals, as well as further analyses of the diets with respect to content of total calories, protein, fat, carbohydrate, water and vitamins.

The entire investigation contibutes to the small fund of information intended to describe in a quantitative way, based on fundamental physiologic facts, the nutritional needs of human pregnancy. This long-neglected field has deservedly received more attention during the last five years, but even yet only a beginning has been accomplished toward answering some important questions vital to the health and preservation of mothers and to the welfare of infants and children.

SURVEY OF LITERATURE

Estimates of the nutritional and dietary needs of human pregnancy have been attempted through four main avenues of approach. First, the quantities of inorganic and organic constituents deposited during prenatal life have been determined by chemical analyses of human fetuses at different stages of development. The data obtained with this means of investigation will be discussed and summarized below.

A second important method for measuring the total nutritional needs of maternity has been through the use of balance experiments. Chemical analyses of intake and outgo show the balance of various elements retained for use during each phase of pregnancy. Whenever results from this method are obtained under fairly ideal conditions with normal subjects, they should express the total dietary needs of maternity, including those of the mother herself, together with the added tasks of reproduction, fetal development and whatever preparation for lactation the organism is capable of providing under such conditions of nutritional status and dietary regimen. The balance experiment has been the tool of investigation employed in the studies described in this bulletin. Along with a discussion of these findings will be found a review of the literature concerned with similar studies during pregnancy.

A study of the dietaries chosen by normal healthy women who are successfully performing the tasks of reproduction represents another method for estimating the probable food needs of pregnancy. This method is conveniently applied to groups, such as in institutions, families and children, but has been less used for pregnancy. Records for large numbers of individuals are necessary in order to warrant the application of the statistical procedures commonly used in making interpretations for dietary studies. Records of dietaries obtained in conjunction with metabolism studies may be more accurately evaluated even though few in numbers. Therefore the facts obtained in the effort to characterize the diets chosen by the women of this study add materially to the accumulation of recorded dietaries and deserve to be discussed from this point of view also.

A fourth means of studying metabolism during pregnancy has involved blood chemistry determinations under various physiological conditions. Little reference to this method will be made in this report. The literature on blood studies alone is a vast field in itself and since less related in procedure and significance to balance experiment, any attempt to cover the topic at this time could be only fragmentary.

CHEMICAL ANALYSES OF THE HUMAN FETUS

Chemical analyses of the human fetus were carried out as early as 1858 (236). In 1877 Fehling (65) reported the water, ash, fat and protein content of 21 fetuses. Some ten years latter Brubacher (27) published figures for the ash, calcium, magnesium, phosphorus, iron and silicon content of two others and Bunge the mineral content of one (29). In 1894, Giacosa (75) reported the potassium, sodium, calcium, magnesium, iron, phosphorus and chlorine content of one fetus. Around 1900 a number of reports came from different laboratories. Michel (152) studied the ash, nitrogen, calcium, magnesium, phosphorus and chlorine content of 6 fetuses and the nitrogen of 1 other. Hugounenq (102, 103) made complete mineral analyses of 7 fetuses of varying ages, Gaube (74) the mineral analyses of two others. De Lange (52) studied the potassium, sodium, calcium, magnesium, iron, phosphorus and chlorine content of one premature, while Camerer (32) and Sœldner (208) made complete mineral determinations on 6 mature fetuses.

Nothing more appeared in print until 1917 when Langstein and Edelstein (119) published data for the nitrogen and inorganic constituents, excepting sulfur and iron, of two premature infants. In 1921 Givens and Macy (76) analyzed 25 fetuses for total ash, calcium and magnesium, and in 1923 Schmitz (187) reported the ash and calcium content of 20 others. A recent report by Iob and Swanson (104) represents probably the most extensive complete mineral analyses yet published. They present data showing the inorganic constituents of 17 fetuses of varying ages.

The data of all workers are summarized in Table 1, except those for 1 fetus each by Bezold and Giacosa, cited by Hugounenq, and those for 1 fetus by Bunge (29) and 2 by Gaube (74). In the total data of Table 1 are included the analyses for 111 of the 116 fetuses for which figures were available. Of these 116 calcium was determined in 90, magnesium in 65, iron and sulfur in as few as 22 and 15 respectively. Since the preparation of this manuscript an excellent summary table of the analyses of 96 fetuses has been published by Macy and Hunscher (143). It is concerned with total ash, nitrogen, calcium, magnesium, phosphorus and iron. The averages for these elements differ slightly from those presented here, due doubtless to the different amount of analytical data included.

Since few investigators have attempted complete mineral analyses, the information for certain elements, particularly calcium and magnesium is more abundant and the averages more accurate than for others. For this reason the number of analyses on which a figure is based is stated in Table 1 alongside the column for average content at the end of each month of prenatal life. Further, the table shows the average daily deposits which are needed to cover fetal need for the various elements during a given month.

From the figures for average content at the end of a given month it is easily seen that the heaviest fetal demand is during the last three months of prenatal life. During this period approximately ⅔ of the total calcium and phosphorus is laid down, and at the same time almost ¾ of the

Table 1.—The Growth of the Human Fetus. Summary of Published Analyses.

	WEIGHT			ASH			NITROGEN			SODIUM			POTASSIUM			CALCIUM		
AGE	No. of analyses	Ave. total wt. at end of month	Daily gain*	No. of analyses	Ave. total content	Daily deposit	No. of analyses	Ave. total content	Daily deposit	No. of analyses	Ave. total content	Daily deposit	No. of analyses	Ave. total content	Daily deposit	No. of analyses	Ave. total content	Daily deposit
Lunar mo.		gm.	gm.		gm.	gm.		gm.	gm.		gm.	mgm.		gm.	mgm.		gm.	mgm.
2	9	17	0.3	9	0.2	0.004	0	....	....	0	....	....	0	....	....	8	0.06	2.1
3	13	32	1.1	9	1.1	0.040	3	0.1	0.001	2	0.02	0.3	2	0.008	0.1	10	0.11	2.0
4	20	118	3.5	17	1.6	0.020	5	0.7	0.01	3	0.55	20.	3	0.44	15.	18	0.53	15.
5	20	304	6.6	20	5.2	0.13	11	3.2	0.09	4	1.15	22.	4	0.59	6.	12	1.97	51.
6	13	600	10.6	13	14.6	0.34	7	8.1	0.17	4	1.52	13.	4	1.08	18.	9	3.51	55.
7	13	1026	15.2	13	26.2	0.41	8	16.3	0.29	4	2.51	35.	4	1.92	30.	9	7.24	137.
8	8	1255	8.2	8	32.8	0.24	6	21.7	0.19	5	2.80	11.	5	2.33	15.	7	8.79	55.
9	3	1710	16.3	3	49.4	0.60	2	39.0	0.62	0	....	....	0	....	....	1	15.14	225.
10	12	2931	43.5	11	85.0	1.27	9	58.6	0.70	10	4.97	38.	10	4.67	43.	11	23.72	306.
Maximum at term		3335			112.5			72.7			5.94			5.63			33.37	
Total No. fetuses all ages	111			103			51			32			32			85		

*Daily gain each month for preceding interim.

Table 1.—The Growth of the Human Fetus. Summary of Published Analyses. (Continued)

AGE	WEIGHT			MAGNESIUM			PHOSPHORUS			CHLORINE			SULFUR			IRON			
	No. of analyses	Ave. total wt. at end of month	Daily gain*	No. of analyses	Ave. total content	Daily deposit	No. of analyses	Ave. total content	Daily deposit	No. of analyses	Ave. total content	Daily deposit	No. of analyses	Ave. total content	Daily deposit	No. of analyses	Ave. total content	Daily deposit	Total No. analyses according to age
Lunar mo.		gm.	gm.		gm.	mgm.		gm.	mgm.		gm.	mgm.		gm.	mgm.		gm.	mgm.	
2	9	17	0.3		gm.	mgm.		gm.	mgm.		gm.	mgm.		gm.	mgm.		gm.	mgm.	
3	13	32	1.1	7	0.015	0.5	0	....	....	00	....	....	0	....	....	0	....	....	33
4	20	118	3.5	3	0.058	1.5	2	0.022	0.5	1	0.02	0.2	0	....	....	0	....	....	45
5	20	304	6.6	10	0.048	....	4	0.68	23.	3	0.56	19.	1	0.081	1.	1	0.040	0.5	85
6	13	600	10.6	8	0.100	1.8	6	1.63	34.	5	1.14	21.	2	0.104	1.	2	0.043	0.1	94
7	13	1026	15.2	6	0.173	2.6	5	2.76	40.	4	1.62	17.	1	0.219	4.	2	0.077	1.2	68
8	8	1255	8.2	8	0.306	4.7	6	4.40	58.	5	2.77	41.	1	0.190	....	2	0.082	0.2	73
9	3	1710	16.3	7	0.512	7.4	5	5.55	41.	5	3.16	14.	0	....	....	0	....	....	56
10	12	2931	43.5	1	0.452	....	1	9.42	138.	0	....	....	0	....	....	1	0.131	0.9	12
Maximum				10	0.703	9.0	11	14.00	168.	11	5.05	34.	8	0.589	5.	9	0.395	9.4	112
at term		3335																	
Total No.					0.886			18.68			6.45			0.604			0.937		
fetuses,																			
all ages	111			60			40			34			13			17			578

*Daily gain each month for preceding interim.

nitrogen, sulfur and iron is deposited. On the other hand, the total magnesium content changes very little during these last three months, while the increments of sodium and potassium also proceed at less rapid rates.

In Figures II and III the rates of acquisition of minerals and nitrogen are illustrated in comparison to that of total fetal growth as expressed by gain in weight. The curves emphasize the fact that the period of heaviest metabolic demand for the mother is during the last trimester of pregnancy. Attention is called to the relative spurt in growth at the seventh month of prenatal life, and to the subsequent slowing of growth shown in the eighth month, for every element except magnesium for which the acceleration comes one month later. The number of analyses available for the seventh and eighth months are similar in totals and for each element. They also compare favorably in numbers to those for the fourth, fifth and sixth months. It is particularly unfortunate in this connection that so few data are available for the ninth month. These charts show also the tendency for certain constituents to be deposited in comparable amounts. Thus sodium, potassium and chlorine fall into one class, magnesium, sulfur and iron into another, while calcium and phosphorus are similar in quantity.

The range of the variations in the composition of different fetuses at term is of added interest here. The averages in Table 1 are not wholly in accord with a series computed by Lusk (135) and cited frequently (149, 173, 192, 231). His figures for total ash, nitrogen and phosphorus content at maturity are appreciably higher than those presented in the above table, due to the fact that his computations were based on original data for one fetus analyzed by Michel (152). One might be justified in adopting the highest figure reported for any given age if it could be assumed that such represented more nearly optimal content and that reported variations below the maximum represented degrees of deficient development. Some of the low figures could doubtless be attributed to inadequate maternal nutrition. At any rate, the maximum content found for each element at maturity is shown in Table 1 for comparison with the averages presented for this age. Possible causes for variations are discussed below.

The weight of the 10 full-term fetuses used in these analyses varied only from 2476 to 3335 grams, a difference of less than 40 percent, reckoned from the lowest figure, while the ash content showed a range of 54 to 112 grams, or a difference of over 100 percent. Increments in total ash content did not parallel gains in weight. There was little range in the sodium, potassium and chlorine content of the newborn, the maximum difference being around 20 percent. On the other hand, as pointed out by Givens and Macy (75), the calcium content at term may vary from 13.08 to 33.27 gram, a difference of more than 150 percent. Iron content at maturity varied from 0.266 to 0.937 gram, a difference of 250 percent. Likewise the phosphorus varied from 8.96 to 18.68, the magnesium from 0.277 to 0.886, and the total nitrogen from 46.8 to 72.7. Hunscher and coworkers (106, 143) have estimated that the fetal accumulation of nitrogen represents less than 50 percent of the total consumed by fetus, fetal structures, hypertrophy of genital organs and development of breasts. Doubtless similar relations of varying degrees hold for the other elements retained during pregnancy. These same workers have recently estimated the extra-fetal needs for calcium and phosphorus as one-half gram each (143).

Wide variations in fetal composition are reported by the same investigator, thus ruling out the possible explanation that the discrepancies were due always to differences in the technique employed by individual workers. The inevitable conclusion seems to be that the fetus may arrive at term endowed with different reserves of these minerals or with varying degrees of development attained during prenatal life (83, 139, 210). Doubtless

FIGURE II

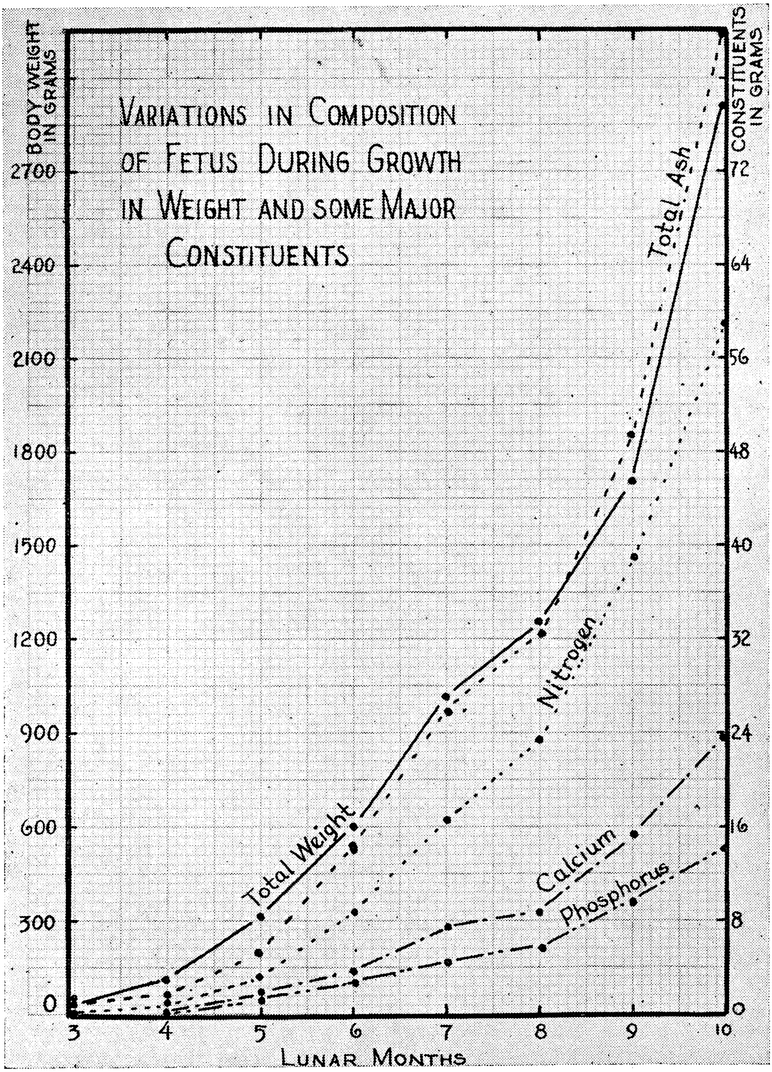


Fig. II.—Rate of fetal growth and variations in content of some major constituents. Composite curves based on published fetal analyses.

FIGURE III

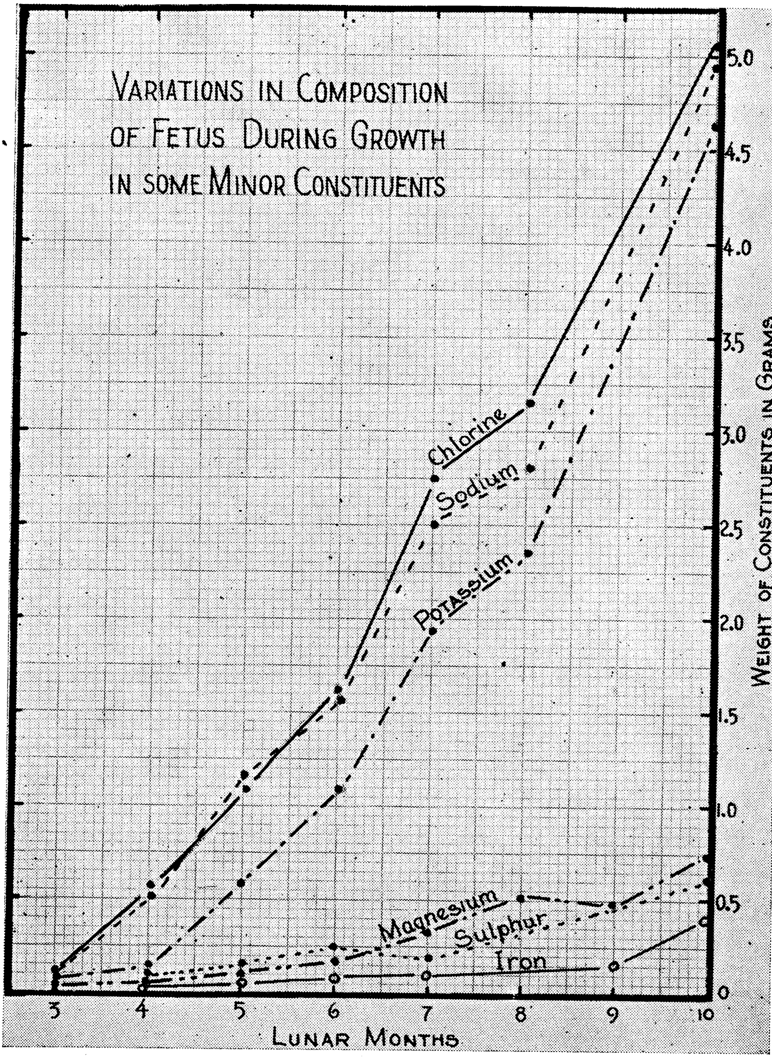


Fig. III.—Fetal growth with respect to accretions of certain inorganic constituents. Composite curves based on published fetal analyses.

such deficiencies explain the greater susceptibility of some infants to rickets, anemia and other manifestations of abnormal metabolism during the first year of post natal life. That these deficiencies may result from the poor nutrition of the mother is shown by the analyses of Booher and Hansmann (26), who were able to correlate variations in the mineral content of the femur of babies with the quality of the diet chosen by the mothers during pregnancy.

Thus the data in Table 1, while revealing the elements which may be present in widely different amounts, point out those for which a possible shortage for embryonic growth may occur and hence those which deserve special attention in the diet and nutrition of mothers during gestation. It is suggestive, for example, that consistently lower figures for calcium are reported by Schmitz (187) than by other workers. His study was carried out in Europe soon after the World War and may have included chiefly the offspring of a generation of undernourished mothers. This possibility of differences in prenatal endowment may explain also the higher figures reported by the workers around 1900 as compared to those reported two and three decades later by Givens and Macy and by Iob and Swanson. The former represented an era of more liberal dietary habits.

The irregular figures for average composition found in the seventh and eighth months, as seen in Table 1, may be due in part to the small or large contributions from the data of early workers to these averages. However, Hugounenq's figures for the composition of fetuses younger than seven months of age were much higher than even those of his contemporaries, while his data for composition of the newborn agree well with those reported by Camerer, Michel and others. This variance in the younger groups may be attributed to errors in reckoning age (75).

The computations in Table 1 estimating the necessary daily deposit of the various elements from month to month are pertinent to the findings in balance experiments discussed beyond. The intake-outgo balance of the mother must be equal to or greater than this daily deposit if her diet is supplying fetal needs. It must exceed this deposit if the maternal diet is adequate for any other than fetal needs alone.

Thus it may be seen that the fetal gain during the last three months of gestation may amount to less than 1 gram daily for nitrogen, but should be 0.2 to 0.3 gram daily for calcium and 0.1 and 0.2 gram daily for phosphorus. Acquisitions of sodium, potassium and chlorine are of similar magnitude during times of maximum retention, 20 to 40 mgm. daily. On the other hand only 4 to 6 mgm. daily of magnesium, sulfur and iron are deposited daily. However, the number of analyses reported for sulfur and iron are too few to lend much weight to the average figures shown for these elements. Furthermore, evidence exists to show that storage of iron in the livers of newborn is continued the first few days after birth, coming from destroyed blood available for bilirubin formation (3, 77).

The rates of deposition of sodium and chlorine tend to reach a maximum by the middle of pregnancy, those for potassium, phosphorus and magnesium proceed rather uniformly throughout gestation, while those for nitrogen, iron and calcium exhibit more rapid tendencies in the last months.

BALANCE EXPERIMENTS DURING PREGNANCY

The earliest balance experiments during pregnancy were concerned with nitrogen only and that during the last 7 to 10 days prior to parturition. These began with Zacharjewsky (250) in 1893 and since then some five other investigators have limited their observations to nitrogen only (82, 188, 202, 203, 247). Bar (10, 11) studied phosphorus in addition to nitrogen in 3 women.

Most extensive of the early metabolism studies was that of Hoffstrœm in Finland (97), who followed the retentions of nitrogen, calcium, magnesium, phosphorus and sulfur during the last 23 weeks of one pregnancy. From his data he was able to compute the "rest material" which his case stored in excess of the needs peculiar to the progress of gestation.

Landsberg, in Germany (117) carried out nitrogen, phosphorus and sulfur balances on 6 women and later (116) nitrogen, phosphorus, calcium and magnesium on 14 others. The intake for his group was the most liberal of any reported for ordinary self-chosen diets. Likewise, the storage of all elements was well in excess of fetal requirements. Thus his data on retention of minerals are in contrast to those reported for women in recent studies.

Macy, Hunscher and coworkers of Detroit (104, 106, 144, 145) have studied intensively the calcium and phosphorus as well as nitrogen utilization of three women during pregnancy and of the same three women during lactation and the non-reproductive periods. These women were capable of heavy milk production and chose diets liberal in quantity and in keeping with their metabolic burden. In general, however, their retentions were poor and unsatisfactory. The same investigators have also reported calcium, phosphorus and nitrogen retention for one woman on a specified diet (142). By such a controlled dietary regimen excellent storage of all elements, particularly calcium, was recorded.

Coons and Blunt have reported nitrogen, calcium, phosphorus, magnesium and iron balances on nine Chicago women (40, 41). In general most of their subjects exhibited poor retentions of calcium and magnesium and, to a less extent, of phosphorus. Although the diets were not unusual in quality, the retentions for nitrogen and iron were fair in comparison to fetal needs alone. Toverud and Toverud of Norway (231) studied calcium, phosphorus and magnesium retention in 29 women receiving institution diets. They found poor retention of all elements, particularly of calcium. Twenty-three women received supplements or regulated diets in some form, many of them following balances on self-chosen diets. The supplements varied for different individuals, but included "Calbifos," "Vigantol," cod liver oil, egg yolk, and extra milk. Best improvements in retentions were found with increases in mineral intake, such as by the addition of a liter of milk daily.

Thus records for 1 to 5 balances per subject covering 1 to 5 elements for a total of 90 women have been published. As yet, however, the results are too much at variance to justify final conclusions concerning the needed dietary quantities of minerals, and the amounts of supplements or accessory factors desirable for promoting maximum utilization of these elements. It is noteworthy that, in contrast to early investigators, all of the recent studies have shown a tendency to low balances (41, 144, 231), particularly of calcium, phosphorus and magnesium. The variations in retention suggest the need for studies of the entire metabolism of pregnancy in each subject. Knowledge of the total metabolism in each and every case would facilitate greatly the interpretation of erratic or unusual balances for certain elements.

To this end the present study adds 25 balances on six women living under conditions somewhat different from those already reported. Nitrogen, sodium, potassium, magnesium, calcium, phosphorus, iron, chlorine and sulfur retentions have been studied. These data comprise the first to be published for sodium, potassium, chlorine and acid-base computations, and the second for iron retentions during pregnancy (40, 42).

Further discussion of the details in the literature will be reserved for consideration in connection with the corresponding elements covered in this project.

SUMMARY OF FACTS REVEALED BY LITERATURE

1. The literature affords data on the analyses of 50 to 100 fetuses for total ash, nitrogen, calcium or magnesium content. Most of these include the ages of 4th to 10th months of prenatal life and show the markedly increased needs of the fetus for growth material during the 7th to 10th months.

2. Data for fetal content of sodium, potassium, phosphorus, chlorine, sulfur and iron are less numerous and complete for the various ages, but are nevertheless suggestive of the trend of fetal needs.

3. Balance experiments during pregnancy have been limited chiefly to nitrogen, calcium, phosphorus and magnesium with observations for these respective elements in 50, 63, 66 and 53 subjects each. Prior to the present study, sulfur and iron retention had been measured in 7 and 9 women respectively, and sodium, potassium, chlorine and acid-base had been measured in none.

4. The need for complete mineral metabolism studies during pregnancy is evident from the gaps in our information on certain phases of this subject. The existing number of unanswered questions relating to nutrition procedures which will protect the mother and at the same time produce offspring with optimum physical development and growth potentialities stimulate further interest in certain phases of metabolism during pregnancy.

EXPERIMENTAL PROCEDURES

The procedure followed in making biochemical metabolism studies varies with the aims of the investigation, with the type of subjects, and with the conditions under which they are living. Methods have been reported for use with infants (48, 98, 237), with children (31, 35, 194), and with hospitalized patients (12, 16, 168). Procedures with individuals living in their own homes and receiving the usual varied diet have been employed most frequently with women during pregnancy (38, 54, 231). This last method, with such variations as used by the writer for the studies described in this report, will be presented here somewhat in detail.

METHODS FOR COLLECTIONS

Each metabolism period consisted of four days during which all intake was measured and sampled (as described below), and urine and feces collected and preserved for analysis. Sundays and holidays and usually Saturdays and Mondays were avoided as affording non-representative diets, and perhaps some unusual lag in eliminations due to irregular meals or other habits of hygiene.

The investigator went to the home at every meal time, weighed the foods to be eaten after they were cooked and immediately before serving, and prepared composite food samples, representing one-tenth of the daily intake, to be used for analyses. Collections of excreta on hand were removed to the laboratory with each home visit. Obviously during the balance experiments the women had no meals outside the home, and remained in and about the house except for such brief absences as would not interfere with the collections of excreta. Otherwise, the routine of daily habits, household activities and family cares were unchanged and fairly constant from period to period.

Equipment Used in the Home. A list of the equipment used in the home will aid the reader in understanding the methods employed. Figure I illustrates some of these articles.

For food collections:
- 1 tested Hanson scale, 500 gm. capacity, 2 gm. graduation, rotating dial.
- 1 torsion balance, sensitive to .1 gram.
- 1 set of brass weights in covered box.
- 1 pair forceps for weights.
- 2 one-quart tightly covered aluminum pails.
- 4 one-liter pyrex beakers, cut down to be enclosed within pails.

For convenience in handling food:
- 1 lb. small shot in two bottles.
- 2 or 3 droppers.
- 1 silver spoon and knife.
- 1 set of salt and pepper shakers.
- 1 bottle of distilled water.
- Filter paper cut in sections, about 2x3 inches.
- Wax paper cut in sections, about 4x5 inches.
- Blank mimeograph forms for weighed diet records.

For drinking water:

- 4 2- or 3-liter glass-stoppered bottles.
- 1 glass measuring cup, preferably conical.
- 1 three-inch glass funnel.

For urine:

- 1 cup, one-liter capacity, acid-proof baked enamel with handle and outpour.
- 1 4-inch funnel with wide delivery and short stem.
- 2 glass-stoppered or screw-top jugs with handle.

For feces:

- 4 1-quart covered aluminum pails, as above.
- 12 1-liter pyrex beakers cut down, as above.
- 1 medium-weight board to fit inside commode.
- 1 fireless cooker, double compartment.
- 2 flat ice bags.
- 1 gallon pail covered, for transporting ice.

Washed carmine and gelatine capsules, size 0.

Except for some of the containers all of this equipment was placed in the home at the beginning of the metabolism period and remained there until the end or the 5th day.

Collection of Samples. The scales for measuring food and taking samples were tested periodically, and weights up to and including 200 gram pieces were calibrated. With some planning, all equipment could be kept in very compact arrangement, thereby avoiding confusion in home or opportunity for meddling by minors. The items listed for convenience with food contributed to the accuracy, speed and cleanliness with which various articles of diet could be handled. A minimum of time was lost between weighing the foods to be eaten and removing samples for analyses in order to reduce errors from moisture changes, variations in mixing, and such like. The subjects themselves were responsible for collection of water samples.

The pails and cut-off beakers used for collecting food and feces facilitated removal to laboratory without loss from accidental breakage in transit and permitted prompt drying without possibilities of errors in transfer. The beakers provided flat bottoms to hasten evaporation and lessen tendency to pack. Deep pyrex crystallizing dishes to fit inside the pails could be used instead of the cut-off beakers.

The medium-weight board was cut in size and shape so as to rest inside the commode just above the water level but enough below the level of the seat to accommodate the aluminum pail containing the pyrex glass inset. This arrangement aided materially in maintaining a comfortable posture and degree of relaxation during the collection of stools. It helped to prevent variation in the usual habits of elimination and to avoid the necessity for resort to laxatives.

The fireless cooker provided a portable refrigerator which aided in the preservation of samples from the time of collection until the next meal time when the investigator could remove them to the laboratory. The space inside this refrigerator accommodated the jug and funnel for urine, the pails containing feces or incomplete daily food composites, and the ice bags filled with cubes from an electric refrigerator. Solid CO_2 was not found to be a practical refrigerant in this case. The supply of ice was replenished each meal time or as often as environmental temperatures necessitated. The bottom of the fireless cooker had extra insulation in the form of several layers of paper which could be renewed from time to time. Thus, it was possible to maintain an inside temperature of 46 to 50°F. The bottle for urine always contained a layer of toluol as additional precaution for preservation.

Every effort was made to lessen the unpleasantness and disagreeable conditions associated with the tasks of collecting in order to maintain a normal mental attitude and therefore normal state of digestion and metabolism on the part of the subject, and to provide for the fullest possible cooperation throughout the series.

Weighing and Care of Samples. All of the food to be consumed was weighed, using the Hanson scale, and while it was being eaten the food composite was prepared by the investigator. Butter, sugar, salt, salad dressing, crackers, cookies, confections, and articles of diet used daily in small amounts were weighed up into marked containers in quantities sufficient to last most or all of the metabolism period. This helped to avoid accumulation of error from multiple small weighings or accidental losses from each service of food as well as to conserve time at each meal. Egg white and yolk were weighed separately except when well beaten together, as were also constituents of salads, certain types of desserts, and other dishes not easily mixed to a homogeneous mass before being eaten or sampled. Milk and other beverages including coffee were weighed regularly rather than being measured by volume.

The food composites prepared for analytical purposes represented 1/10 of the intake for each food item. These were weighed directly into the pyrex container which had been counterpoised on the torsion balance, using the small shot for this purpose. The glass container was well oiled inside with a portion of the butter to be used for sample, and each one served to hold the food composite for the entire day. It was found that if dry foods such as bread and vegetables were composited first, liquids last, and fruit or other gummy material, meats, and fatty particles distributed over all parts of the composite, particularly near the top and edges, and if no mixing was done before drying, the dried mass could be removed and broken apart for grindng with less difficulty than if well mixed and allowed to dry in a compact, fairly homogeneous but hard mass.

Both food and feces were dried at 80°C. or lower to prevent charring. The water bath method of drying gave a more uniform and easily pulverized sample than was obtainable in a ventilated gas dehydrater, electric drying oven, or by other methods tried out. The feces were covered and mixed with alcohol and dried as rapidly as possible, dividing the large stools between two containers if necessary to hasten dessication. A second application of alcohol just before the final drying often separated a gummy residue which was difficult to remove from the container and hence introduced errors in the recovered sample.

Carmine was used throughout these studies for markers at the beginning and end of the metabolism periods. The amount needed averaged about 0.5 gram or the content of size 0 capsules. Dark alkaline stools required more and light fermentative stools less. The capsules were taken 5 to 15 minutes before the first breakfast of the weighed diet, and again at the end of the period before the first breakfast following the last day of weighed diet. Laxatives were avoided as far as possible and actually were seldom used. Thus, the normal gastro-intestinal rate and time of exposure of food mass to digestion and absorption was maintained. Mineral oils complicate drying and analytical procedures and milk of magnesia or other salts introduce errors (16). Plain water enemas were corrected for through the drinking water sample, and aliquots of laxatives ingested orally were added to the food composite.

Aliquots of each 24-hour collection of urine were composited for the entire period. Certain determinations, to be described later, were made on the daily collections. Total nitrogen and chloride analyses were made on the composite as soon as made up and the remainder of it acidified with 10 to 25 cc. of concentrated HCl per liter of urine for preservation until additional mineral analyses could be carried out.

Before storage both food and feces were pulverized by hand in a porcelain mortar, fine enough to pass a 30-mesh brass sieve in the case of feces and a 20-mesh sieve in the case of food. They were stored subsequently in glass containers. Porcelain spoons and spatulas were used throughout in making transfers and handling the ground material. These precautions were necessary to prevent contamination with iron as were many others, including the redistillation of alcohol used in drying feces, the use of iron-free concentrated HCl, and the rinsing of all containers with HCl and distilled water immediately preceding their use for collections and samples.

CHEMICAL ANALYSES

In most instances it will be enough to refer briefly to the methods of analyses employed for the various chemical constituents of food, feces and urine, since the original description of these procedures are easily accessible in the literature. Exception will be made in the case of the method for iron which embodied details from various sources. All chemical determinations were made in triplicate and repeated if necessary to obtain well-agreeing results. At the end of this section will be found a table showing the approximate sizes of sample required for the different methods, since this information is frequently lacking elsewhere and often influences the choice of an analytical method.

Calcium and Magnesium. For calcium and magnesium the material was burned off over a gas flame, finished in an electric muffle furnace, usually over night, and dissolved in 4 N HCl. The calcium was separated from the magnesium and determined gravimetrically according to the method of McCrudden (137, 138) as modified by Shohl (199). Thus the calcium was precipitated as calcium oxalate with sodium acetate to buffer the solution and methyl red to control the pH for separation of the two elements. After standing over night the precipitate was filtered onto a prepared Gooch crucible and ignited to CaO.

For magnesium the filtrate was acidified and concentrated on an electric hot plate to 50 cc., and the magnesium precipitated with an excess of saturated disodium phosphate according to the procedure described by Epperson (60). After 8 to 16 hours, not longer, the precipitate was filtered off and washed, dissolved and reprecipitated in the same container and same manner and allowed to stand 4 hours. The magnesium ammonium phosphate was then filtered onto a prepared Gooch crucible, dried at 120°C., ignited to bright red heat, about 1000° C., and weighed as magnesium pyrophosphate.

Phosphorus. All ashing for phosphorus determinations was done by the wet oxidation methods of Neumann (158), using first a mixture of sulfuric acid and nitric acids, and finishing with repeated small additions of nitric alone. From this ash solution, diluted to 75 cc., phosphorus was precipitated as ammonium phosphomolybdate at a temperature of 65° C. The yellow precipitate, collected on filter paper, was dissolved into beakers with the use of ammonium hydroxide. From this solution the phosphorus was precipitated as magnesium ammonium phosphate in a manner similar to that described for magnesium, and finally weighed as the pyrophosphate.

This procedure has been much studied by various laboratories and many precautions have been suggested (60, 93, 134, 136, 141). As with all other analytical procedures for the various mineral constituents of biological materials, the chief difficulty is with the ashing. In this laboratory it was found impossible to dispense with the wet oxidation method advocated by Neumann (158). Dry ashing even with low heat in the electric muffle gave variable results which were always low. The use of MgO and $Mg(NO_3)_2$ for ashing also gave variable results, but frequently higher than the adopted procedure. In the use of the Neumann method, the end point of complete oxidation was often difficult to determine. Excessively fat food samples often required 6 to 8 hours for complete oxidation. The time was shortened some two or more hours by allowing the sample to stand over night in contact with the digestion acid mixture before heat was applied. Rapid heat gave straw colored solutions, reduced volumes, and silica dissolved from the container, as well as frequently low results. The repeated additions of the sulfuric-nitric acid mixture, as suggested by Peters and Van Slyke (164), quickly resulted in too much sulfuric acid in the solution, particularly in the case of foods. Testing the accuracy of a procedure by known solutions in the presence of extraneous organic matter, such as sugar, or by the use of the recovery method (80) cannot be always relied upon for phosphorus since the element can occur combined with various complex molecules. Repeated analyses of the same sample on different occasions and by different workers afforded the only reliable test of accuracy for figures for a given sample.

Volumetric determination by means of titration of molybdate at the end of the first precipitation likewise gave inconsistent results. It was found, also, that losses occurred in filtering off the molybdate precipitate if the room temperature was above 32°C., unless the wash water, 5% NH_4NO_3, was cooled sufficiently to keep the temperature of the mass on the filter paper below 25° C. In the winter season this precaution was unnecessary.

Sodium and Potassium. Sodium and potassium were obtained as combined chlorides of sodium and potassium according to the procedure described by Hawk (90) and by Treadwell and Hall (232). The material had been ashed at a low temperature in an electric muffle, dissolved in HCl, and the alkaline earths, phosphorus, and iron had been removed with the use of barium chloride and ammonium carbonate according to the method of MacKay and Butler described by Peters and Van Slyke (164). From the combined sodium and potassium chlorides, potassium was separated as perchlorate, dried, and weighed according to the method of Scott (191). Platinum dishes were used for ashing and for the final precipitations of sodium.

Sulfur. The determination of sulfur was also complicated with ashing difficulties. The procedure finally adopted was that of Benedict (19). The material was weighed into 30 cc. porcelain dishes, covered with a solution of $Cu(NO_3)_2$ containing $KClO_3$, and allowed to stand 12 hours or longer. After the sample was carefully dried, the heat was gradually raised, first in the drying oven, then on the electric hot plate, and finally over a bunsen burner at red heat. From the HCl solution of the ash the sulfur was precipitated with barium chloride, filtered onto a weighed Gooch crucible, dried, ignited over a hot flame, and weighed.

Chlorine. Ashing for chlorides was done by the open Carius technique (164) with the modification that the ashing mixture after the addition of nitric acid and excess $AgNO_3$ was allowed to stand 12 hours in the cold and then digested slowly on a water bath 8 to 12 hours. The excess of $AgNO_3$ was titrated with NH_4CNS and the chlorides calculated indirectly by difference, according to the method of Volhard (235). In the case of urine, chloride determinations were made on the fresh sample.

Under the above conditions, longer digestion did not give higher results, indicating that the liberation of chlorides was as complete as the method afforded. The results agreed fairly well, few repetitions being necessary. These and other methods for chlorides in tissues have been the subject of recent investigation and criticism (217, 218).

Acid-base. The data from the analyses for sodium, potassium, calcium and magnesium of the base-forming elements and for sulfur, chlorine and phosphorus of the acid-forming were used in the computations of the acid-base balance in the intake, urine and feces. The algebraic sum of these gave the base balance or amounts of excess base retained by different subjects at the various stages of pregnancy. In calculations of the potential acidity and alkalinity, chemical equivalents of 2 each were used for calcium, magnesium and sulfur, 1.8 for phosphorus (164, 198) and 1.0 for sodium, potassium and chlorine. The final results were expressed as cc. of 0.1 N acid or alkali.

Iron. The methods employed for the determination of iron in biological materials have received much critical attention of late. The flood of recently published papers on this topic constitute *a priori* charges against present available procedures for iron analyses and attest current dissatisfaction with these methods.

Certain volumetric methods are still used with food, feces and urine (12, 176, 225). The Thompson colorimetric method (226) in some form has been widely tested, having had various modifications purported to stabilize the color produced. Efforts have been made to intensify the iron thiocyanate color with acetone mixtures (30, 73, 124, 148, 204, 207). Other variations have had to do with the separation of the colored compound from the interfering salts (165), using amyl alcohol (110), glycocoll (30, 84, 125, 248) and other organic solvents (171). No colorimetric method seems satisfactory from every point of view, such as convenience, simplicity, briefness, and an unfailing degree of accuracy at the hands of all workers.

However, with careful application, some methods are sufficiently accurate to indicate tendencies in iron metabolism. An adaptation of the thiocyanate and amyl alcohol procedure has been employed by the writer in this study, and the method with some discussion of details and precautions is presented here. Valuable suggestions as to technique have come from different sources (6, 57, 58, 94, 215) and nothing original is claimed in the application of the procedure.

For food and feces the samples were weighed into low, flat-bottom, platinum evaporating dishes of about 60 cc. capacity, and 1 cc. of standard iron solution containing 0.1 mg. of iron was added to one sample in each triplicate series. All were burned off carefully over a gas flame, then placed into a cold muffle which kept them below visible red heat over night or for at least 8 hours.

The loose white ash was dissolved in 2 cc. of 4 N HCl, allowed to stand for 1 hour, and transferred to 150cc. beakers. No filtering was required with these samples when the ash obtained was of the desired character, and only occasionally was one filtered. To each sample was added 1 cc. of 40 percent NaOH and the solution was boiled very gently for 1 hour on an electric hot plate, keeping the volume at 50 cc. or above. Then the volume was concentrated to 10-15 cc., allowed to stand over night and transferred to volumetric flasks, 50 cc. for foods and 100 cc. for feces, with the aid of 2 or 3 2-cc. portions of N/4 H_2SO_4 to dissolve the precipitate formed. After diluting to volume, 5 cc. were removed from each flask and the acidity titrated and computations made to determine the adjustment necessary with 1:4 H_2SO_4 to give an acidity equivalent to 90 cc. of N/4 NaOH in a 10 cc. portion. A standard was prepared containing approximately the same amount of iron as the unknown, with the same reagents in the same volume and the acidity titrated as for the unknown.

For developing the color, 10 cc. or an aliquot containing about 0.02 mgm. of iron was pipetted from each flask to a well stoppered 60 cc. separatory funnel. The amount of 1:4 H_2SO_4 calculated above as necessary to adjust the acidity was added to the funnel, then ½ cc. of 4 percent potassium persulfate and enough water to make the volume 15 cc. After standing 1 minute or more, 10 cc. of iso-amyl alcohol from one burette and 5 cc. of KCNS (20 percent) from another were added in the order named, and immediately the contents were shaken well for about 20 seconds and allowed to separate. The aqueous portion was run off through the funnel stem and the colored amyl alcohol layer carefully poured from the top into the colorimeter cup. The color in the standard was developed simultaneously with the unknown and in the same manner. Readings in the colorimeter were made at once.

In the case of urine, 100 cc. volumes were concentrated, dried carefully, organic matter burned off and ash solutions made in the same manner as for food and feces. Because of the larger quantities of salts from the urine, acid rather than alkali hydrolysis of the pyrophosphates was employed in order to avoid further increase in salts by the successive neutralizations accompanying alkali hydrolysis. For this the ash solutions were transferred to 150 cc. beakers, 5 cc. of concentrated HCl added, the mixture boiled gently for 20 minutes, the volume concentrated and transferred to a 50 cc. volumetric flask. The amount of iron in urines was so small that 0.1 mgm. of standard iron in solution was added to each sample, and 0.2 mgm. to the third one in each triplicate series.

For developing color in this mixture, a 10 cc. portion was transferred to a separatory funnel and to this was added, in the order named, 1 cc. of concentrated HCl, 2 drops of $KMnO_4$, 10 cc. of amyl alcohol, and 5 cc. of KCNS. Shaking, separating, and reading colors was carried out as above. More exact adjustment of acidity for production of color in urine samples seemed unnecessary since the total amount of iron was negligible in its effect on the iron balances.

Most of the unusually high figures for iron in foods and feces represent an average of six concordant determinations, instead of triplicates, having been checked by both the acid and the alkali methods of hydrolyses described above.

Unfortunately, the above procedures are not error-proof. Frequent repetitions were necessary as a result of accidents or faulty variations in minor details of technique. Some practical suggestions for avoiding contamination and improving the accuracy of determinations are listed below:

1. All apparatus, including pipettes, burettes and volumetric flasks were carefully calibrated, particularly the pipettes and flasks used in series.
2. Iron-free chemicals were used as far as possible. One lot of NaOH, flaked, and two lots of HCl were found to be practically free from iron. The KCNS always contributed more iron contamination to the sample than all other reagents combined. Some of this could be removed by first preparing a saturated solution, filtering it, and then diluting to the desired concentration. Blank determinations on reagents were run almost daily, and the quantity of reagents employed

in each sample measured accurately and kept constant to facilitate iron corrections and regulations of acidity.

3. To prevent contamination from iron-laden dust particles and exposed surfaces, all pipettes and other volumetric apparatus were kept under glass covers such as inverted tall cylinders, or in glass specimen jars. The mouths of reagent bottles were kept covered with beakers or other glass vessels. The samples, while being allowed to stand for extraction or over-night, were kept covered with glass, such as inverted beakers.
4. HCl was used freely to rinse apparatus, including platinum dishes between each series of samples or before each use. During the handling of samples after ashing, such as in making transfers and developing color, the finger tips were rinsed frequently with dilute HCl and dried on filter paper.
5. Nothing iron was allowed to come in contact with samples or apparatus. Preliminary ashing was done in the open room with asbestos boards resting on perforated glazed crockery cylinders and over brass burners. Electric hot plates, wooden transport trays and the floor of the electric muffle were kept covered with fresh clean asbestos sheeting. Paper toweling was used freely to keep apparatus and samples from coming in contact with dusty surfaces on drying racks. All exposed iron piping and fixtures in the room were covered with aluminum paint. Desk tops, table tops and similar surfaces in the room were wiped off daily or oftener with a wet towel.
6. Work was not carried on in the presence of drafts nor near open windows or other conditions contributing to circulation of dust. This precaution greatly increased the objections to the method using amyl alcohol, since in such an atmosphere the comfort and mental and physical efficiency of the worker are soon lowered to levels incompatible with accurate work such as matching colors in the colorimeter.

Other precautions were concerned with the prevention of irregularity in the behavior of the chemical mixtures used. The recovery method seems to be the most satisfactory way, at present, for testing an analytical iron procedure (80). Blank tests using purified chemicals do not measure the effects of ashing; and the recovery method does not measure the completeness or effectiveness of an ashing procedure. These facts emphasize the necessity for well-agreeing triplicates, at least one recovery sample in each series, repeated determination on the same sample at different times, and frequent blank tests on reagents, in order to reveal some of the systematic errors in technique. Other precautions, such as the following, to insure uniform maximum color development, have been applied regularly to the iron determinations reported in this series.

1. The color was developed in not more than four samples at once, including the standard. All were read within five minutes after the KCNS was added. In addition they were left, often, to stand after reading to see whether any showed rapid fading.
2. No glassware, particularly beakers for hydrolysis of pyrophosphates, was used if there were any signs of etching.
3. Colorimeter readings on the amyl alcohol layer were made at the same temperature at which it was separated from the aqueous portion. Cooling frequently resulted in slight supersaturation which produced a faint cloudiness. For this reason, also, drafts were avoided, as were readings too near a cold north window in winter.
4. A north daylight was always used for the colorimeter. This was reflected from an open space of grass and trees with no buildings

nearer than two city blocks. Readings were made only between 10:00 a. m. and 3:00 p. m. to avoid appreciable fluctuations in light incidence and quantity.

The H_2SO_4 medium of acidity was preferred to the HCl for color development, particularly in warm weather when the former, after alkali hydrolysis, seemed to give more stable colors. It is likely that the application of these procedures to materials with markedly different ash, such as those much higher in phosphorus, might necessitate the adjustment of acidity to other concentrations. The color from the ash of certain food composites showed a greater tendency to fade than did others and this was probably due to an ash of different character.

Yellow contaminated the thiocyanate color when the amount of KCNS or of acid was too great, often when wet ashing with nitric acid was used or when the sample had been heated too intensively during ashing. Uneven temperatures in different parts of the electric muffle were a source of much difficulty in this respect. Some grades of amyl alcohol gave yellow color which could be removed partly by distillation of the alcohol before use. Only colorless fresh iso-amyl alcohol should be used, and it should contain no alcohols of greater solubility than amyl, no aldehydes or reducing agents. Normal-amyl alcohol gives a purer quality of red color but has a more disagreeable odor than does iso-amyl.

Next in importance, after the achievement of a loose, white, carbonate-containing ash with no evidence of fusion, is the prevention of color fading. Since this has been attributed to the presence of pyrophosphate, the above procedures aim first to lessen the amount of such phosphates present by hydrolysis with acid or alkali (6, 57, 215). The same is accomplished by some workers with wet oxidation instead of dry ashing (12, 73, 84). The second condition to be attained is the removal of the iron thiocyanate from most of the interfering salts by the use of amyl alcohol (110). Procedures (58) calling for the removal of the phosphorus before determining the iron were found to be impractical since this method does not remove possible interfering salts other than phosphates, always removes traces of iron with the bulky molybdate precipitate and does not provide for the removal of the pyrophosphate form of phosphorus, which is not precipitated by the molybdate solution and which gives the most interference with color stability.

Nitrogen and Miscellaneous Determinations. Total nitrogen was determined by the Arnold-Gunning Macro-Kjeldahl procedure using $CuSO_4$ as a catalyst (90). Determinations on urine were made in duplicate daily and in triplicate on the composite for the period. Other daily determinations in urine were: creatinine and titrable acidity by the methods of Folin and Hawk respectively (71, 72, 90); ammonia; and pH, estimated colorimetrically. Specific gravity and tests for sugar and albumin were made daily. Records of the volumes of water intake and outgo through urine and feces were made daily for estimation of water balance in the studies of acid-base equilibrium.

Total Calories. Total calories in foods were determined by the use of the oxycalorimeter (20). This procedure measures the amount of oxygen required for the combustion of a given sample. For comparison, total calories were computed from the content of protein, fat and carbohyrate. The results agreed fairly well except in the diets containing most fat. In these the divergence of results, from 1 to 5 percent, appeared due to inaccuracies in the method used for determination of fats (39).

Fat, Carbohydrate and Protein. Fat was determined with the Soxhlet extraction apparatus (8). It was found necessary to extract the food sample with warm water to remove soluble carbohydrate and dry again to constant weight in order to obtain consistent results and figures from which the computed total calories agreed reasonably well with those found by the oxycalorimeter method (20). Protein was estimated from total nitrogen using the 6.25 factor (108), and carbohydrate was obtained by difference after the fat and protein content were known.

Size of Samples. Table 2 presents a summary of the approximate amounts of material needed for analyses according to the procedures listed. Such a compilation aids in estimating the quantities of material to be preserved for a series of analyses and is convenient reference in the selection of a method to be used.

TABLE 2. Quantities of Materials Needed for Samples.

Mineral Constituents	Food composite [2]		Urine		Feces [2]		Method used
	Sample	Constituent [2]	Sample	Constituent [2]	Sample	Constituent [2]	
	gm.	gm.	cc.	gm.	gm.	gm.	
Calcium	10	0.025	200	0.03	1	0.03	McCrudden-gravimetric
Magnesium	10	0.007	200	0.01	1	0.009	$MgNH_4PO_4$
Phosphorus	2	0.005	25	0.01	1	0.015	Neumann-gravimetric
Sodium	5	0.04	100	0.2	2	0.04	Lindo-Gladding
Potassium	5	0.03	100	0.15	2	0.03	Perchlorate
Chlorine	3	0.04	10	0.03	1	0.003	Open Carius Volhard
Sulfur	4	0.005	10	0.003	2	0.008	Benedict-gravimetric
Nitrogen	2	0.04	20	0.04	1	0.05	Macro-Kjeldahl
Iron, in mgm.	3	0.10	100	0.015	0.5	0.20	Thiocyanate-amyl alcohol.
Total calories	2	10.cal.					Oxycalorimeter
Fat	2	0.40					Soxhlet extraction

[1] Dry weight.

[2] Approximate amount of element present in sample of size indicated.

OTHER EXPERIMENTAL CONDITIONS

Sunshine. Inasmuch as the primary aim of this series of investigations was a determination of the extent of the calcium and phosphorus retentions in southern women during pregnancy, daily records were kept of the approximate amounts of sunshine and of cloudy and rainy weather in this locality during the time of the balance experiments. The findings were checked with official reports of the nearest weather bureau to determine whether or not the seasons during which these studies were made were typical for this section (233).

Subjects. Six women, varying in ages from 21 to 31 years, served as subjects for a total of 25 metabolic periods covering 101 days. All

TABLE 3a-. Summary of Case Histories of Subjects (Mother).

Case	MOTHER										
	Parity	Age	Height	Weight [1]	Weight [2] deviation	Final weight	Total gain	Duration of gestation	General health	Date of Parturition	Success in lactation [3]
		yrs.	inches	lbs.	lbs.	lbs.	lbs.	weeks			
I	I	27	62	108	—13	140	32	40	Frequent constipation.	1- 3-32	+++
II	I	27	61	110	— 9	134	24	38	Slightly anemic. Supplemented diet.	1- 6-32	+++
III	II	28	66	131	— 4	165	34	40	Indigestion. $NaHCO_3$ regularly. Constipation. Active.	5-23-32	++++
IV	III	31	71	187	+30	202	15	40	Active. Outdoors much. Occasional slight edema.	6-19-32	++++
V	I	21	65	120	— 8	145	25	40	Worked first 6 mo. Nervous strain at last external to conditions of gestation.[5]	6- 7-32	+
VI	I	23	66	113	—20	148	35	40	Some nausea at first. Frequent diarrhoea. Occasional edema. Outdoors much.	7-18-32	++++

[1] Pre-pregnant weight.

[2] Deviation from commonly used standards (Woods).

[3] ++++ denotes success; +++ some difficulty; + failed after 6 weeks.

[4] Total gain divided by 7.

[5] Nervous breakdown, 2 months post-partum.

TABLE 3-b. Summary of Case Histories of Subjects (Infant).

	INFANT						
	Birth weight		Monthly gain of first 7 months	Dentition		Muscular achievement	
				First tooth	No. at 8 mos.	Sitting age	Walking age
	lbs.	ozs.	ozs.	mos.		mos.	mos.
I	8	12	23	8	2	4	10.5
II	8	0	20	6	4	4	10.5
III	9	12	20	5.5	4	6	12
IV	8	0	27	4	8	6	12
V	8	4	25	6	8	6	13
VI	7	0	21	5.5	6	4	11

were primiparae, except one duo-para and one tripara. All the balances save three were made in the last half of gestation. In one subject a balance period came four weeks prior to the beginning of gestation and another two weeks after. One balance in another subject came during the 18th week. Table 3 summarizes some of the facts pertaining to case histories.

The activities of these women varied little from their usual routine. They did their own housework, only Case IV having regular help. Most of them found time for several minutes in the open daily, and three of them had one to two hours in sunshine each day looking after gardens. The time spent outdoors was usually in the early morning or late afternoon, rarely during the four hours in the middle of the day. The two women serving in the late fall, Cases I and II, were deprived of much outdoor activity and sunshine during November and December on account of intermittent rainy and cloudy weather.

All of the subjects were college women, four of them graduates, and all capable of a high degree of intelligent cooperation. They represented the average middle class home, as indicated by the fact that four of them were wives of faculty members, one the wife of a student, and one of a local merchant.

Their interest was indicated by the fact that half of the group were unsolicited volunteers, and also by the willingness exhibited by all from the beginning and throughout. Their sincerity was evident repeatedly during the studies by their concern as to details and their faithfulness in reporting small errors and accidents. The investigator refrained from making educational comment regarding the choice of food, either during the home visits or from period to period after results of analyses were known.

SUMMARY OF DESCRIPTION OF EXPERIMENTAL PROCEDURES

1. A method for carrying out balance experiments with women living in their own homes and performing their usual household activities is described here.

2. A list of equipment needed for the home in such a study is included together with descriptions of special apparatus found useful in this work.

3. The method employed for determination of iron has been described somewhat in detail.

4. A summary of case histories of the 6 experimental subjects affords an introduction to the discussion of the results of the biochemical studies, including 25 balance experiments, found in the following sections.

RESULTS AND DISCUSSION

The findings respecting the different minerals, along with the literature pertinent to each, will be treated first. A consideration of the acid-base balance will precede the results pertaining to iron. Discussion of nitrogen metabolism will be followed by an estimate of the adequacy of the diets as a whole. This series concerned with nutrition during normal pregnancy, although greatly lacking in the data needed to reveal certain phases of metabolism, is believed to represent the most complete description published for any one group of women.

CALCIUM

Literature. Prior to 1929 reports of calcium balance studies in pregnancy had been recorded by only two investigators, Hoffstrœm (97) and Landsberg (116), and these included 37 balances on 10 women. During the last few years the number of such studies has increased materially, as will be seen from the summary in Table 4. This summary does not include 2 balances on one case receiving a restricted calcium intake, reported by Bauer (15), three balances on a case of osteomalacia during pregnancy, reported by Dieckmann (53), nor three cases referred to, one by Macy (144) and two by Dieckmann (53), for which no figures were given.

Various experimental conditions prevailed in the studies recited in Table 4. Hoffstrœm's was a continuous observation from the 17th week to the end of gestation. Toverud's group represented women living in an institution and on the regulated diets; various supplements, such as cod liver oil, vigantol, minerals, milk and eggs were added to the usual ration. Three of the women of Macy's series (144, 106) were studied during a pregnancy subsequent to heavy lactation, hence were accustomed to uncommonly high intakes, while the other subject was on a fairly constant and superior diet, including cod liver oil and 750 grams of orange juice daily (142). Women in both of the studies reported by Coons (40, 41, 42, 43) were taking ordinary self-chosen home diets, only one diet of the present series being supplemented in any way. The investigations reported in this bulletin add 24 balances on 6 women, making a total of 160 balances on 60 women for which figures have been published.

The recently published results from various laboratories have agreed in emphasizing that on the usual home diet, unsupplemented with extra vitamin D or minerals, it was difficult for the maternal organism to attain a satisfactory positive calcium balance. The average calcium intake as well as retention on the usual diet has been considerably less than that recorded by earlier investigators. From the table it will be seen that Hoffstrœm's subject ingested an average of 1.712 grams of calcium per day and had a storage of 0.213 grams daily. Landsberg's women averaged much higher. The study reported by Coons and Blunt, 1929, showed an average intake of only 1.096, with a storage of 0.096.

Table 4. Summary of Calcium Balances During Pregnancy in the Human Mother.
Daily Averages

Investigator	Date	Place	Total no. of observations		Calcium intake			Calcium retention		
			Women	Periods	Minimum	Maximum	Mean	Minimum	Maximum	Mean
					gm.	gm.	gm.	gm.	gm.	gm.
Hoffstrœm	1909	Finland	1	23	1.013	2.385	1.712	—0.330	0.951	0.203
Landsberg	1914	Germany	14	14	1.821	2.935	2.670	0.016	0.829	0.423
Coons-Blunt	1930	Chicago	9	23	0.603	1.624	1.096	—0.114	0.286	0.096
Macy et al.										
Ordinary diet	1930	Detroit	3	12	1.531	2.693	1.922	—1.276	0.278	—0.103
Regulated diet	1931	Detroit	1	2	1.550	1.930	1.740	0.620	0.640	0.630
Toverud	1931	Norway	29							
Ordinary diet			16	27	0.582	1.692	1.033	—0.426	0.680	0.069
Regulated diet		”	23	30	0.807	2.157	1.524	—0.263	0.895	0.335
Weighted averages										
Prior to 1930.			15	37	1.013	2.935	2.074	—0.330	0.951	0.286
Recent studies			42							
Ordinary diet			28	62	0.603	2.693	1.228	—1.276	0.680	0.046
Regulated diet			24	32	0.807	2.157	1.538	—0.263	0.895	0.353
This study	1932	Oklahoma	6	24	0.809	2.375	1.418	0.059	0.448	0.279

Most significant is the fact that all of the later studies, except the one herein reported, have been with women living in the northern latitudes of the temperate zone, and frequently in the midst of the unfavorable conditions of the crowded city or institution. With these groups there was a tendency, decidedly more manifest in some individuals than in others, for the calcium retentions from the unsupplemented diets to be negative, or a positive one too low to meet the supposed fetal requirement. Toverud and Toverud found that cod liver oil did not improve the calcium and phosphorus balances so much as did an increase in the intake of these elements to levels of 1.6 and 1.8 grams daily respectively, accomplished through the addition of milk and eggs to the diet. As was shown in the introduction, the fetus alone demands on an average 0.22 gram of calcium daily during the ninth month and 0.31 gram daily during the tenth

Table 5. Calcium Retention by Southern Women during Pregnancy.
Daily Averages

Subject and period	Month of year	Week of Pregnancy	Calcium intake	Calcium outgo			Balance
				Urine	Feces	Total	
			gm.	gm.	gm.	gm.	gm.
I-0	Feb.	— 4	0.726	0.049	0.554	0.603	0.123
I-1	Apr.	2	0.697	0.043	0.536	0.579	0.118
I-2	Sept.	27	1.253	0.082	0.977	1.059	0.194
I-3	Oct.	31	1.199	0.060	0.805	0.865	0.334
I-4	Nov.	34	1.291	0.057	1.021	1.078	0.213
I-5	Dec.	38	1.242	0.076	0.838	0.914	0.328
II-1	Oct.	26	1.796	0.357	1.068	1.425	0.371
II-2	Nov.	30	1.773	0.280	1.032	1.312	0.461
II-3	Dec.	34	1.820	0.371	1.176	1.547	0.273
II-4	Jan.	38	1.920	0.260	1.393	1.653	0.267
III-1	Mar.	33	0.902	0.306	0.390	0.696	0.206
III-2	Apr.	37	1.441	0.391	0.634	1.025	0.426
IV-1	Apr.	31	1.022	0.594	0.369	0.963	0.059
IV-2	May	35	1.236	0.557	0.459	1.016	0.220
IV-3	June	39	1.562	0.545	0.529	1.074	0.488
V-1	Mar.	28	0.886	0.164	0.408	0.572	0.314
V-2	Apr.	33	0.809	0.161	0.516	0.677	0.132
V-3	May	38	1.033	0.102	0.615	0.717	0.316
V-4	May	39	1.071	0.099	0.595	0.694	0.377
VI-1	Feb.	18	2.375	0.294	1.970	2.264	0.111
VI-2	Mar.	23	2.085	0.207	1.516	1.723	0.362
VI-3	Apr.	27	1.449	0.267	1.031	1.298	0.151
VI-4	May	31	1.748	0.201	1.334	1.535	0.213
VI-5	June	35	1.861	0.191	1.211	1.402	0.459
VI-6	June	38	1.558	0.135	1.111	1.246	0.312
Average							
This series*			1.418	0.242	0.897	1.139	0.279
Chicago series			1.096	0.232	0.768	1.000	0.096

*Exclusive of Period I-0.

FIGURE IV

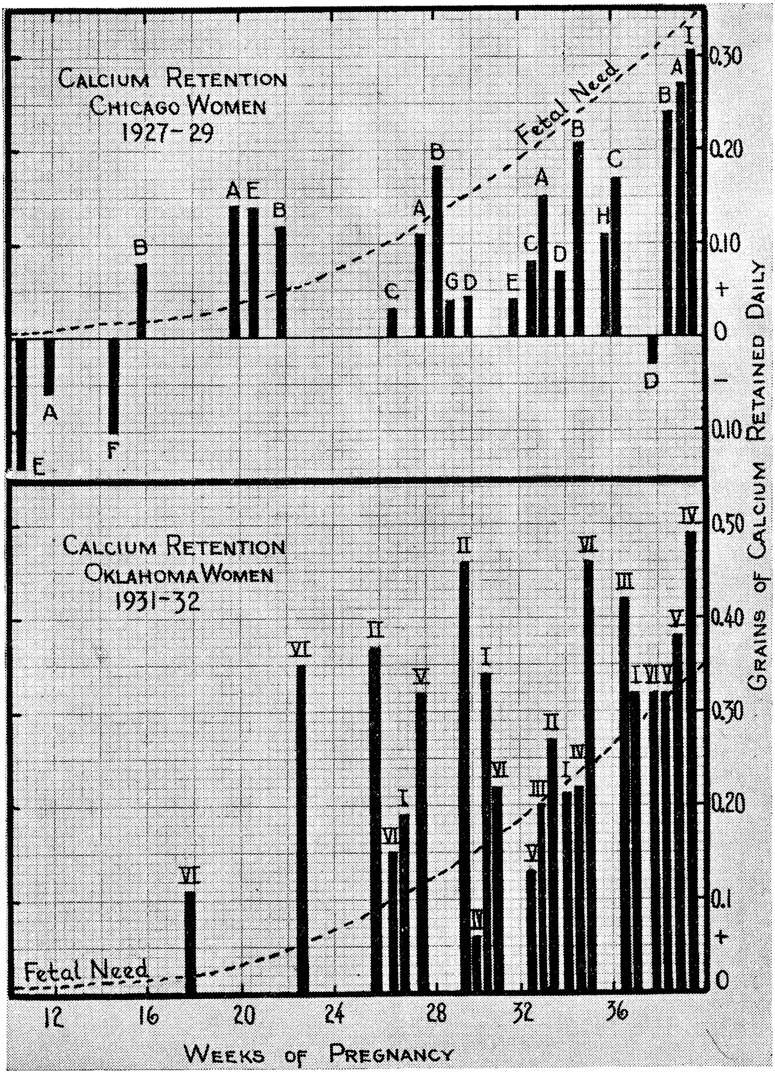


Fig. IV.— The superior retention of calcium by Oklahoma women in contrast to inadequate storage by a Northern group.

month of gestation (Table 1). Givens and Macy (76) estimated that about 0.21 gram of calcium daily is needed during the last three months of gestation.

The present investigation was prompted by the desire to know to what extent such low calcium retentions would be exhibited by normal women receiving their usual unsupplemented home diets but living under more favorable conditions with reference to the quantity and quality of sunshine. Pertinent in this connection is the very recent report of Eliot and coworkers of Yale (55, 56) that the sunshine of Puerto Rico is practically 100 percent effective in preventing rickets in children, almost 600 of whom were examined.

Calcium Retention by Oklahoma Women. Table 5 shows the data from the intake-outgo analyses for calcium in each experimental period. The time of the balance experiment and the week of pregnancy is shown for each period. In the first column the Roman numeral indicates the case number, the arabic numeral the period number. For example, there were six balance periods for Case VI, only two for Case III. At the end of the table is a summary of the average intake, excretion and balances of all the periods except I-0. These means have been compared to those for the Chicago group previously studied by the author using the same methods of collection and analytical technique.

Figure 4 shows the balances for calcium in relation to the fetal demand as estimated from fetal analyses and in comparison with a similar chart taken from the study with the Chicago women (41). It demonstrates how frequently the northern women failed to store enough to meet this need while many of the present series stored much in excess of this estimated demand.

It will be seen from both the table and the chart that not one negative balance was found in the calcium studies for the Oklahoma group, although some of the diets (Cases I and V) were none too high in this element. The highest calcium retention recorded for any individual in the earlier series was 0.306 gram daily, but this figure was exceeded in 12 of the 24 balances of the southern group. The lowest calcium retention found for Oklahoma women during pregnancy was 0.059 gram daily, but 7 of the 23 Chicago balances were less than this amount. The average calcium retention was approximately three times as high in the southern group.

Doubtless some of the improved calcium storage was due to the higher dietary calcium of these women as shown by the averages for each group. However, a comparison of individual periods showed that a high intake did not always assure good storage (Periods II-3, VI-1 and VI-4, as compared to Periods I-3 and V-1). Moreover, a higher percentage of the dietary calcium was retained by these southern women, amounting to more than a fourth of the intake in 9 of the balances and averaging 21 percent for the entire group as compared to an average of 9 percent for the Chicago group. Tabulation of storage rates at different levels of intake in the two groups (Table 6) demonstrates that the Oklahoma women attained far better utilization at all levels. Although low intakes were more frequent in the Chicago group, at no level did the quantity retained average as well as it did for the southern women on intakes of less than one gram daily. This table shows, also, that the Oklahoma group exhibited almost as good metabolic performance on intakes of 1.0 to 1.3 grams daily as they did on intakes above 1.6 grams. These facts are of interest in connection with the minimum of 1.6 grams of calcium daily which has been proposed as a standard requirement for pregnancy (143).

Table 6. Relation of Calcium Intake to Storage.

No. of balance periods	Range of daily calcium intake	Range of daily calcium storage	Average daily storage
	gm.	gm.	gm.
		Oklahoma women	
4	Below 1.0	0.118 to 0.314	0.192
8	1.0 to 1.29	0.059 to 0.377	0.255
4	1.3 to 1.59	0.151 to 0.488	0.344
8	1.6 and up	0.111 to 0.461	0.290
		Chicago women	
7	Below 1.0	—0.114 to 0.238	0.062
10	1.0 to 1.29	—0.138 to 0.306	0.102
4	1.3 to 1.59	0.038 to 0.168	0.116
2	1.6 and up	0.143 to 0.144	0.144

Bauer's finding (17) that the bone trabeculae constitute an important storehouse for easily mobilized calcium salts in both the adult and the young animal suggests that the retention of dietary calcium might vary with the presence of different amounts of reserve at the beginning of gestation as well as with the requirements of the fetus and of maternal tissues undergoing hypertrophy. There is no reason to believe, however, that the subjects of recent studies, living in northern climates and showing low retentions of calcium, had available reserves greater than those of very early subjects accustomed to higher intakes of calcium but nevertheless exhibiting good retentions during pregnancy. Nor does it seem likely that the women of the present series, accustomed to fairly adequate diets plus an abundance of sunshine, could have entered gestation with more impoverished calcium reserves than did the women of the northern United States, and that this condition necessitated greater retentions from the diet by the southern women during the period of pregnancy.

A glance at Figure IV reveals that only one of the 24 calcium balances (IV-1) obtained during pregnancy was significantly below the probable fetal requirement, a deficiency exhibited even more markedly by 14 of the 23 balances for northern women. In fact, the retention in the present group was frequently two to three times as much as the apparent fetal demand.

Another interesting difference evident from Figure IV is with respect to the increased retentions from diet near the end of pregnancy. The retentions were markedly improved in the Chicago group during the last two months of gestation, presumably as a result of the urgent physiological demands of this period in the absence of maternal calcium reserves acquired in the earlier weeks. In the southern group retention early in pregnancy provided these available depots and lessened the need for relatively greater storage from diet near the end of gravidity when fetal demand was actually greatest (Cf. Table 1). An exception was Case IV, whose early retentions were low; and her records illustrate the expected increase in storage as gestation progressed.

Coefficients of digestibility for nitrogen and ratios of the weight of dry food to feces to be described later (Table 25) show that digestion of food was more complete and absorption greater in the southern women, indicating thereby conditions conducive to better calcium utilization. That the improved calcium retention was not due to better calcium-phosphorus ratios in the intake will be shown in the discussion under phosphorus (Table 9). Also, acid-base balance computations showed no significant or consistent role of this factor (Table 16).

The balances for Case II, since not particularly unusual, have been presented along with the others and included in the final averages. The diet of this subject differed in respect to the amount and sources of vitamin D, in that she was receiving at the beginning of the study and regularly throughout the intervals as well as the metabolism periods, 3 teaspoonfuls of cod liver oil (Squibb's) daily, only irradiated whole wheat bread and precooked irradiated cereal, and 1 to 2 eggs daily. The diet was superior in other respects, including regular servings of liver, approximately an ounce of wheat germ preparation and over 700 grams of milk daily. With the exception of one period at the 30th week, her retentions of calcium showed no advantage over those of other women (Periods III-2, IV-3, and VI-5). Unfortunately, blood calcium was not determined to indicate whether or not, with sunlight superimposed on the various dietary sources of vitamin D, she may have been receiving some excess of this factor, thus accounting for the higher excretions of this element particularly in the last two periods after time had elapsed for a cumulative effect of overdosage (129, 130). Certainly there was no indication from these balances that this subject was greatly benefited by the increased supply of vitamin D. This is in accord with the findings of Toverud (231) that cod liver oil did not markedly improve the retention of calcium. None of the other women received vitamin D from any except natural food sources and the action of sunlight. The data for Period II-4 are not comparable with others since they represent only a two-day period because the study was interrupted by the onset of labor, coming in approximately the 38th week of gestation.

Case IV presented an interesting study in that up to the end of the first metabolic period she had gained less than two kilograms in weight since the beginning of gestation, although there had been no nausea or ill health to interfere. Her overweight condition (Table 3), about 30 pounds at the beginning of pregnancy, suggested that up to that time she had been transferring needed fetal materials from her own reserves and hence made low retention from her diet. Such was indicated in the low nitrogen retentions and presumably was the explanation for low calcium also, since the baby appeared normal in bone development at birth, had four teeth at the end of four months, eight at the end of eight months, and otherwise showed supernormal physical growth in early post-natal life.

Sunshine. A few comparisons will serve to substantiate the hypothesis that the better calcium retentions were due to the sunshine superior in quality and quantity found in this section. During the entire time when the subjects of this study were under observation, the nearest U. S. Weather Bureau, Oklahoma City, 70 miles away, recorded an average of 61 percent of the possible sunshine hours, compared to 53 percent for Chicago for the period when those studies were in progress (41, 233). However, since metabolism periods in each series were concentrated in certain months of the year, an average of the actual sunshine hours per month, weighted for the frequency of balance experiments occurring in a given month, furnishes a better comparison of the quantity of the sunshine available.

These averages were 172 hours per month for Chicago and 251 hours per month for Oklahoma, an increase in Oklahoma of 46 percent over that for the northern locality during the respective experimental periods. Moreover, 18 of the 23 metabolic studies in Chicago were made during the seven months of low ultraviolet light, September to March inclusive, described by Tonney and his coworkers (229, 230).

Young and Dorrough (249) found that at Denton, Texas, 200 miles south of Stillwater, with the same longitude and a similar humidity, the sunlight in 1931, through January, February, March and April, daily between the hours of 10 to 11 a. m. and 2 to 3 p. m., showed a total of 8, 24, 21 and 31 erythema hours per month respectively, as compared to 0, 2, 7 and 12 hours for these months in 1927 in Chicago's unobscured sunshine such as found at the Navy Pier (230). These figures were obtained from actinic readings using the oxalic acid-uranyl sulfate method (5), but they do suggest that the available sunlight for this section may be of three-fold potency or better as compared to that of the smoky city during the corresponding months of the year. Extensive studies on the potency of sunlight received in the southern United States are few (51, 55, 56, 122).

The sun's altitude at noon for this part of Oklahoma varies from 77° 20′ maximum to 40° 20′ minimum. The antirachitic effect of sunlight from an altitude below 35° is said to be negligible (228). The balance experiments of this series extended from late September to July 1 and hence included periods of both maximum and minimum sun's altitude.

On the whole the subjects of this investigation represented about the average woman of the southern United States as regards access to sunshine, standing between the class of city dwellers with little or no outdoor freedom and the more fortunate group of active farm women whose duties require that a large proportion of their time be spent outdoors at all hours of the day. The group serving as subjects for these experiments occupied a mid-way position geographically, receiving less total hours of sunshine than residents of the more arid sections of Texas, New Mexico and Arizona, and more than those of the states bordering the Gulf and lower Mississippi with heavy rainfall, fogs and high humidity.

Food materials used widely for human consumption are notably lacking in vitamin D. Exceptions are the fish liver oils from the waters of the north temperate zone. Prior to this modern period of city dwelling and use of processed foodstuffs of various kinds, man of the temperate zone dwelt more in the open and was dependent on sunshine and sun-treated foods to regulate his calcium and phosphorus metabolism (227). In tropical climates he developed varying degrees of pigmentation of the skin as a protection against an excess of the light factor in his metabolism. It is not unreasonable to suppose that these sources of vitamin D will still suffice for human needs in regions and situations where full advantage of them is possible.

The importance of these biochemical findings in relation to the problems of the control of rickets, protection of women during pregnancy, and similar questions is obvious. It is a relief to those interested in maternal and infant welfare to know that women in some sections are able, apparently, to make the calcium of the diet supply the needs of gestation. One may well ask: "How much cod liver oil do southern women need during pregnancy? Do they ever need viosterol? May there not be some other deficiency in the diets of women of this section which is more deserving of the attention of research workers?"

SUMMARY OF SIGNIFICANT FINDINGS REGARDING CALCIUM RETENTION.

1. In 25 experimental periods, covering 100 days on six women, no negative calcium balances were found, although the unsupplemented diets had an average calcium content of only 1.40 grams of calcium daily.

2. Storage amounted to 2 to 3 times the estimated fetal need and was in striking contrast to recently published low retentions observed in similar studies elsewhere.

3. The excellent calcium retention was not attributable wholly to superior diets, whether rated according to total calcium content, sources of calcium, distribution of foodstuffs in the diets, Ca:P ratios, or the uniform preponderance of acid or base.

4. Access of the women to large amounts of antirachitic efficient sunshine seemed to be the factor affording the most plausible explanation of the unusual results obtained.

5. These findings show that it is possible for women to retain from their ordinary unsupplemented diets sufficient calcium to supply the needs of gestation.

PHOSPHORUS

Published Reports. One hundred fifty experiments on 61 women showing phosphorus retention during pregnancy were reported by six investigators prior to 1931. These have been summarized in Table 7. Four of the reports covering 56 balances were published prior to 1915 and hence antedate our present knowledge of nutrition and of adequacy of diet. As was found true above for calcium, most of the balances were determined under conditions unfavorable to the utilization of this element, such as a lack of sunshine and of normal retention of calcium. These facts justify the study of phosphorus retention by women receiving larger amounts and better quality of sunshine. Furthermore, as Macy has emphasized, it is "the accumulation and recording of many and diverse data on the same individual and on different individuals in various stages of gestation that will ultimately reveal the functions and physiological processes peculiar to the progression of the human reproductive cycle" (144).

From Table 7 it is seen that on intakes averaging 1.5 to 2.0 grams of phosphorus daily, storage ranged from 0.15 to 0.30 gram. Better retention occurred when the diet was regulated by the addition of extra minerals, milk, or supplements of cod liver oil or other forms of vitamin D (142, 231). There was a total of 17 negative balances for phosphorus, most of them concentrated in the groups reported by Macy, Toverud and Coons. With the exception of Bar's three women (11) the Chicago group showed the poorest utilization of dietary phosphorus.

Attention is called to the agreement between different groups as to the average Ca:P ratio of the diet and to the marked disparity in the ratio of Ca:P stored. Negative balances for one or both elements do not permit the calculation of the Ca:P ratio of the storage in many individual instances, but ratios for a group have been based on average retentions given in this and in Table 4.

TABLE 7. Summary of Phosphorus Balances During Pregnancy in the Human Mother.
Daily Averages

Investigator	Date	Place	Total No. of Observations		Phosphorus intake			Phosphorus retention			Ca :P ratio	
			Women	Periods	Min'm	Max'm	Mean	Min'm	Max'm	Mean	Intake	Storage
					gm.	gm.	gm.	gm.	gm.	gm.		
Bar	1905	Paris	3	11	5.75	4.30	3.34	—0.29	1.14	0.337	[1]	....
Hoffstrœm	1909	Finland	1	23	1.149	2.862	1.952	—0.150	0.591	0.331	0.88	0.62
Landsberg	1912	Germany	6	8	1.768	1.938	1.859	0.225	0.364	0.303	[1]	....
	1914	"	14	14	2.186	3.102	2.710	0.097	1.274	0.612	0.98	0.71
Coons-Blunt	1930	Chicago	9	23	0.942	2.210	1.431	—0.140	0.529	0.161	0.76	0.62
Macy et al.	1930	Detroit	3	12	1.459	2.966	2.247	—0.162	0.528	0.259	0.87	[2]
Macy Regulated diet	1931	"	1	2	1.76	1.99	1.88	0.17	0.39	0.28	0.92	2.25
Toverud	1931	Norway	29									
Ordinary diet			16	27	0.711	1.976	1.221	—0.611	0.736	0.202	0.84	0.34
Regulated diet			23	30	0.711	2.633	1.678	—0.345	1.136	0.411	0.91	0.81
Weighted Averages												
Ordinary diet			52	118	0.711	4.30	2.099	—0.611	1.274	0.293	[1]	....
Regulated diet			24	32	0.711	2.633	1.690	—0.345	1.136	0.403	0.91	0.88
This study	1931	Oklahoma	6	24	1.050	2.556	1.632	0.025	0.767	0.299	0.86	1.21

[1] Data lacking.

[2] Negative balances.

Phosphorus Metabolism in Oklahoma Women. The retention of phosphorus by Oklahoma women, like that of calcium, showed no negative balances (Table 8). However, the range in the quantities ingested and stored was wide, 1.050 to 2.556 grams of dietary phosphorus, with 0.025 to 0.767 grams retained. The best retentions for each subject tended to coincide with the periods of highest phosphorus intake as well as with the early part of pregnancy. These early high storages were not dependent upon fetal demand at this period, but, along with the high nitrogen and to a less extent good calcium retention, they may be taken to represent an accumulating of maternal reserves in preparation for the later demands of gestation and of lactation. In other words, there seemed to be an impulse to store certain minerals and nitrogen in advance of the demands

TABLE 8. Phosphorus Retention by Southern Women during Pregnancy.
Daily Averages

Subject and period	Week of pregnancy	Phosphorus intake	Phosphorus outgo			Balance
			Urine	Feces	Total	
		gm.	gm.	gm.	gm.	gm.
I-0	— 4	1.226	0.581	0.322	0.903	0.323
I-1	2	1.448	0.631	0.314	0.945	0.503
I-2	27	1.431	0.655	0.449	1.104	0.327
I-3	31	1.374	0.754	0.433	1.187	0.187
I-4	34	1.402	0.704	0.512	1.216	0.186
I-5	38	1.388	0.684	0.454	1.138	0.250
II-1	26	1.986	0.846	0.620	1.466	0.520
II-2	30	2.083	0.801	0.612	1.413	0.670
II-3	34	2.067	1.075	0.675	1.750	0.317
II-4	38	1.754	1.022	0.645	1.667	0.087
III-1	33	1.371	0.879	0.323	1.202	0.169
III-2	37	1.791	0.898	0.522	1.420	0.371
IV-1	31	1.562	1.046	0.491	1.537	0.025
IV-2	35	1.281	0.781	0.340	1.121	0.160
IV-3	39	1.612	0.960	0.304	1.264	0.348
V-1	28	1.182	0.618	0.338	0.956	0.226
V-2	33	1.050	0.473	0.435	0.908	0.142
V-3	38	1.261	0.588	0.430	1.018	0.243
V-4	39	1.271	0.675	0.418	1.093	0.177
VI-1	18	2.556	0.964	0.825	1.789	0.767
VI-2	23	2.345	0.998	0.977	1.975	0.370
VI-3	27	1.528	0.863	0.557	1.420	0.108
VI-4	31	1.827	0.792	0.778	1.570	0.257
VI-5	35	1.844	0.801	0.663	1.464	0.380
VI-6	38	1.753	0.726	0.637	1.363	0.390
Average						
This series*		1.632	0.802	0.531	1.333	0.299
Chicago series		1.431	0.718	0.552	1.270	0.161

*Exclusive of Period I-0.

of the fetus during late gestation. Goss and Schmidt (79) have shown that this impulse may proceed independently of the existence of a developing embryo. The amounts retained in this early period varied largely with the available dietary supply (Cases VI and III) as well as upon the depletion of these reserves at the beginning of gestation (39).

Certain facts suggest, however, that the phosphorus metabolism was less improved by sunlight than was that of calcium. The diets of the Oklahoma women contained, on an average, 12 percent more phosphorus and 28 percent more calcium than those of the Chicago women. The average phosphorus retention in this series showed only 86 percent improvement over the northern group, while the calcium was almost 200 percent better. These women stored 18 percent of the dietary phosphorus, the Chicago group 11 percent, and correspondingly 21 and 9 percent of the dietary calcium.

Calcium:Phosphorus Ratio. The different improvement in phosphorus retention as compared to that of calcium is manifest chiefly in the Ca:P ratio of the amounts stored (Table 9). The diets contained a ratio varying

TABLE 9. Relation of Phosphorus to Calcium and Nitrogen.

Subject-Period	Week of pregnancy	Ca:P ratio		N:P ratio	
		Intake	Retention	Intake	Retention
I-0	— 4	0.59	0.38	6.7	1.5
I-1	2	0.48	0.23	5.5	3.4
I-2	27	0.87	0.59	5.7	3.5
I-3	31	0.87	1.79	7.2	6.0
I-4	34	0.92	1.14	7.0	5.3
I-5	38	0.93	1.31	6.3	7.3
II-1	26	0.90	0.71	5.9	4.7
II-2	30	0.85	0.69	6.9	5.6
II-3	34	0.88	0.86	7.1	9.4
II-4	38	1.09	3.07		
III-1	33	0.67	1.21	8.4	8.3
III-2	37	0.80	1.15	7.2	6.1
IV-1	31	0.65	2.36	7.6	7.2
IV-2	35	0.96	1.38	7.6	8.1
IV-3	39	0.97	1.40	7.2	4.4
V-1	28	0.75	1.39	8.5	7.6
V-2	33	0.77	0.93	7.7	4.9
V-3	38	0.82	1.30	7.9	11.7
V-4	39	0.84	2.13	7.1	6.2
VI-1	18	0.93	0.14	6.9	4.1
VI-2	23	0.89	0.98	8.2	14.4
VI-3	27	0.95	1.40	8.8	13.2
VI-4	31	0.96	0.83	6.7	7.1
VI-5	35	1.01	1.21	6.5	7.1
VI-6	38	0.89	0.80	5.7	2.7
Average					
This series		0.86	1.21	7.1	9.4
Chicago series		0.76	0.62	7.7	13.4

from 0.59 to 1.01 with an average of 0.86; the retention ratios varied from 0.14 to 2.36, with an average of 1.21, proceeding independently of the ratios furnished in the intake. These averages may be contrasted with the ratios cited in Table 7 for the Chicago group, 0.76 in the diet and 0.62 in the storage. In fact the tendency, as shown in that table, was distinctly for an average retention ratio below that for intake. In the present series the reverse was true, with 17 of the 24 individual balances showing a storage ratio well above the intake ratio. Three balances in the Chicago group and 14 in the present series showed retention ratios above 1.00, and the highest retention ratio observed in the former group, 1.35 was paralleled or exceeded in 9 of 24 balances with southern women. The higher ratios for this series resulted from the high calcium and relatively less high phosphorus retentions. Such ratios may or may not be optimal for human pregnancy.

These facts are in accord with the findings reported by Mull and Bill (155, 156) that the serum calcium of the blood of pregnant women exhibited seasonal variation but that serum phosphorus showed no such changes.

The ratio of the Ca:P in the amounts retained during pregnancy tended to follow a fairly definite curve, corresponding to, but much below, that shown by fetal analyses with a tendency to highest ratios near the end of gestation. The data on fetal analyses summarized in Table 1 show, for total composition, a Ca:P ratio rising from 0.80 at the 4th month to 1.27 by the 6th month, 1.58 by the 8th month and finally to 1.62 by the end of prenatal life. The storages reported for this series of women approach these ratios more nearly than any hitherto reported, except Macy's subject on the regulated diet (142).

Interrelations of Phosphorus and Nitrogen. Phosphorus is used for storage along with nitrogen as well as with calcium. Almost nothing is known of the magnitude of gestational need for phosphorus exclusive of those of the fetus. Macy and Hunscher allow 0.5 gram for extra-fetal needs (143). Reserves for lactation are not easily estimated. One means of determining hypothetically the relative amount of phosphorus needed is by calculations based on the ratios of Ca:P and N:P in the tissues acquired during a given period. The Ca:P represents the ratio commonly found in the bones of such an animal at the age in question, and the N:P ratio the relative quantities of these elements in the soft parts of the body. Ratios around 17:1 have been applied to accretions of nitrogen and phosphorus in human tissues (4, 17, 63), and 2:1 has been used as the proximate ratio of Ca:P in the bones of infants (49, 209). These figures are not at variance with those cited above for the fetus, because the content of total, not skeletal phosphorus alone, is considered in the data for composition of the fetus. Doubtless several errors result when the 17:1 and 2:1 ratios are applied to retention during pregnancy because the gains are both maternal and fetal and are proceeding at different rates. The maternal tissues requiring nitrogen and phosphorus resemble in their development the muscle building resulting from the exercise of mature muscles, whereas the nitrogen used for fetal development is appropriated for rapid increase in cell numbers with their nucleoproteins and in all kinds of phosphorus-containing tissues. Since the Ca:P ratio in bones is not constant throughout fetal life, approaching 2:1 as a maximum, and since lower ratios of N:P would be required during stages of rapid development of fetal brain, nerve and parenchymatous tissue, the amount of phosphorus estimated necessary by application of the 17:1 and 2:1 ratios would, in all probability, be too low.

However, keeping in mind the limitations of the procedure, the hypothetical phosphorus balance or deficiency has been computed for the two groups of subjects. In 12 periods too little phosphorus was retained to supply the equivalents for both calcium and nitrogen. A similar deficit occurred in 10 metabolism periods of the Chicago group. In view of the fact that nitrogen retentions by the southern group were also below average (43), the apparent phosphorus deficiency would be augmented. It may be assumed, therefore, that the amounts of phosphorus retained by the Oklahoma group were improved by way of larger calcium retentions, but evidence of a more direct effect of sunlight or vitamin D on phosphorus metabolism is lacking.

SUMMARY OF FINDINGS IN PHOSPHORUS STUDIES.

1. Balances for phosphorus, as for calcium, showed retentions coniderably in excess of fetal needs and revealed no negative retentions.

2. The Ca:P ratios in the diets exceeded 1:1 in two instances and then only slightly, whereas the ratios in the amounts retained varied from 0.23 to 3.07, demonstrating thereby the selective power of the organism in the appropriation of these elements from the diet.

3. In spite of the fairly large amounts of phosphorus stored, the quantities were calculated to be too small to supply equivalents for calcium in the skeleton and for nitrogen in the soft tissues of the growing fetus.

MAGNESIUM

Literature. Kruse, Orent and McCollum (112, 113, 114, 159) have shown conclusively the need of the animal organism for magnesium. Although much of the biochemical literature dealing with this element has been concerned with the effect of feeding excessive amounts and with the "antagonistic" action of the magnesium toward calcium (62, 123, 151, 196), more pertaining to the actual physiological requirements of the human subjects has been reported of late (13, 96, 220, 239).

The studies of McCollum and associates show the effects of marked deficiency of magnesium on the nervous muscular mechanism of the animal as well as in lowering serum magnesium. Hirschfelder (96) has described clinical symptoms in human subjects exhibiting low serum magnesium and Cramer (45) has produced experimental evidence of the degenerative renal damage or nephritic changes produced by synthetic diets very low in magnesium. The latter investigator calls attention to the clinical use of magnesium sulfate in warding off uremic convulsions in children (23). Watchcorn (239) observed that serum magnesium tended to be low during prgenancy.

Relations of magnesium to child nutrition were discussed in the report of the Committee on Growth and Development of the White House Conference on Child Health (240). Peters and Van Slyke (163) and more recently Shohl (196) have presented summaries of the literature on various phases of the biochemistry of this element.

Since the element has been shown to be indispensable to animal nutrition, it would seem that more information is needed concerning factors affecting its utilization, the variations in human requirement under different physiological conditions such as in growth and pregnancy, and more data relating to the quantities present in average and superior dietaries. Furthermore, greater emphasis is being placed on the interrelations of calcium, magnesium and phosphorus in metabolism and on the need for a knowledge of the behavior of each in order to facilitate a more accurate understanding of the metabolism of the other two.

Only four investigators reporting mineral balances during human pregnancy have included magnesium (Table 10). The subjects of all of these showed wide fluctuations in retention in spite of fair agreement

TABLE 10. Summary of Magnesium Balance Studies during Human Pregnancy.
Daily Averages

Investigator	Date	Place	Total No. of obervations		Magnesium intake			Magnesium retention			Ratio Ca :Mg	
			Women	Periods	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Intake	Retention
					gm.	gm.	gm.	gm.	gm.	gm.		
Hoffstrœm	1909	Finland	1	23	0.177	0.395	0.282	—0.176	0.188	0.013	6.1	15.
Landsberg	1914	Germany	14	14	0.338	0.512	0.457	0.002	0.159	0.056	5.9	7.5
Coons-Blunt	1930	Chicago	9	23	0.236	0.810	0.375	—0.311	0.081	—0.085	2.9	....
Toverud	1931	Norway										
Ordinary diet			16	27	0.186	0.688	0.341	—0.047	0.255	0.099	3.0	0.7
Regulated diet			23	30	0.186	0.688	0.368	0.000	0.322	0.132	4.1	2.5
Weighted Averages			53	117	0.177	0.810	0.357	—0.311	0.322	0.041	4.2	5.8
This study	1932	Oklahoma	6	23	0.285	0.471	0.394	—0.003	0.154	0.063	3.6	4.6

as to the quantities ingested. The subjects of Coons and Blunt and of Toverud showed negative balances in 52 and 18 percent of the experimental periods respectively. Toverud's group on the regulated and supplemented diets, particularly those containing additions of cod liver oil and other forms of vitamin D, usually showed improvement in magnesium retentions with no negative balances and a higher average storage. The retention seemed improved simultaneously with better calcium storage and in the absence of increased magnesium intake, thus indicating that certain factors essential to utilization were common to the two, an observation made also by Swanson on infants (220).

Givens and Macy (76) have demonstrated the low requirement of the fetus for magnesium. Their figures show that not over 0.003 gram daily is needed during the last half of gestation. This small amount should be obtainable easily from the mother's food intake. They report approximately 24:1 as the ratio of the Ca:Mg content of the average mature fetus. However, the Ca:Mg ratio in breast milk is approximately 7:1 (146) and if these elements are stored during gestation in preparation for lactation, then the maternal organism needs magnesium in a greater proportion and larger total amount than the figures for fetal composition would indicate.

Magnesium Retentions by Oklahoma Women. Table 11 shows the individual balances for the women of this study and also indicates the Ca:Mg ratios of the intake and storage. Data are lacking for Periods I-0 and I-1 because material was not saved from these for analysis.

The most noteworthy fact about the magnesium retentions was that all balances were positive except one which amounted practically to equilibrium. These positive balances are in contrast to the 12 negative out of 23 total for the Chicago group for which the technique of collection and analysis was identical (38, 41).

Storage in the present series varied from 0.010 to 0.154 gram daily with the exception of the negative balance noted above. The average storage was 0.063 gram daily, which was well above the —0.085 average storage by the Chicago group. It was much less than that shown by the Norway women on the supplemented diets, but was ample for the 0.003 gram daily calculated necessary for fetal development during the last five months.

The amount of magnesium retained seemed unrelated to any stage of gestation because high and low balances occurred in succession and in cycles in the same woman. Good magnesium retention tended to parallel good calcium storage, thus demonstrating that the two are not always antagonistic and that magnesium was not being substituted for calcium in a deficiency of the latter.

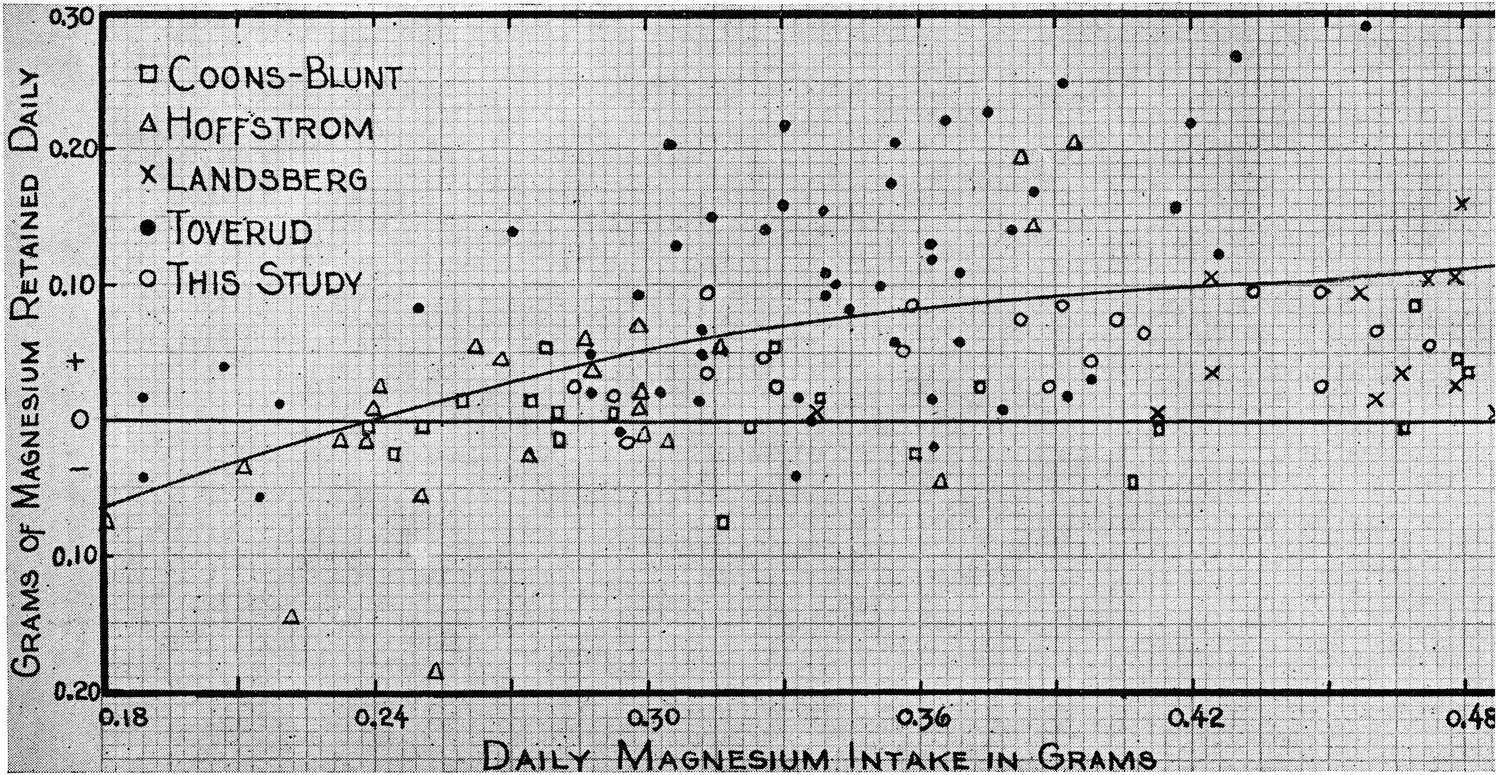
The intakes ranged from 0.285 to 0.523 with an average of 0.394 gram daily. This was very close to the average magnesium intake found for groups studied previously (Table 10), hence the better storage is not to be explained on this basis. However, better storage occurred on the higher magnesium intakes reported for this group of women. Retentions on intakes below 0.3 gram seldom approached the average, while intakes of 0.5 gram or over seemed abundant under the conditions of this experiment. Figure V shows the retention of magnesium in relation to intake. The data included are for the present series as well as all similar figures from balance experiments reported in the literature. The composite curve as well as the scatter diagram shows low or negative balances when the intake was below 0.30 gram of magnesium daily, but there was little improvement in retention from intake above 0.45 gram. It is evident from this chart that the intake of magnesium is one of the factors controlling its retention. Macy and Hunscher (143) estimate that 0.30 gram daily can be regarded a minimum requirement for pregnancy, but this standard seems low in comparison with the relatively higher standards which they propose for calcium and phosphorus and in the light of the excellent metabolic performance on the higher magnesium intake in this group.

An average of 16 percent of the intake was retained, although individual cases retained as much as 30 percent of the quantity ingested. Hoffstrœm's woman retained an average of 17 percent of the intake during the last 23 weeks of pregnancy, Landsberg's group 12 percent, while Toverud's group on the diets improved with additions of calcium, phosphorus or vitamins, but not magnesium, stored an average of 36 percent of their magnesium intake. From Table 10 it will be seen that this superior storage was not due to the higher average intake.

TABLE 11. Magnesium Retention by Oklahoma Women During Pregnancy.
Daily Averages

Subject-Period	Week of pregnancy	Mg intake	Mg Outgo			Mg balance	Ratio — Ca :Mg	
			Urine	Feces	Total		Intake	Storage
		gm.	gm.	gm.	gm.	gm.		
I-2	27	0.327	0.096	0.206	0.302	0.025	3.8	7.7
I-3	31	0.359	0.089	0.188	0.277	0.082	3.3	4.1
I-4	34	0.294	0.102	0.195	0.297	—0.003	4.4	.....
I-5	38	0.315	0.111	0.169	0.280	0.035	3.9	9.4
II-1	26	0.446	0.103	0.246	0.346	0.100	4.0	3.7
II-2	30	0.510	0.104	0.252	0.356	0.154	3.5	3.0
II-3	34	0.521	0.135	0.343	0.478	0.043	3.5	6.3
II-4	38	0.523	0.093	0.279	0.372	0.151	3.7	1.8
III-1	33	0.285	0.121	0.141	0.262	0.023	3.2	8.9
III-2	37	0.395	0.155	0.159	0.314	0.081	3.6	5.2
IV-1	31	0.446	0.124	0.294	0.418	0.028	2.3	2.1
IV-2	35	0.385	0.129	0.226	0.355	0.030	3.2	7.3
IV-3	39	0.435	0.123	0.214	0.337	0.099	3.6	4.9
V-1	28	0.326	0.079	0.162	0.241	0.092	2.7	3.4
V-2	33	0.291	0.064	0.217	0.281	0.010	2.8	1.3
V-3	38	0.381	0.057	0.248	0.305	0.076	2.7	4.1
V-4	39	0.352	0.081	0.220	0.301	0.051	3.0	7.4
VI-1	18	0.471	0.101	0.320	0.421	0.051	5.0	2.2
VI-2	23	0.462	0.091	0.301	0.392	0.070	4.4	5.2
VI-3	27	0.325	0.091	0.184	0.275	0.050	4.4	3.0
VI-4	31	0.399	0.073	0.283	0.356	0.043	4.4	4.9
VI-5	35	0.405	0.098	0.230	0.328	0.077	4.6	6.0
VI-6	38	0.409	0.050	0.291	0.341	0.068	3.8	4.6
Average		0.394	0.099	0.233	0.332	0.063	3.6	4.6

ig. V.—Compilation of published data on the retention of magnesium during human pregnancy showing relation of storage to in



Definite individual tendencies were exhibited by the women of this study with regard to the paths of excretion. Urinary magnesium exceeded 0.13 gram daily in only one instance, while Cases V and VI excreted habitually less than 0.1 gram daily by way of urine. Shohl estimated (196) that approximately one-fifth to one-half of the magnesium salts were excreted daily by way of the kidney. An average of approximately one-fourth was excreted through urine by these women as compared to an average of one-third in the urine of the Chicago group. In that series the urine rarely contained less than 0.1 gram per 24 hours, while the average was 0.126 compared to an average of 0.099 for the present group. Thus, there was a reduction of urinary magnesium accompanying general good storage.

Only Case III of the Oklahoma series showed fairly equal distribution between the two paths of excretion studied. She had been ingesting considerable quantities of $NaHCO_3$ daily and excreted a very alkaline urine. Hence, the high urinary magnesium found was not needed for acid-base equilibrium in the urine. The urinary calcium of this subject was also high. The high urinary phosphorus was expected to accompany the ingestion of alkali. It will be noted that relatively high urinary excretions of all minerals studied was characteristic of Cases III and IV (Table 20) (Figure VII).

The ratio of Ca:Mg retained was quite independent of the ratios in the intake or of the stage of gestation. The retention ratio was variable from woman to woman and from period to period in the same subject. The ratio stored tended to be above that in the intake, both for individuals and for the average, which was 4.6:1 stored as compared to 3.6:1 ingested. This would indicate relatively less metabolic demand for the quantities of magnesium supplied by the diets than for the calcium.

SUMMARY OF FINDINGS CONCERNING MAGNESIUM METABOLISM

1. Evidences of exerimental and clinical deficiencies of magnesium in metabolism have been described in the literature, and a new significance is thereby attached to the question of what constitutes an adequacy of this element in the diet, particularly in that of the pregnant woman.

2. The magnesium balances were all positive except one which amounted to equilibrium. The storage rates were higher and more regular than those reported for any other studies during pregnancy, except those of Toverud.

3. Storage tended to be related to the intake of magnesium, and 0.35 to 0.45 gram daily seemed to represent a safe range for dietary magnesium in this and in most of the studies reported.

SULFUR

Literature. Peters and Van Slyke (163) have pointed out that the present information on sulfur metabolism is very incomplete and as yet somewhat unrelated. Not enough studies showing the clinical significance of variations in the diet and in metabolism are available for formulating even tentative conclusions as to its complete role in human nutrition. The physiological and chemical aspects of the subject have been reviewed by Lewis (127, 128). A number of established facts as well as more recent unconfirmed findings serve to stimulate further interest in the investigation of the part played by sulfur in human metabolism.

Among other facts are those relating to the existence and indispensability of certain sulfur-containing amino acids, especially cystine (126), and the metabolic significance now attached to glutathione, methionine and ergothionine (101, 128). Variations in the partition of urinary sulfur between inorganic sulfate, ethereal or conjugated sulfate and "neutral" sulfur under different physiological and dietary conditions have long been studied as one avenue of approach to the subject (69, 245, 246).

Smith in his review (205) has given particular attention to the possible influence of solar rays on sulfur metabolism and refers to the abnormal metabolism of sulfur in pellagra noted by some investigators (109, 216). He suggests that an inadequate supply of the sulfur-containing amino acid, cystine, may, by rendering the skin hypersensitive to solar radiation, play a part in the production of pellagra. More recently Payne and Perlzweig (162) reported markedly reduced cystine content of the fingernails of 14 pellagrins showing severe dermatitis. Lewis (127) referred to the selective absorption of ultraviolet rays by cystine, as noted by Ward (238), and called attention to the high sulfur content of melanins, 3 to 4 percent for one of these pigments as compared to 5.7 percent for human hair and 2.2 for tortoise shell (163). However, very recently, Lewis failed to find abnormal variations in the sulfur content of the skin of pellagrins (125), and questions the general applicability of the above findings. Smith also suggested that it was of interest to note that the cystine content of the foods advocated by Goldberger for preventing pellagra was roughly comparable to the prevention value of these foods. The same observation, however, could be applied to a number of other factors in these foodstuffs.

Abderhalden and coworkers noted a weak or negative nitroprusside reaction for glutathione in the tissues of pigeons suffering from vitamin B deficiency, although the cystine content of the tissues was normal (1, 127). Sherman and Smith (195) have summarized the conflicting evidence on this point and concluded that vitamin B and cystine are not interrelated in the process of oxidation in the tissues.

Very recently vitamin B_2 or G, which plays a part in the prevention of pellagra, has been identified as a lacto-flavine (25, 88, 115) and contains nitrogen and possibly sulfur. Harris (88), commenting on the easy destruction of flavins by visible light, refers to the practice of Hogan and coworkers of irradiating the ration to produce pellagra-like lesions in rats. Correlating these facts with the seasonal incidence of pellagra, he suggests that the flavine may be largely destroyed either in the food or in the subject by the increased exposure to light. It is true (9, 36, 205) that pellagrins do not manifest the typical skin lesions on the deficient diet of the winter months until after exposure to the more potent sunlight of April and May, and that such lesions are improved by protection from sunlight.

Laurens (121) pointed out that animals on a vitamin B (complex) deficient diet succumbed readily to the effects of irradiation while those without the light treatments continued to survive. Light et al. (129, 130) reported pellagra-like symptoms in third and fourth generation rats receiving a moderate overdosage of vitamin D and the alleviation of the effects of overdosage by higher vitamin B intake. These relations were noted as early as 1923 by Hopkins (100) and have also been referred to by Harris (89). Lowered glutathione content of red blood cells has been observed following increased vitamin D administration (92) and decreased glutathione content of tissues followed dietary deficiencies of cystine (147).

As implied in the beginning, these various findings suggest possible interrelations between vitamin G deficiency, solar radiation, sulfur metabolism, and pellagra. At least they are not unrelated to the subject in hand, namely, the metabolism of sulfur in southern women during pregnancy. As will be shown below, the sulfur retention was found to be low in all cases and even negative in over half of them. Although more studies on such women are needed before an explanation can be assigned, the findings are in striking contrast to those for other minerals, particularly calcium, magnesium and phosphorus. The results for sulfur were unexpected findings in the course of complete mineral analysis of intake and output for the determination of the acid-base balance.

Sulfur retention during pregnancy has been measured by only two other workers. Landsberg (117) obtained 8 balances on 6 women. The range of daily intakes and excretions with means were as follows: food, 1.599 to 1.804 grams, average 1.701; urine, 1.226 to 1.544 grams, average 1.370; feces, 0.099 to 0.168 gram, average 0.133; and retentions, 0.120 to 0.286 gram, average 0.198. His data showed that not one negative balance occurred. The diets consumed by his women were high in good quality protein, approximately 100 grams in 2400 calories, coming chiefly from 1 egg, 1500 grams of milk, 100 grams of beef, 60 grams of pork, with vegetables and fruits lacking. Accordingly, the diets, compared to other dietaries, were high in total sulfur, as will be seen below.

Sulfur output in the urine and feces during pregnancy was studied in detail by Hoffstrœm (97) for his one case, but since only the average sulfur content of the diet for the period was estimated, it is not possible to say how many negative balances occurred or whether there were wide fluctuations in the quantities retained. The average daily intake was 1.101, the retention 0.086 gram. Urinary sulfur ranged from 0.635 to 1.109, averaging 0.848 gram; fecal sulfur from 0.104 to 0.261, averaging 0.167 gram daily. Thus, both intake and retention were much below those for Landsberg's cases, although the amount in the feces was comparable, being slightly higher in Hoffstrœm's case.

Hoffstrœm estimated the total needs of gestation, including placenta, amniotic fluid, fetus and adnexa, for sulfur as 1.181 grams with 0.878 for the fetus alone. The figures for fetal sulfur were taken from Camerer and Sœldner (32, 208). The total requirement of pregnancy would call for a retention of about 0.004 gram daily throughout the period. On the other hand, if it be assumed that 14.5:1 is the average N:S ratio for tissue protein (245, 246) and 72.2 grams the nitrogen content of the mature human fetus (152), then approximately 5 grams total or 0.02 gram of sulfur daily throughout gestation would be required for soft tissues alone. Some small additional quantities would be needed for bony structure and hypertrophy of maternal muscles.

However, the retention by Landsberg's women was much in excess of either of these hypothetical requirements and there was no indication of deficient storage by Hoffstrœm's case.

This Series of Sulfur Balances. The balances for total sulfur in this series of Oklahoma women are shown in Table 12, together with the N:S ratios of the intake and output and the quantities of urinary sulfur per kilogram of body weight. Averages are also included from Landsberg's and Hoffstrœm's cases cited above. Sulfur balances for Case III were not determined because the collections could not be used for acid-base balance studies. Unweighed portions of sodium bicarbonate were ingested daily, thus rendering impossible a knowledge of the sodium intake. Analyses for sodium, potassium and chlorine, as well as sulfur, were omitted.

At once evident from the table is the frequent negative retention of sulfur, occurring in 12 out of the 20 balances. The magnitude of both positive and negative balances was small, less than 10 percent of the intake except in one instance. Nine of the 20 balances amounted to 3 percent or less of the intake and hence represented practical equilibrium, since such small percentages are within the range of experimental error for an intake-outgo study. The average retention amounted to 0.004, which, although comparable to the gestational demands computed by Hoffstrœm, was much below the actual retention reported for either Hoffstrœm's or Landsberg's cases, 0.086 and 0.198 gram daily respectively.

Some of this lower retention in the present series must be attributed to the lower intake of total sulfur. In every period save one (VI-1), the intake was less than the average for Hoffstrœm's case, and all were far less than that for any one period reported by Landsberg. The average intake was below that estimated for the case reported by Sandiford and coworkers (182), as was also the urinary sulfur. Fecal sulfur and sulfur storage were not measured in that case. On the other hand, data for total sulfur tell nothing of the nutritional quality of the sulfur contained in the diets.

Positive retentions of this element were found only for Cases I, II and VI. Best retentions were recorded for the last two whose diets were not only higher in sulfur but supplied larger proportions of milk and eggs (Table 27), in addition to being superior in minerals and vitamins, particularly vitamin B. On the other hand the highest retention for a given individual did not always occur in the period of her series character-

TABLE 12. Sulphur Retention During Pregnancy.
Daily Averages

Case-Period	Week of pregnancy	S intake	Sulfur Outgo			S Balance	N:S Ratio			Urinary S. per kilo body wt.
			Urine	Feces	Total		Intake	Urine	Feces	
		gm.	gm.	gm.	gm.	gm.				gm.
I-2	27	0.569	0.461	0.096	0.557	0.012	17	16	11	0.08
I-3	31	0.654	0.552	0.085	0.637	0.017	15	14	12	0.09
I-4	34	0.588	0.544	0.093	0.637	—0.049	17	14	11	0.09
I-5	38	0.605	0.550	0.086	0.636	—0.031	14	11	11	0.09
II-1	26	0.862	0.626	0.118	0.744	0.118	14	13	11	0.12
II-2	30	0.844	0.682	0.116	0.798	0.046	17	13	12	0.12
II-3	34	0.835	0.678	0.125	0.803	0.032	17	15	13	0.11
IV-1	31	0.729	0.672	0.105	0.777	—0.048	16	14	12	0.08
IV-2	35	0.642	0.594	0.100	0.694	—0.052	15	12	13	0.07
IV-3	39	0.737	0.626	0.120	0.746	—0.009	16	14	12	0.07
V-1	28	0.627	0.579	0.078	0.657	—0.030	16	13	13	0.09
V-2	33	0.596	0.514	0.100	0.614	—0.018	14	12	13	0.08
V-3	38	0.636	0.542	0.111	0.653	—0.017	16	11	12	0.08
V-4	39	0.550	0.460	0.094	0.554	—0.004	16	14	13	0.07
VI-1	18	1.355	1.123	0.260	1.383	—0.028	13	11	8	0.20
VI-2	23	0.944	0.754	0.146	0.900	0.044	20	16	13	0.13
VI-3	27	0.813	0.699	0.119	0.818	—0.005	17	15	13	0.11
VI-4	31	0.969	0.643	0.277	0.920	0.049	12	13	7	0.10
VI-5	35	0.773	0.551	0.148	0.699	0.074	16	14	11	0.08
VI-6	38	0.799	0.678	0.157	0.835	—0.036	12	11	10	0.10
Average		0.757	0.626	0.127	0.753	0.004	16	13	12	0.09
Hoffstrœm		1.101	0.848	0.167	1.015	0.086	11	12	5	....
Landsberg		1.701	1.370	0.133	1.503	0.198	10	9	7	....

ized by highest intake (I-4, VI-1 and VI-5). Doubtless diets containing considerable quantities of fruits and vegetables, as in Case IV, supplied much of their sulfur in the form of inorganic sulfate, thereby affording proportionately less of the biologically valuable protein-bound sulfur. All diets were more or less restricted in meat proteins, that of Case II probably least so. Retentions for total nitrogen were also low (43), as will be shown later (Table 23).

Table 12 shows that the N:S ratio of the diets varied only from 12 to 20, averaging 16, which was, however, higher than for previously reported studies, hence was indicative of less protein-bound sulfur in the diets of the Oklahoma group.

The urinary sulfur was correspondingly low in this group while total fecal sulfur compared closely with that reported by either of the early investigators, especially that by Landsberg. The N:S ratio in the urine ranged from 11 to 16, averaging 13. Ratios of 13 to 16 constitute a normal range (127), and may represent catabolism of tissue proteins as in fasting. From Table 12 it will be seen that ratios of 11 and 12 in the urine were invariably associated with negative sulfur balances, although frequently with good nitrogen retention (Periods I-5, V-3 and VI-1) (Table 23), and ratios of 15 and 16 in three of four instances paralleled sulfur retention (Periods I-2, II-3 and VI-2). The fact that N:S ratios in the urine and feces were always lower than those in food (except VI-4) shows a proportionally higher utilization or retention of the nitrogen of the diet than of the sulfur. Since the nitrogen retentions were below average for such individuals, the deficiency in sulfur utilization is further exaggerated. More data are needed to determine what constitutes an adequate intake for normal adults.

The N:S ratio in the feces varied only from 11 to 13, except in three periods for Case VI. In Period VI-1 the high intake of sulfur was doubtless responsible for more fecal sulfur. The large amount lost by way of feces in Periods VI-4 and VI-6 were traceable to frequent stools and diarrheal tendencies (See discussion under Iron).

Aside from the large number of negative balances, the most interesting part of the data in Table 12 is the correlation of urinary sulfur with the body weight as shown in the last column. Acting on the assumption that the negative balances were due largely to the lower sulfur intakes, it was desirable to know whether the amounts of urinary sulfur per kilogram of body weight would show any consistent tendency and hence possibly represent wear and tear quota, or minimum maintenance requirements. The pregravid body weight has been used for the computations, although weights more representative of the mass of active tissue would have increased the accuracy of the ratios.

It will be noted that when the urinary sulfur was as low as 0.07 gram per kilogram of body weight, negative balances always occurred, even though these were not of similar magnitude in every case. Negative balances also tended to be the rule and were larger when the amount was as low as 0.08 to 0.09 gram per kilogram of body weight. These quantities probably represent just less than or very near to the wear and tear quota, since as much as 0.11 to 0.12 gram per kilogram of body weight seemed ample to permit storage (Cases II and VI). This tentative conclusion seems further justified in view of the disconcerting effects possible as a result of the varied character of the diets affording different qualitative sulfur contents and the variations in the anatomical build and body composition and hence metabolic intensity represented by the weights used for reference.

Whether or not more adequate sulfur intake would satisfactorily improve the sulfur metabolism of such subjects awaits further study. It is unique that the retention of this element was so deficient as compared to the excellency of that for other minerals. It is of further interest that most of the negative balances occurred in the spring of the year (Table 5). In view of the above findings and those cited from the literature implying probable interrelations of sulfur metabolism, irradiation and certain dietary factors, sulfur metabolism in human subjects seems worthy of more intensive investigations, experimentally as well as clinically.

SUMMARY OF OBSERVATIONS CONCERNING SULFUR METABOLISM

1. An unusual series of sulfur balances, of which over one-half of the number were negative, stimulates interest in the metabolism of this element and makes more studies imperative before full interpretation of the results reported here is possible.

2. Low sulfur intakes, averaging 0.757 gram daily, resulted in balances averaging only 0.004 gram per day.

3. The amount of urinary sulfur per kilo of body weight as correlated with the negative balances was a fairly constant quantity and suggested that the women were receiving approximately a minimum maintenance quota of this element.

4. The results of this series, supported by fragmentary evidence in the literature, implies probable interrelations between sulfur metabolism, irradiation or exposure to sunlight, and certain dietary influences. These have been discussed somewhat at length.

CHLORINE

Literature. Blood chlorides have been studied under numerous clinical conditions, including pregnancy, and these are fully discussed by Peters and Van Slyke (163). So far as the writer is aware, no balance experiments on chlorides in pregnancy have been reported. The chief aim for including chloride studies in this series was the measurement of the acid-base balance. Some irregularities were noted in certain phases of the chloride metabolism and these will be referred to here.

The Nutrition Committee of the Medical Section of the White House Conference (240) estimated the fetal demands for this element from reported analyses of human fetuses, as summarized by Czerny and Keller (47). The content of chlorine seemed to diminsh with age, 2.54 grams per kilo at 7 months as compared to 1.70 grams per kilo, approximately 5 grams total, at maturity. It was pointed out that the chlorine content of the fetus was about 0.25 percent; of the newborn, about 0.18 percent; and of the adult, 0.12 percent. Iob and Swanson (107) discussed these changes as manifest in the fetuses analyzed by them. The explanation offered by both groups of writers was that chlorine occurs chiefly in body fluids, and that the water content decreases with age. This growth change explains the slow increment of chlorine in the total fetus during the last months of pregnancy, as was apparent from the summary of analyses in Table 1. This would suggest relatively less need for chlorine in the later months of pregnancy, more in the early months.

Retention of Chlorides in Pregnancy. The intake, outgo, and retention of chlorine found for the 20 balances of this study are shown in Table 13. It will be observed that the metabolism of this element showed wide variations. There were three negative balances, but in instances of high retentions the amount was almost ⅓ of the intake (Period V-3) and was equivalent to 30 percent of the fetal content. Good retention was not dependent upon high intake (Cases V and VI). The intakes varied from

3.73 grams to 9.676, with a mean of 5.822 grams daily. The retentions ranged from —0.236 to 2.170, with a mean of 0.877 gram daily. From the table it will be seen that the negative balances occurred on intakes not greatly different from those permitting good retention in the same individual (Periods I-2 and I-5; also VI-4 and VI-6). Consecutive periods, V-3 and V-4, showed at one time the highest retention recorded for chlorine and in the subsequent period one of the lowest positive balances found. Hence the stage of pregnancy did not explain the irregular rates of retention.

In the instance of the consecutive periods cited above, the fecal chlorides remained practically the same, but the urinary output in the last period increased 27 percent in spite of almost 10 percent decrease in intake. The unaccounted water balance (See Table 17) in Period V-3 was higher than in Period V-4, while the urine volume was larger in the last period. The differences in retention during the two periods was probably, therefore, a matter of an accumulating slight edema in the third period, with a disappearance of the same in the fourth period, and of the resulting greater chloride excretion which accompanied the increased urine output.

Similar explanation can be given for the negative balance in Period VI-6. A visible slight edema in the extremities existed at the beginning

TABLE 13. Retention of Chlorine During Pregnancy.

Daily Averages

Case-Period	Week of pregnancy	Cl intake	Cl outgo			Cl balance
			Urine	Feces	Total	
		gm.	gm.	gm.	gm.	gm.
I-2	27	3.995	4.125	0.106	4.231	—0.236
I-3	31	3.739	3.178	0.106	3.284	0.280
I-4	34	4.224	3.330	0.111	3.441	0.783
I-5	38	3.960	3.252	0.171	3.423	0.537
II-1	26	4.849	4.788	0.076	4.864	—0.015
II-2	30	5.448	4.679	0.120	4.799	0.649
II-3	34	5.607	3.744	0.064	3.808	1.799
IV-1	31	6.568	4.740	0.078	4.819	1.749
IV-2	35	6.301	4.514	0.064	4.578	1.723
IV-3	39	9.676	8.156	0.271	8.427	1.249
V-1	28	6.493	5.751	0.043	5.794	0.699
V-2	33	5.030	4.089	0.071	4.160	0.870
V-3	38	6.752	4.516	0.066	4.582	2.170
V-4	39	6.122	5.737	0.065	5.802	0.320
VI-1	18	8.257	7.220	0.207	7.427	0.830
VI-2	23	6.854	6.011	0.251	6.262	0.592
VI-3	27	6.075	4.315	0.197	4.512	1.563
VI-4	31	5.764	4.093	0.335	4.428	1.336
VI-5	35	5.128	4.082	0.230	4.312	0.816
VI-6	38	5.588	5.617	0.132	5.749	—0.161
Average		5.822	4.797	0.138	4.935	0.877

of the last period and the disappearance of it before the end of the experiment was coincident with an increased fluid output as well as with a chloride excretion in excess of that in the intake. Since various degrees of water retention are possible in such individuals, doubtless many of the fluctuations in chlorine balances find explanation in some such abnormal behavior of metabolism, namely, slight dehydration or water retention for which there were no outward signs.

Although the amount of fecal chlorine failed to have any appreciable effect on the final balance when moderate retention occurred, the quantities lost by this path were larger when more frequent stools occurred (Periods VI-4 and IV-3).

High retention of chlorides might be apparent from the balance figures when abnormal sweating caused appreciable undetermined losses of this element through the skin (221, 222). However, the negative balances occurred in October and July and during a time when environmental temperatures were above normal for the season. The high storages found for Case IV occurred in March and April, while that of Period V-3 was found in May during inclement weather with temperatures not unlike those for Period V-4. That of Period II-3 occurred during late December (Table 5). Hence losses through sweat do not seem to account for the occasional high storage. Reference to this question is made again in the section on acid-base balance.

SUMMARY ON CHLORINE METABOLISM

1. The balances for chloride intake and outgo during pregnancy were variable and irregular and unrelated to diet or stage of pregnancy. Three negative balances occurred, while some positive ones amounted to more than one-fourth of the intake or of the total fetal content.

2. Fetal demand seemed to affect the retention to only a minor extent, while irregularities in hydration and urinary sodium chloride excretion are believed responsible for most of the variations in storage.

SODIUM AND POTASSIUM

Literature. The chief aim in determining sodium and potassium balances in this series was, as for chlorine, the measurement of the acid-base balance of mineral retained. No balance experiments during pregnancy have been concerned with these two elements. Shohl, in a recent review (197), has emphasized the possible physiological interrelations of these elements with others more frequently studied and shows the need for a knowledge of mineral metabolism as a whole in order to permit correct interpretation of the metabolic exchanges of any one of them.

The White House Conference Sub-Committee on Nutrition (240) estimated the K_2O content of the newborn as 1.98 grams per kilo of body weight, equivalent to approximately 4.89 grams of potassium in the mature fetus. The Na_2O content was estimated as 2.12 grams per kilo of body substance, or approximately 4.73 grams of sodium in the newborn. These are in accord with the average figures presented in Table 1, which includes more recently published data.

Actual retentions by growing children as well as accretions computed from body composition have indicated retentions of 0.025 gram daily for sodium and of 0.020 gram daily for potassium (198, 240). Sodium, like chlorine, is found chiefly in body fluids, potassium in the tissues.

Peters and Van Slyke (163) have discussed the upper and lower limits of intake of these elements by man, as well as the paths of excretion. They also point out that the greater part of the 3 to 6 grams of sodium in the ordinary diet daily comes from added NaCl, while the 2 to 4 grams of potassium comes from the various organic and inorganic potassium salts of the animal and vegetable tissues employed as food.

Retentions of Sodium and Potassium during Pregnancy. The 20 balances for both sodium and potassium are presented in Table 14. The Na:K ratios in intake, urine and storages are also given. There was only one negative balance for these two elements. Period VI-6 revealed sodium loss along with the negative chlorine balance noted above, the two occurring in almost equivalent quantities following a condition of slight edema.

Except in three of the 20 balances the sodium retentions were fairly constant, from 1 to 2 grams daily, but were irregular with reference to any stage of pregnancy. Low retentions appeared at the end as well as at the beginning of gestation (Cases V and VI). The average storage was 1.265 grams daily with an average intake of 4.907 grams.

The amounts of potassium retained were more variable, ranging from 0.163 to 1.004, with a mean of 0.508 gram daily. The wide variations in potassium retention suggest that the unaccounted losses of this element through sweat may have been responsible for the apparently high figures for retention (163), since Swanson and Iob (221) have reported for infants that the greatest error in balance experiments from losses through sweat were found for potassium. Doubtless such losses have introduced some error for most of the elements studied in this series (68, 222, 223, 224). However, the highest retention of potassium occurred in November (Case II-2) and probably cannot be explained in this way. The high positive balance for Period VI-5 occurred in June and hence may be less accurate than others because of lack of data for losses through perspiration. The three lowest potassium balances (Periods I-3, V-2 and VI-3) were each associated with lowered potassium intakes. The storage of both of these elements, like that for chlorine, seemed ample for fetal needs. Doubtless some of both the sodium and potassium retained represented temporary retention in maternal tissues rather than deposition in a developing fetus.

The ratio of Na:K stored was fairly constant, approximately 2.5 for Cases IV and V, but quite irregular in other subjects. The ratio in the urine was below 1.66, usually considered normal, in all but five of the periods and was below 1.0 in six instances. The ratios in the urine tended to parallel those in the intake and suggested, therefore, that the higher proportion of potassium in urine had its origin in food rather than in a condition of acidosis requiring an increased elimination of the alkali. The ratios of the amounts stored were with two exceptions above those of the intake, suggesting either a selective demand for sodium or a greater fluctuation of the sodium content of the body than of potassium due to hydration and dehydration processes. The storage rates for sodium and potassium were not parallel to those of the alkaline earths, calcium and magnesium, except that occasionally high retentions of potassium and calcium were coincident (Cases II, IV and VI).

The intakes of sodium ranged from 3.622 to 7.578, averaging 4.907 grams daily. This was only slightly above the usual 3 to 6 gram intake cited by Peters and Van Slyke (163). The potassium intakes ranged from 2.296 to 4.470, averaging 3.630 grams daily, likewise comparing favorably with the 2 to 4 grams range named by these authors. These facts further indicate that the diets of these women were not unusual in quantity, nor in the quality affording the proportionate parts of these elements.

TABLE 14. Retention of Sodium and Potassium by Women during Pregnancy.

Daily Averages

Case-Period	Week of pregnancy	Na intake	Na outgo			Na balance	K intake	K outgo			K balance	Ratio — Na:K		
			Urine	Feces	Total			Urine	Feces	Total		Intake	Urine	Stored
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.			
I-2	27	3.859	2.281	0.240	2.521	1.338	3.533	2.890	0.376	3.266	0.267	1.1	0.8	5.1
I-3	31	4.029	2.042	0.280	2.322	1.707	3.112	2.664	0.285	2.949	0.163	1.3	0.8	10.6
I-4	34	3.622	2.176	2.292	2.468	1.154	3.502	2.834	0.308	3.142	0.360	1.0	0.8	3.2
I-5	38	3.986	2.713	0.295	3.008	0.978	3.293	2.461	0.327	2.788	0.505	1.2	1.1	2.0
II-1	26	4.537	2.939	0.253	3.192	1.345	4.470	3.461	0.299	3.760	0.710	1.0	0.8	1.9
II-2	30	4.746	2.939	0.295	3.234	1.512	4.335	2.999	0.332	3.331	1.004	1.1	1.0	1.5
II-3	34	5.230	3.140	0.221	3.361	1.869	4.350	3.558	0.310	3.868	0.482	1.2	0.9	3.9
IV-1	31	5.096	3.786	0.210	3.996	1.100	3.563	2.491	0.617	3.108	0.455	1.4	1.5	2.4
IV-2	35	5.434	3.620	0.262	3.882	1.552	4.100	3.015	0.429	3.444	0.656	1.3	1.2	2.4
IV-3	39	7.266	4.934	0.563	5.497	1.769	4.411	3.214	0.519	3.733	0.678	1.6	1.5	2.6
V-1	28	5.360	4.275	0.082	4.357	1.003	3.160	2.540	0.234	2.774	0.386	1.7	1.7	2.6
V-2	33	3.977	3.298	0.131	3.429	0.548	2.296	1.656	0.391	2.047	0.249	1.7	2.0	2.2
V-3	38	5.084	3.103	0.170	3.273	1.811	3.366	2.167	0.445	2.612	0.754	1.5	1.4	2.4
V-4	39	4.609	3.802	0.172	3.974	0.636	3.167	2.172	0.366	2.538	0.629	1.5	1.8	1.0
VI-1	18	7.578	5.490	0.435	5.925	1.653	3.927	2.954	0.639	3.593	0.334	1.9	1.9	4.9
VI-2	23	5.285	4.008	0.317	4.325	0.960	4.250	3.262	0.564	3.826	0.424	1.2	1.2	2.2
VI-3	27	4.795	2.992	0.292	3.284	1.736	2.911	2.306	0.439	2.745	0.166	1.6	1.3	9.4
VI-4	31	5.054	2.684	0.581	3.265	1.789	3.869	2.527	0.708	3.235	0.634	1.3	1.1	2.8
VI-5	35	4.273	2.632	0.469	3.101	1.172	3.708	2.305	0.479	2.784	0.924	1.2	1.1	1.2
VI-6	38	4.313	4.104	0.344	4.448	—0.135	3.370	2.491	0.397	2.888	0.482	1.3	1.6	*
Average		4.907	3.348	0.294	3.642	1.265	3.630	2.698	0.424	3.122	0.508	1.3	1.3	3.4

*Negative balance.

SUMMARY OF FINDINGS CONCERNING SODIUM AND POTASSIUM

1. The sodium and potassium balances were all positive save one. The amounts retained, like those for chlorine, were far in excess of fetal needs and were unrelated to the progress of gestation.

2. It is doubtful whether losses through sweating introduced appreciable variations in the results. Such errors as existed were probably fairly constant from period to period.

3. In general the retentions were fairly constant as were also the Na:K ratios in the diets chosen and in the urine excreted.

ACID-BASE BALANCE

Significance. A number of factors have prompted the desire to measure the acid-base balance in the metabolic periods of this study, and it was to this end that the various analyses for intake and outgo of sodium, potassium, chlorine and sulfur were undertaken in addition to those for calcium, magnesium, and phosphorus which constituted the primary aim of the project.

It was important to know whether the acid-base balance of the intake would afford an explanation for the excellent calcium retention observed from the beginning with each subject. It was also desirable to characterize definitely in respect to acid-base content as well as in many other phases these apparently usual diets on which such good metabolic performance occurred.

It was noteworthy, also, that no data existed on which to base calculation of the total amounts of acid- and base-forming elements needed by the human mother at different stages of gestation. As has been indicated by the results in the preceding sections, fetal requirements alone do not constitute the total nutritional needs of gestation. Rowe and his associates (178) have studied acid elimination by pregnant women. The clinical phases of acid-base equilibrium as revealed by blood chemistry studies, and of acidosis during both normal and abnormal states of gestation, have received considerable attention at the hands of investigators (163, 198).

With the exception of Landsberg's women (116, 117) on liberal diets and possibly Hoffstrœm's one case (97) for whom occasional balances were also negative, none of the balance experiments previously reported for pregnancy (41, 144, 231) have been with women qualifying as "normal" in their mineral metabolism, particularly calcium and phosphorus. They were, therefore, not suitable subjects for acid-base balance studies involving a composite picture of the total mineral metabolism.

The discovery, then, of apparently normal subjects justified the attempt to follow out a study of all minerals contributing to the acid-base equilibrium.

By normal subjects is meant in this connection women who, in addition to showing adequate retention of the major elements concerned, present an uncomplicated health record throughout gestation. In such subjects there will be, in the course of an average uneventful pregnancy, no early nausea to deplete the chlorides or other mineral reserves or cause fasting acidosis. There will be few or none of the digestive disturbances common to pregnancy and relieved by the frequent use of bicarbonates or other alkalinizing compounds. There should be no toxemias involving upsets in water and electrolyte metabolism, even though these are not contemporary to the balance experiment itself. There will certainly be no prematurity to imply abnormal exchange between the maternal organism and the fetus. The women who were subjects of this experiment qualified admirably in these respects. Occasional irregularities, such as light edema or tendency to diarrhoea, have been noted in the preceding pages.

Literature. Actual measurements of acid-base balance in growing infants showed a retention of 56 cc. of 0.1 N excess base daily on the average (198). This same investigator postulated that the diet of the pregnant woman should provide at least 150 cc. of 0.1 N excess base daily. His estimate was based on calculations from existing data on the composition of the human fetus. These showed an average deposit of 85 cc. of 0.1 N excess base daily during the last 100 days of gestation. Since then three other publications (76, 107, 187) have appeared dealing with the composition of the fetus.

Shohl, cited above (198), computed from Camerer's total ash content of the mature fetus (32) that it contained 11,970 cc. of 0.1 N acid and 18,680 cc. of 0.1 N base, or 6710 cc. of excess base. The average composition figures shown in Table 1 for the fetus at term amount to 9911 cc. of 0.1 N acid, 15,774 cc. of base, or 5863 cc. of 0.1 N excess base. The data for the maximum content of each element, shown also in Table 1, total 13,030 cc. of acid, 21,409 cc. of base, or 8379 cc. of 0.1 excess base. Thus, the values given by Shohl lie between these average and maximum data. Therefore the new data necessitate no appreciable change in his estimate of fetal needs for relative amounts of acid- and base-forming radicals.

Acid-Base Balances in Women during Pregnancy. The metabolic behavior of the individual elements have been discussed separately in the preceding sections as well as in a previous publication (42). Table 15 shows the contributions of the different elements to the acid-base balance expressed in terms of 0.1 N acid and alkali.

Of the base-forming elements in the intake, sodium contributed most, potassium second and magnesium least. Likewise, chlorine played the leading role for the acid-forming elements in the intake, with phosphorus second, and sulfur least. The same relations hold for excretion through the urine except that frequently magnesium alternates rank with calcium. In the feces, however, calcium contributes most, magnesium second and potassium or sodium least of the base-forming group. Most of the acid-forming elements in the feces consist of phosphorus. The relative composition of the material retained is more like the intake, sodium leading, calcium and potassium alternating for second rank, and with either chlorine or phosphorus providing most of the acid-forming elements. Negative balances, where they did occur, were insignificant compared to the total electrolytes retained.

In none of the avenues of intake or excretion does chlorine occur in amounts equivalent to the sodium present. Since phosphorus alone frequently parallels the calcium in equivalent amounts, it must be assumed that much of the sodium and potassium present occurred in other combinations, such as with organic acid radicals. The negative balance found for sodium was almost exactly equivalent to the negative chlorine retention of the same period, confirming the assumption that the poor storage was the result of dehydration and elimination of sodium chloride after a mild edema.

The chemical equivalent association of phosphorus and calcium was apparent, especially in the amounts retained (Cases IV and V), but in 6 periods, including all those of Case II, the amount of total phosphorus retained was equivalent to the combined quantities of calcium and magnesium. The quantities of nitrogen retained need to be reckoned in this connection.

TABLE 15-a. Contributions of Different Elements to Acid-Base Balance; Daily Averages in Terms of 0.1 N Alkali.

Case-Period	Wk. of pregnancy	CALCIUM					MAGNESIUM					SODIUM					POTASSIUM				
		Intake	Urine	Feces	Total outgo	Balance	Intake	Urine	Feces	Total outgo	Balance	Intake	Urine	Feces	Total outgo	Balance	Intake	Urine	Feces	Total outgo	Balance
		cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
I-2	27	625	41	488	529	96	269	79	169	248	21	1678	992	104	1096	582	903	739	96	835	68
I-3	31	598	29	402	431	167	296	74	154	228	68	1752	888	122	1010	742	796	681	73	754	42
I-4	34	644	28	510	538	106	242	84	160	244	—2	1576	946	127	1073	503	895	725	79	804	92
I-5	38	620	38	418	456	164	258	91	139	230	28	1733	1180	128	1308	425	842	629	84	713	129
II-1	26	896	178	533	711	185	367	85	200	285	82	1973	1278	110	1388	585	1143	885	76	961	182
II-2	30	885	140	515	655	230	418	85	207	292	126	2063	1278	128	1406	657	1108	767	85	852	256
II-3	34	908	185	587	772	136	429	111	282	393	36	2274	1365	97	1462	812	1112	910	79	989	123
IV-1	31	510	297	184	481	29	367	102	242	344	23	2216	1646	91	1737	479	911	637	158	795	116
IV-2	35	617	278	229	507	110	316	106	186	292	24	2363	1574	114	1688	675	1049	771	110	881	168
IV-3	39	780	272	264	536	244	358	101	176	277	81	3159	2146	245	2390	769	1128	831	133	954	174
V-1	28	443	82	204	286	157	274	65	133	198	76	2330	1859	35	1894	436	808	649	60	709	99
V-2	33	404	81	257	338	66	240	53	179	232	8	1729	1434	57	1491	238	587	423	100	523	63
V-3	38	516	51	307	358	158	314	47	204	251	63	2210	1349	74	1423	787	861	554	114	668	193
V-4	39	534	49	297	346	188	290	67	181	248	42	2004	1653	75	1728	276	810	555	94	649	161
VI-1	18	1186	147	983	1130	56	388	83	263	346	42	3295	2387	189	2576	719	1004	755	163	918	86
VI-2	23	1041	103	757	860	181	380	75	247	322	58	2298	1743	138	1881	417	1087	834	144	978	109
VI-3	27	723	133	515	648	75	267	75	151	226	41	2085	1301	127	1428	657	744	590	112	702	42
VI-4	31	872	100	666	766	106	328	60	232	292	36	2067	1167	252	1419	648	989	646	181	827	162
VI-5	35	928	95	604	699	229	333	80	189	269	64	1858	1144	204	1348	510	948	589	122	711	237
VI-6	38	778	67	555	622	156	336	41	239	280	56	1875	1784	150	1934	—59	862	637	101	738	124

TABLE 15-b. Contributions of Different Elements to Acid-Base Balance; Daily Averages in Terms of 0.1 N Alkali.

Case-Period	Wk. of preg-	CHLORINE					SULFUR					PHOSPHORUS				
		Intake	Urine	Feces	Total outgo	Bal-ance	Intake	Urine	Feces	Total outgo	Bal-ance	Intake	Urine	Feces	Total outgo	Bal-ance
		cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
I-2	27	1128	1162	31	1193	—65	355	288	60	348	7	830	380	261	641	189
I-3	31	1054	896	30	926	128	408	344	53	397	11	798	437	251	688	108
I-4	34	1191	939	31	970	221	367	339	58	397	—30	813	408	297	705	108
I-5	38	1117	917	48	965	152	378	343	54	397	—19	805	396	264	660	145
II-1	26	1367	1350	21	1371	—4	538	390	74	464	74	1152	490	360	850	302
II-2	30	1536	1319	34	1353	183	526	425	72	497	29	1208	465	355	820	388
II-3	34	1581	1056	18	1074	507	521	423	78	501	20	1199	624	391	1015	184
IV-1	31	1852	1337	22	1359	493	455	419	65	484	—29	906	607	285	892	14
IV-2	35	1787	1273	18	1291	496	400	371	62	433	—33	743	453	197	650	93
IV-3	39	2729	2300	76	2376	353	460	390	75	465	— 5	935	557	176	733	202
V-1	28	1831	1622	12	1634	197	391	361	49	410	—19	685	358	196	554	131
V-2	33	1418	1153	20	1173	245	372	321	62	383	—11	609	274	252	526	83
V-3	38	1904	1273	19	1292	612	397	338	69	407	—10	731	341	249	590	141
V-4	39	1726	1618	18	1636	90	343	287	59	346	— 3	737	392	243	635	102
VI-1	18	2328	2036	58	2094	234	845	700	162	862	—17	1483	559	479	1038	445
VI-2	23	1933	1695	71	1766	167	588	470	91	561	27	1360	579	566	1145	215
VI-3	27	1713	1217	55	1272	441	507	436	74	510	— 3	886	500	323	823	63
VI-4	31	1625	1154	95	1249	376	604	401	173	574	30	1059	459	451	910	149
VI-5	35	1446	1151	65	1216	230	682	344	92	436	46	1070	464	385	849	221
VI-6	38	1576	1584	37	1621	—45	498	423	98	521	—23	1017	421	369	790	227

Table 16 shows the final acid-base balance expressed as 0.1 N alkali for total base and excess base in the intake, retention and paths of excretion. Ratios of base to acid are presented in the last four columns.

From this table it will be seen that all diets supplied a predominance of base-forming elements varying from 561 to 1422 cc. of 0.1 N base daily, with a mean of 1037 cc. The ratios of base to acid show that these values were not so different, relatively. The narrow range of ratios in the intake extends only from 1.2 to 1.5 with a mean of 1.3. More excess base appeared in the stools. The range in ratios was wider and higher, 1.7 to 2.5, mean 2.1, but these were unrelated to the variations in the ratios of the diets.

The best calcium retention was found in the case of diets affording a potential alkalinity of more than 1000 cc. of 0.1 N fixed base daily (Periods II-2, IV-3 and VI-5). The poorest retentions were found in the case of those containing 800 cc. or less of 0.1 N excess base (Periods IV-1, V-2 and VI-3). However, not all diets supplying a potential alkalinity of more than 1000 cc. daily of 0.1 N base in excess of acid gave optimal storage, implying that factors other than the acid-base balance of the diets were important to calcium retention. The ratio of base to acid in the intake bore no relation to the quantities of calcium stored. Nor was good calcium storage associated with high base retention, except insofar as the large amounts of calcium retained contributed to the excess of base stored.

Base-forming elements always predominated in the quantities stored but unlike the excess in the intake showed considerable variation from period to period and from person to person, without any apparent relation to the stage of pregnancy. The storage, as expressed in volumes of 0.1 N excess base daily, ranged from 59 to 770 cc. with a mean of 436. In all but 2 of the 20 balances the amount was greater than the 150 cc. of 0.1 N base daily allowed for the fetus by Shohl (198). The retention was over three times this allowance in half of the experimental periods. The retention ratios of base to acid ranged from 1.2 to 5.8 and were independent of intake. The storage ratios were usually above 2 in those balances which were augmented by excellent calcium retention.

The relative quantities of acid and base in the urine were more variable than in food or feces. In four instances, acid-forming elements actually predominated over the 4-day period and the excess base was low or negligible in nine others. This variation was to be expected from the part played by urine in the elimination of electrolytes and in contributing to the maintenance of normal acid-base equilibrium in the body tissues and fluids. Fluctuations in factors contributing to the acid-base economy were reflected in the urine as shown by the number of negative base balances, by the absence of constant ratios of base to acid in the urine (Table 16) and by the lack of correlation with the acid-base proportions in the intake. In other words the urine was the avenue by which the kidney, through regulation of losses of electrolytes, served to stabilize the retention against the variations of diet, digestion and metabolic demand.

The question of organic acids in the urine arises in this connection. It is regrettable that these were not determined routinely. The preponderance of fixed base in certain urines (Case IV) was not paralleled by the findings for titrable acidity, free ammonia and pH estimations. The facts suggest, however, that appreciable quantities of organic acids were being excreted, coming either from fruits and vegetables or from some endogenous source peculiar to the stage of pregnancy (178, 186). More information is needed regarding the fate of various organic acids in different physiological states and the actual potential basicity in the human organism of many foodstuffs containing quantities of organic acids. Some work

TABLE 16. Total Base Retained by Women During Pregnancy; Daily Averages in Terms of 0.1 N Alkali.

Case-Period	Wk. of preg-nancy	INTAKE		URINE		FECES		RETENTION		RATIO—Base :Acid			
		Total base	Excess base	Total base	Excess base	Total base	Excess base	Total base	Excess base	Intake	Urine	Feces	Reten-tion
		cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.				
I-2	27	3475	1162	1851	21	857	505	767	636	1.5	1.0	2.4	5.8
I-3	31	3442	1182	1672	—5	751	417	1019	770	1.5	1.0	2.2	4.1
I-4	34	3356	985	1783	89	876	490	697	406	1.4	1.1	2.3	2.4
I-5	38	3453	1153	1938	282	769	403	746	468	1.5	1.2	2.1	2.7
II-1	26	4379	1322	2426	196	919	464	1034	662	1.4	1.1	2.0	2.8
II-2	30	4474	1204	2270	61	935	474	1269	669	1.3	1.0	2.0	2.1
II-3	34	4723	1422	2571	468	1045	558	1107	393	1.3	1.2	2.2	1.5
IV-1	31	4004	791	2682	319	675	303	647	169	1.2	1.1	1.8	1.3
IV-2	35	4345	1415	2729	632	639	362	977	421	1.5	1.3	2.3	1.7
IV-3	39	5425	1301	3349	102	818	491	1258	708	1.3	1.0	2.5	2.3
V-1	28	3855	948	2655	314	432	175	768	459	1.3	1.1	1.7	2.5
V-2	33	2960	561	1991	243	593	259	376	59	1.2	1.1	1.8	1.2
V-3	38	3901	869	2001	49	699	362	1201	458	1.3	1.0	2.1	1.7
V-4	39	3638	832	2324	27	647	327	667	478	1.3	1.0	2.0	3.5
VI-1	18	5873	1217	3372	77	1598	899	903	241	1.3	1.0	2.3	1.4
VI-2	23	4806	925	2755	11	1286	558	765	356	1.3	1.0	1.8	1.8
VI-3	27	3819	713	2099	—54	905	453	815	314	1.2	1.0	2.0	1.6
VI-4	31	4256	968	1973	—41	1331	612	952	397	1.3	1.0	1.8	1.7
VI-5	35	4067	1069	1908	—51	1119	577	1040	543	1.3	1.0	2.1	2.1
VI-6	38	3851	760	2529	101	1045	541	277	118	1.2	1.0	2.1	1.7

is being carried on in this direction (22, 28, 64, 66, 160, 183, 184, 185). For example, the limited ability of human subjects to oxidize tartaric and citric acids has been demonstrated (67, 166, 167, 189, 190) as has been the fact that excessive intakes of organic acids may give rise to increased endogeneous production of uric acid (37). One comment based on Saywell's work (185) was that "each edible fruit really needs to be tested in the crucible of the human body"; and one might add: "in several different human bodies under varying physiological conditions."

Thus, more facts are needed for the correct evaluation of the potential acid and base qualities of fruits and vegetables as well as for correct interpretation of acid-base studies.

SUMMARY OF FINDINGS IN COMPUTATION OF ACID-BASE BALANCE

1. The need for acid-base balance studies in pregnancy has been indicated by the clinical and biochemical significance of blood chemistry observations on such subjects, and by the results of acid-base studies in children as well as the total absence of such data for pregnancy in women.

2. Sodium, potassium and chlorine were the main contributors to the acid-base system of inorganic elements in the diets and urine excretions; phosphorus and calcium furnished a much smaller quota, with a tendency for magnesium and sulfur to contribute least.

3. Sodium and calcium, phosphorus and chlorine, furnished most of the acid-base equivalents retained; sulfur and magnesium furnished a neglibible amount.

4. An excess of base-forming elements was retained in each of the 20 experimental periods. The amounts ranged from 59 to 770 cc. of 0.1 N alkali and came from diets affording a preponderance of base varying from 561 to 1422 cc. of 0.1 N alkali.

5. For good calcium retention it seemed desirable that the diets provide 800 to 1000 cc. of 0.1 excess base daily in conjunction with other factors favorable to calcium utilization.

6. Organic acids in the foods and urine confused the acid-base metabolic picture considerably.

WATER BALANCE

Purpose and Scope. A form of water balance was recorded throughout these studies, and although incomplete it is presented here for what it contributed to an understanding of the total base balance. An accurate measurement of water balance would take into account that coming from drink, from preformed water in foods, and from the processes of metabolism. In the outgo would be considered that in urine, fecal moisture, sweat, insensible perspiration, and loss through lungs. In this study only the water in drink, food, urine and feces has been recorded since conditions of the experiment prohibited the measurement of other losses. The inclusion of the findings in this report seems justified in view of the fact that water gained or lost through avenues except sweat would be fairly constant for any given individual, hence result in data at least comparable from period to period.

Among other reasons for these records of certain phases of water balance, the chief one was to determine the relation, if any, to variations found in the acid-base balance of the minerals retained. Additional reasons were: to determine whether wide fluctuations were to be explained by edemas, and whether the acid-base balance of the intake seemed sufficient to influence the output of water and electrolytes. Such records show variations in urine volume in relation to water intake, and aid in explaining fluctuations in mineral retention due to dehydration, edema or losses through sweat (163). Water intoxication has special significance in pregnancy because of its relation to the onset or cause of toxemias and eclampsia. Data for the moisture content of feces serve to mark periods when the stools were normal for a particular individual as compared to the prevalence and extent of diarrhoeas at other times. Finally, it was hoped that such records would reveal habits and possible requirements in the matter of gross liquid intake for women during pregnancy.

Literature. An excellent summary of the various phases of water metabolism is presented in Part III, Nutrition, of the Report of the Committee on Growth and Development of the White House Conference on Child Health (240) and also by Chaney and Ahlborn (34) and by Gortner (78). It was estimated that in the infant the urine furnished 60 to 75 percent of the water leaving the body, the feces 2 to 15 percent and the lungs 20 to 35 percent. The losses through insensible perspiration have been estimated as 400 to 800 cc. per day in the adult; while the loss through

TABLE 17. Relation of Daily Water Balance to Environmental Temperatures and Retention of Total Electrolytes.

Subject and period	Wk. of pregnancy	Temperature		Total calories from diet	Water intake from —			Water outgo in —			Unaccounted balance from intake	Retention of electrolytes	
		Mean	Days above 86° F.		Food	Drink	Total	Urine	Feces	Total		Total	Excess base
		°F.			cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc. of 0.1 N	cc. of 0.1 N
I-2	27	75	2	2164	1312	1197	2509	1637	98	1735	774	898	636
I-3	31	62	0	2249	1307	1221	2528	1655	88	1743	785	1268	770
I-4	34	57	0	2136	1320	1154	2474	1914	88	2002	472	988	406
I-5	38	41	0	2299	1347	1357	2704	2045	109	2154	550	1024	468
II-1	26	63	0	2752	1420	1075	2495	1899	69	1968	527	1406	662
II-2	30	63	0	2880	1450	1153	2603	1902	84	1986	617	1869	669
II-3	34	47	0	2870	1435	1082	2517	1950	69	2019	498	1818	396
III-1	33	59	0	2702	1040	2654	3694	2370	195	2565	1129	*....	*....
III-2	37	57	0	3093	1305	2897	4212	2800	152	2952	1260	......	......
IV-1	31	69	0	2980	1185	2272	3457	1975	201	2176	1281	1125	169
IV-2	35	70	0	2744	1565	2497	3052	1980	109	2089	1973	1533	421
IV-3	39	74	0	3585	1890	1857	3747	1942	215	2157	1591	1808	708
V-1	28	52	0	2461	1265	1071	2336	1775	45	1820	516	1077	459
V-2	33	67	0	2293	742	1348	2090	1450	62	1512	578	793	59
V-3	38	72	0	2601	902	1494	2396	1265	104	1369	1027	1944	458
V-4	39	75	1	2459	1002	1744	2776	1875	65	1940	836	856	478
VI-1	18	55	0	2982	1885	994	2879	2292	187	2479	400	1565	241
VI-2	23	53	0	3043	2010	832	2842	2109	167	2276	566	1174	356
VI-3	27	63	0	2674	1517	1421	2938	1530	126	1656	1282	1316	314
VI-4	31	67	1	2592	1640	1278	2918	1727	251	1978	940	1507	397
VI-5	35	78	2	2378	1605	1492	3097	1582	201	1783	1314	1537	543
VI-6	38	78	3	2170	1427	1089	2616	1992	117	2109	407	436	118

*Undetermined.

lungs alone, varying with the humidity, has been estimated as 250 to 350 cc. per day. The water coming from food as a result of metabolic chemical changes was estimated as 12 cc. per 100 calories. This means that about 400 cc. daily of the intake were not accounted for by the method of this study. Likewise some 800 cc. plus sweat are not included in the total output reported here. Therefore, if the water content of the body remained the same throughout each period, one would expect some 500 to 1000 cc. of unaccounted balance daily for each period.

Acidifying influences in metabolism are known to increase water output (163) and alkalinizing ones to favor water retention. Edemas occur on low protein diets and in certain vitamin deficiencies (195). Thus dehydration might be associated with the elimination of alkalies corresponding to the relief from acidosis, whereas water retention could mean also curtailed elimination of sodium, potassium and chlorine.

It has been estimated that the water requirement is roughly 1 gram per calorie, or 3000 cc. per day for the average man.

Water Balances. Table 17 shows the water intake and outgo and the unaccounted water balance. In accessory columns are shown also the season, daily temperature maximums, and retention of total electrolytes and excess base. The term "unaccounted" balance is used advisedly to designate the excess of intake over outgo, since the figures cannot represent true retention.

Sweating occurs when environmental temperature goes above 30° C. or 86° F. The daily temperatures from official weather bureau reports for this section (233) show how many days of each metabolic period the temperature went above this point. From these data the mean temperature has been computed for each period and recorded in Table 17. Included also is the number of days of each period with maximum temperatures above 86° F. A tendency to a minimum of sweating would occur in these subjects because of the inclination to avoid exertion or violent and prolonged exercise.

From this table it will be observed that the amount of the unaccounted balance shows no relation to the total intake of electrolytes except in general for Case IV. The deficit was as low as 400 cc. in two periods and above 1000 cc. in three, with the exception of Case IV. The figures compare favorably with the 500 to 1000 cc. amounts expected to occur unaccounted for by the methods used. The irregularities for Case IV can be explained partly by the large size, hence greater surface area for more excessive total losses through insensible perspiration and sweating. The urine volume was fairly constant for a given individual, but this was due to the habitual amounts of water intake rather than to impaired kidney function. The variations found in the specific gravity of the urine (see section on Nitrogen) testify to this conclusion regarding renal capacity.

For each individual the lowest excess base retention tended to be associated with low unaccounted water balances. This was due, doubtless, to the tendency to greater water output which accompanied the more acid conditions in metabolism.

It will be noted that the moisture content of the feces for Case VI was higher than for other subjects, thus supporting previous statements regarding the number and unusual frequency of stools. During Period VI-4 the feces possessed the highest moisture content of any in the series; but the lowest mineral retentions were exhibited during Period VI-3, associated with a lower intake. Hence even a slightly curtailed intake may be of more serious consequence to the retention of minerals than mild diarrhoea or frequent stools.

The habitual water intake in these diets totaled roughly 2100 to 3700 cc. daily, with the majority of intakes around 2500 cc. The average was 2748 cc. and is comparable to the average of 2618 calories per day (Table 28). Thus the habitual water intake of these women measures up roughly to the standards which have been postulated, namely 1 gram of water for every calorie of intake. The data show that only Cases II and V failed in certain periods to take enough water to meet these requirements. The quantities listed take no account of the water from chemical changes of food in the body, which source would add some 250 to 300 cc. more to each total intake, making it to equal or exceed the calorie intake. The moisture content of the food for Cases I, II and VI approached or exceeded the daily amount taken in the form of drinking water. This would be expected on diets containing liberal amounts of milk.

IRON

Previous Studies on Iron Metabolism in Pregnancy. The needs for iron in pregnancy may be grouped under three classes, in time order of demand: (a) the maternal organism, particularly for placenta, adnexa and circulation therein; (b) fetus, for circulatory system and normal iron content of tissues; (c) fetal reserves, such as in the liver, for post-natal growth. It is believed that the last is acquired mainly in the last three months of prenatal life. Variations, then, in the iron content of the new born, 0.266 to 0.937 gram (See Table 1 and discussion), may be attributed chiefly to the completeness or lack of fetal reserves, since these are acquired last. This is in accord with the conclusions of MacKay (139, 140) and of Strauss (210). Measurement of adequate iron retentions throughout pregnancy should indicate something of the magnitude of these needs in toto.

Gladstone (77) failed to find evidence of progressive depositions of iron in the livers of fetuses during the last four months of intra-uterine life, but did find the iron content markedly increased in 1 to 10 weeks after birth. This, he believed, resulted from post-natal intravascular destruction of blood arising from the adjustment of the levels of high red cell count at birth to the conditions of oxygen tension surrounding extra-uterine life. On the other hand it is noteworthy that the total iron content of livers of mature infants analyzed by him varied from 18.22 to 144.67 mg. This wide difference indicates that some livers were better equipped with iron reserves than were others, a fact to be explained perhaps by differences in maternal supply and utilization or some other error in maternal metabolism.

The importance of iron metabolism in relation to the nutrition of mother and children was emphasized by the Committee on Growth and Development of the Child, Medical Section of the White House Conference on Child Health (240). The Committee estimated that the average daily transfer from mother to fetus, necessary during the first two-thirds of pregnancy, would amount to 0.4 mg., and during the last third to 4.7 mg. daily, or a total of 0.5 mg. of iron for fetus alone. The average figures shown in Table 1 also suggest this accelerated acquisition of iron by the fetus during later months.

Iron retention during human pregnancy has been reported in only one other study (40) and that consisted of 23 balances on 9 women. These data showed that the women did not retain iron during the last trimester at the ten-fold rate which is suggested by fetal composition figures. The study did not show that on a fairly adequate ordinary diet permitting good early storage of iron the total retention throughout gestation seemed sufficient to supply at least the needs of the fetus.

In other words, with iron, as with calcium and nitrogen, when conditions are favorable for storage, maternal reserves seem to be replenished in advance of the period of highest fetal demand. In the case of iron, doubtless the liver and spleen provided these maternal mobile depots (24). Thus it becomes unnecessary for the mother to assimilate enough iron from her food to supply all of the excessive demands peculiar to the last stages of gestation (29). Furthermore, the ability to economize on iron in metabolism seems to be characteristic of even the non-pregnant female as shown by difficulties in producing nutritional anemia in rats of this sex (153, 174).

On the other hand, there is plenty of evidence that the maternal organism often fails to provide enough iron for her own needs and those of her offspring. Much attention has been given recently to an investigation of the anemias of pregnancy both in laboratory animals and clinical patients (180, 201).

The frequency of anemia in gestating laboratory animals has been observed by Beard and Meyers (18), Smythe and Miller (207), Sure et al. (219), Kojima (111), Mitchell (153), and Van Donk (234). The last named attributed most of this to the hydremia incident to gestation and post-parturition. It is common knowledge, also, that the iron content of nursing young reaches a minimum by weaning time (29, 59, 133, 150, 161) or sooner if the maternal supply of iron has been deficient (139, 140, 210, 213). Strauss and MacKay each found that no matter how anemic the mothers were, their infants were born with normal hemoglobin and red cell count, but that the infants born of anemic mothers do develop severe anemia during the first year of life. This anemia was prevented by adinistering iron to the mothers during pregnancy or corrected by giving iron to the infant. Hence the authors conclude that the anemia of late infancy in their cases was due to deficient storage of iron in the fetus dependent upon an inadequate supply of this element in the diet of the mother. The existence, therefore, of dietary or other conditions which influence unfavorably the metabolism of iron during pregnancy warrants further studies on iron retention during this physiological state.

Recent literature abounds in suggestions as to dietary factors affecting iron metabolism. Frequent reference is made to the anemias, chiefly macrocytic, often associated with sprue, pellagra, beri-beri, and other subtropical diseases (7, 13, 50, 95, 169, 242). Yeast or marmite has been found to be effective in tropical and the macrocytic anemias of pregnancy as occurring in India (81, 242, 243). Strauss (211, 212, 213) found hypogastric secretion in many patients during pregnancy and interpreted this to be evidence of a deficiency of Castle's (33, 214) so-called intrinsic factor. His work (214) suggests also that an extrinsic factor which plays a significant role in blood formation by interacting with the intrinsic may be vitamin G or some closely related substance. Later work of Wills (214) tends to disprove this hypothesis but shows the extrinsic factor to be closely associated with the vitamin G in occurrence (219, 244).

The vitamin balance phenomenon described by Harris (89) and previously observed by Light (130) and Hopkins (100) would indicate a relatively greater requirement for some component of vitamin B when large amounts of vitamin D are available. Positive identification of vitamin G as a flavin (25, 115) which is unstable to light also lends support to this view (88). This suggests further that individuals living in sections which permit access to an abundance of solar radiation might be more susceptible to deficiency of this nutritional factor, probably vitamin G, than residents of northern latitudes. The clinical features of pellagra also suggest this possibility, as mentioned above in connection with sulfur metabolism. Whether or not a similar deficiency of Castle and Strauss's extrinsic factor predisposes to macrocytic anemias or whether repeated gastro-intestinal infection with consequent diarrhoeas reduce the opportunity for absorption of the interaction product of the extrinsic and intrinsic factors (211) are questions which await further investigation. However, the above findings should stimulate an interest in iron utilization by human subjects exposed to an abundance of vitamin D. Pertinent also in this connection is the finding that the iron content of egg yolk decreases with exposure of the hens to sunlight (61).

The higher requirements of pregnancy and lactation for factors of the vitamin B complex would tend to augment any deficiency which might have existed in the diets of these women. A clinical expression of this high requirement is found in the statement of Roberts (170) that "a mother of several children * * * is a likely pellagrous candidate" presenting a type of pellagra which he chose to call "pellagra in multiparae."

The facts and hypotheses cited above point to the existence during pregnancy of a complex nutritional problem with a multiplicity of factors

TABLE 18. Iron Retention during Pregnancy.
Daily averages

Subject and period	Week of pregnacy	Iron intake	Iron outgo			Iron balance
			Urine	Feces	Total	
		mgm.	mgm.	mgm.	mgm.	mgm.
I-2	27	9.45	0.21	6.93	7.14	2.31
I-3	31	10.64	0.11	6.74	6.85	3.79
I-4	34	11.16	0.16	8.71	8.87	2.29
I-5	38	11.76	0.16	8.50	8.66	3.10
II-1	26	17.38	0.24	11.04	11.28	6.10
II-2	30	34.88	0.47	33.57	34.04	0.84
II-3	34	24.14	0.19	21.02	22.11	2.03
III-1	33	11.71	0.20	10.45	10.65	1.06
III-2	37	15.74	0.28	12.81	13.09	2.65
IV-1	31	15.98	0.25	15.70	15.95	0.03
IV-2	35	11.96	0.25	10.95	11.20	0.76
IV-3	39	13.76	0.25	8.55	8.80	4.96
V-1	28	13.17	0.21	8.98	9.19	3.98
V-2	33	14.21	0.21	13.73	13.94	0.37
V-3	38	11.62	0.17	10.37	10.54	1.08
V-4	39	12.73	0.29	8.83	9.12	3.61
VI-1	18	33.58	0.28	26.42	26.70	6.88
VI-2	23	18.32	0.26	15.08	15.34	2.98
VI-3	27	12.19	0.23	9.78	10.01	2.18
VI-4	31	16.27	0.35	15.16	15.51	0.76
VI-5	35	12.16	0.47	10.78	11.45	0.71
VI-6	38	13.62	0.12	11.95	12.07	1.55
Averages						
This series		15.74	0.24	13.05	13.29	2.45
Chicago series		14.72	0.17	11.39	11.56	3.16

tending to concur in poor iron metabolism such as is described below for these Oklahoma women. The excessive demands of pregnancy for iron, the frequently deficient gastric secretion, various clinical manifestations of anemia during pregnancy and in peoples of the subtropics, the possible interrelations of vitamins G and D, together with the peculiar needs of pregnancy for these accessory factors, all contribute to an interest in this investigation of iron retention in southern women and are to be kept in mind during the following discussion of the individual balances.

This Series of Iron Balances. Twenty-two balances on the six women of this study are reported here. Of the total 25 biochemical metabolism periods obtained for calcium and phosphorus, three were not usable for iron determinations. In two of these the collections were not handled so as to be free from iron contaminations (Periods I-0 and I-1). In a third instance (Period II-4) an accidental unremediable error made in weighing the copper-iron supplement to the diet rendered impossible an exact knowledge of the iron intake for that period.

Table 18 shows the iron storage found in the different experimental periods, together with averages of intake and outgo for subjects of the present series compared to those of the Chicago women reported earlier (40).

The storage ranged from 0.23 to 6.88 mg. of iron daily with an average of 2.46 mg. as compared to 3.16 mg. daily for the group previously studied. All balances save two were obtained during the last trimester of pregnancy, during which period the average storage was 2.22 mg. daily compared to the 4.70 mg. estimated necessary for the fetus alone during this phase of gestation (240). Ten of the balances represented a storage of 2.0 mg. or less of iron daily, while six of the 22 showed a storage of less than 1.0 mg. daily. On the other hand, in the Chicago group, only five balances were less than 2.0 mg. and only 2 out of 23 balances were below 1.0 mg. daily.

In Figure VI is shown graphically the extent to which the individual balances failed to meet the supposed fetal demand, and by way of contrast, the iron storage in the Chicago series. It will be observed that the few balances occurring in the early part of the gestation were considerably in excess of bare fetal needs during those months and doubtless provided maternal reserves from which iron could be transferred to the fetus in later months. This confirms a tendency to early storage observed in the previous study and discussed above. It emphasizes the need for iron balances in early as well as late gestation for accurate interpretation of the findings in either phase of pregnancy.

From this chart it will be seen also that in only two instances did exceptionally good storage occur. In one of these (Period II-1) the diet contained a good supply of meat, liver, and eggs in addition to a wheat germ preparation affording appreciable quantities of iron. (See also Table 27.) In the other instance (Period VI-1) the total iron intake was unusually high because the diet, abundant in all respects, contained also liver and whole grain cereals. Thus both diets were exceptional in quantity and quality and the retentions were likewise not typical of the series.

The chart shows the tendency to relatively higher storage exhibited by these women as gestation progressed, but not by the Chicago group for iron retentions. This is interpreted to mean that retentions had been deficient in most cases until the heavy demands of late fetal life resulted in some improved utilization of dietary iron. Such marked increase in storage during the last months becomes unncessary when the maternal reserves have been replenished with transferable material in early pregnancy, as

FIGURE VI

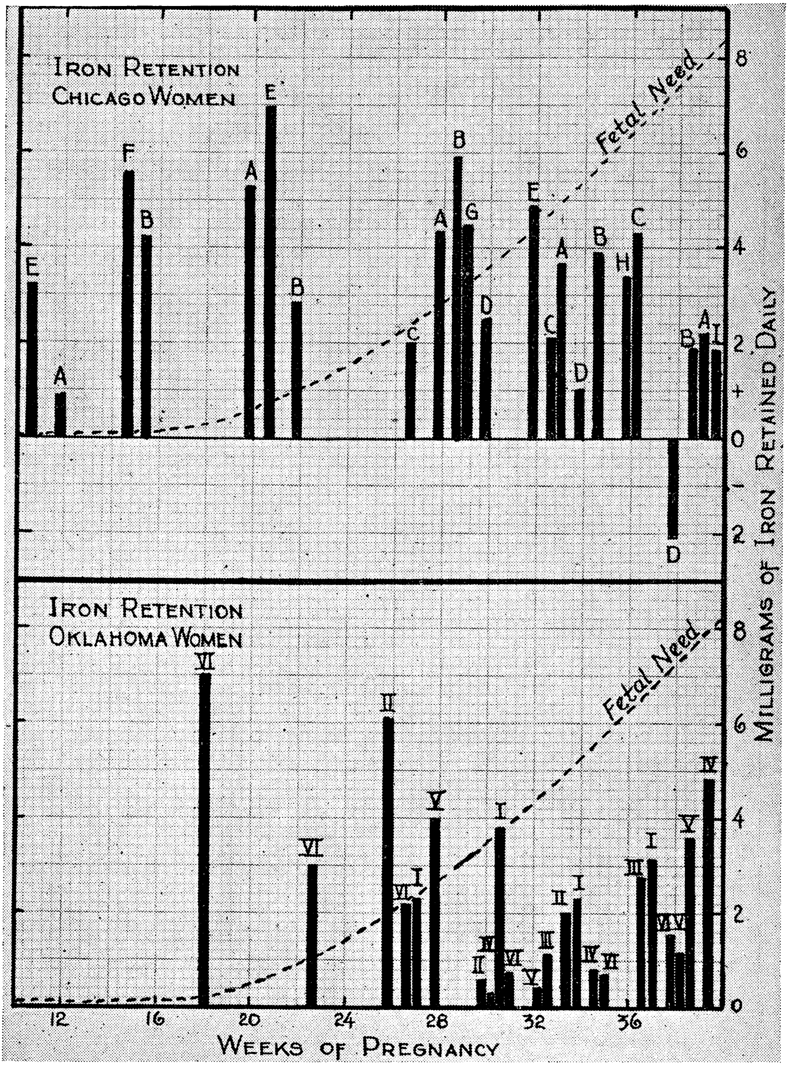


Fig. VI.—Inadequate storage of iron by Oklahoma women as contrasted to better records in a Chicago group.

seems to have been done by most of the Chicago group. A reverse condition illustrating the same physiological principle was noted for calcium retentions. The Chicago group was forced to relatively higher retentions in the last month while the southern women tended to acquire their calcium prior to this period of high fetal demand (Figure IV).

Figure VI shows at a glance the superior retention of iron by the northern group. The different rates of iron storage in the two groups of women were not due to corresponding variations in the levels of intake. In the favored group the average intake was 14.72 mg. and 22 percent of this was stored, while in the present series the average was 15.74 mg. of which 17 percent was stored. In the Oklahoma group 9 balances represented less than 10 percent of the intake, and 8 of them more than 20 percent of the intake, corresponding to 3 and 13 balances respectively at the extreme levels of utilization in the Chicago group.

A comparison of the storages in periods of both series where the dietary iron ranged from 11.5 mg. to 16.0 mg. daily excludes the extreme variations in intake and includes roughly the middle 50 percent of the total periods in either group. The average storage for this representative series was 1.93 mg. daily for the Oklahoma group and 3.36 mg. daily for the Chicago group, while the resulting average intakes were 13.24 and 13.89 mg. of iron respectively.

The quality of the diets, such as the amounts of representative foodstuffs furnishing the dietary iron, was not greatly different in the two groups, as will be seen from the summary in Table 27 in the section on diet (39). Liver appeared in about the same frequency and amount in six periods and with an average of 21 grams daily. The amounts of eggs, meat and green vegetables were also comparable. More iron came from whole grain cereals in the diets of the Chicago women, since those of the present group seldom chose whole wheat bread or other forms of whole grain cereals. The value of iron from this source for human subjects has not been tested as it has for rats suffering from nutritional anemia (174, 175).

In the matter of quality of the diets as related to the rates of iron storage, particular attention is called to Case II. Her diet was regularly supplemented with cod liver oil and other forms of vitamin D, including irradiated cereals. There was also added a wheat germ preparation which contributed 2.0 to 2.5 mg. of iron daily. Some two weeks before her second balance experiment the physician in charge advised 2 teaspoonfuls of saccharated copper-iron solution daily. The subject had a history of mild anemia and had exhibited a slightly lowered erythrocyte count (4,000,000) in the early part of pregnancy. No count was made immediately preceding the addition of the copper-iron solution. This addition to the diet accounts for the higher iron intake of the second period. In the third period the available supply of the preparation lasted for the first two days only, resulting in a lower average intake for the period. The analytical data indicate that the preparation supplied about 15 mg. of iron daily. The amount of the solution ingested was measured by weighing the bottle and contents to the third decimal place before and at the end of the period. An aliquot of the amount consumed was added to the food composite for total analyses but the material was not analyzed separately. Under the conditions of this experiment no beneficial effects in iron retention could be ascribed to the preparation ingested. The response from additions of liver to the diets was more favorable (Periods II-1, VI-1 and VI-2) (cf. Table 27), and suggested a more complicated dietary deficiency common to these women than merely an insufficient intake of iron as found by Strauss for some of his subjects (210).

Poor digestion and absorption did not explain the deficient iron storage by these women. The lower figures for total dry weight of feces as compared to dry weight of food was found to indicate relatively better absorption of total solids in this group, as did also the greater tendency to constipation and the higher coefficients of digestibility for nitrogen for the southern group (see Table 25). Rapid gastro-intestinal rates and failure to digest food do not, therefore, account for the large excretions of fecal iron and lower iron balances in the group as a whole.

Diarrhoea has been shown to cause excessive losses of iron (40, 193). However, only for Case VI of this series was such a condition likely responsible for the iron losses. The stools of this subject frequently numbered three or more daily and contained undigested food particles. This was especially true for Period VI-4 and to a less extent for Period VI-5, as will be seen from the figures, for fecal moisture (Table 25). Her iron balances were low for both periods.

In connection with these differences in storage it is interesting that the Oklahoma women excreted some 50 percent more iron in the urine, averaging 0.24 daily as compared to 0.17 mg. for the women studied previously. While the amount was too small to affect the final iron balance appreciably, the quantities present may signify unfavorable tendencies in iron metabolism. Hanzal and Bing (85) reported finding urinary iron ranging from 0.20 to 0.30 mg. daily, other investigators 0.08 to 0.32 mg. daily (91, 120, 157). However, Lintzel (131, 132) says there was not more than 0.02 mg. of iron per liter in the urine examined by him. In both groups of women studied during pregnancy the magnitude of the urinary iron seemed to be an individual characteristic, independent of the urine volume but surprisingly constant from period to period.

Complete definition of the factors playing a role in the poor iron economy of the southern women awaits further investigation. The biological value of the quality of iron furnished may be a limiting phase. The difficulty seemed to be less one of total iron intake, digestion and absorption, more likely some deficiency associated with retention or utilization in the tissues. Presumably the iron metabolism is interrelated with nitrogen and phosphorus metabolism and indirectly also with some component of the vitamin B complex, perhaps not vitamin G but probably the extrinsic factor of Castle and Strauss (33, 214), presumably deficient in these diets and supplied in some form by liver. Pertinent in this connection is the recently expressed conclusion of Harris (88), referring to reports on the relationship of vitamin B to anemia, that "we have in these findings a further example of the effect of a vitamin B deficiency in bringing about some error in iron metabolism of a nature not yet clearly understood."

Another less probable explanation of the poor iron retention would assume that maternal iron reserves, such as those of the liver, were abundant at the beginning of gestation, or were replenished during the stages prior to those covered by these data. Blood pictures in the mother and in the infants at intervals throughout the first year of life might have clarified the findings more.

Whatever the explanation, the fact remains that on comparable levels of iron intake similar to the usual diet the retention by the southern women averaged almost 50 percent lower than that for some Chicago women during corresponding periods of pregnancy in spite of exactly the same technique for collections and analyses with the same laboratory equipment, chemicals and analyst.

SUMMARY ON IRON METABOLISM

1. Iron retention was found to be quite deficient in this series of metabolic studies. There were no negative balances but approximately three-fourths of the number fell short, many of them far short, of the estimated fetal need.

2. The low retentions were not due to low total iron intake but probably to a deficiency of other dietary factors which promote the utilization of iron.

3. The results are discussed in the light of recent studies on anemia in pregnancy and in subtropical diseases, as well as from the viewpoint of recently discovered but indefinitely determined dietary factors affecting iron metabolism and blood formation.

4. Further studies on this topic appear highly desirable.

TABLE 19. Proportion Retained from Intakes of Various Elements.

Case-Period	Week of pregnancy	Percentage of intake retained										
		Ca	Mg	Na	K	Cl	S	Phos.	Excess base	N	Fe	Total base
I-0	—4	17	*....	....	....	....	....	26	....	6	....	....
I-1	2	17	*....	....	....	....	....	35	....	18	....	....
I-2	27	15	6	35	7	—6	4	23	55	12	24	22
I-3	31	28	22	42	5	12	3	14	65	12	36	30
I-4	34	16	0	32	10	18	—8	13	41	10	20	21
I-5	38	26	13	25	15	14	—3	19	41	22	26	22
II-1	26	21	24	30	16	0	13	26	50	21	35	24
II-2	30	26	31	32	23	12	9	32	56	26	2	28
II-3	34	15	8	36	11	32	5	15	28	22	8	23
II-4	38	14	29	*....	....	....	....	5	*....	....	....	....
III-1	33	23	8	*....	....	....	....	12	....	12	9	....
III-2	37	30	21	*....	....	....	....	21	....	17	17	....
IV-1	31	6	7	22	13	27	—7	16	21	12	2	16
IV-2	35	18	8	28	16	28	—8	12	30	13	6	22
IV-3	39	31	23	25	15	13	—1	22	54	13	36	23
V-1	28	35	27	19	12	11	—5	19	48	17	30	20
V-2	33	16	3	14	11	17	—2	14	11	9	2	13
V-3	38	31	18	36	22	32	—2	19	53	28	9	31
V-4	39	35	14	14	20	5	0	14	57	12	28	18
VI-1	18	5	11	22	9	10	—2	30	20	18	20	15
VI-2	23	17	15	18	10	9	4	16	38	27	16	16
VI-3	27	10	18	31	6	26	—1	7	44	11	18	21
VI-4	31	12	13	35	16	23	5	14	41	15	5	22
VI-5	35	25	20	27	25	16	9	21	51	23	6	26
VI-6	38	20	17	—3	14	3	—5	22	16	10	11	7
Range												
Minimum		5	0	—3	5	—6	—8	5	11	9	2	7
Maximum		35	31	36	25	32	13	35	65	28	36	31
Average		20	15	26	14	15	2	18	41	16	17	21

*Undetermined.

UTILIZATION OF THE MINERALS OF THE DIETS

Relative Retention of Various Minerals. Table 19 summarizes for the various elements studied the percentage of the intake which was retained. The table includes the nitrogen balances which are discussed in a subsequent section as well as in a previous publication (43). In some periods as much as ⅓ of the intake was available for storage in the case of calcium, magnesium, sodium, potassium, chlorine, phosphorus and iron, ¼ in the case of nitrogen, but less than 1/10 of the sulfur intake was so used. From the maximum and minimum as well as the average figure given at the end of the table, it is apparent that the usual range between extremes was approximately 30 percent, except in the case of sulfur. This was less true for nitrogen and potassium than for other elements. The average percentage retentions show a proportionately higher demand for dietary sodium, 26 percent, and a fairly high use of dietary calcium, 20 percent. For all other elements except sulfur approximately 1/6 of the intake was used for storage in most instances.

Sulfur occupied a unique position as regards a low average of percentages retained, frequent low negative balances, and a narrow range of amounts appropriated from intake for storage. Even when positive sulfur balances did occur they were in no way comparable in percentage of intake to the general trend found for other elements. This could mean that the diets supplied such an abundance of this element that only a small proportion was sufficient for storage. Such an interpretation was contradicted by the higher retentions recorded for Landsberg's women and by the large number of negative balances as well as average low intake of the present series. A more plausible interpretation and one consistent with the facts would be that the intake was regularly too near maintenance requirements to permit storage except on the highest diets (Case II).

The widest range in percentage retention of any element was found for iron, and the average storage of this mineral was most erratic from period to period in each individual series. This irregular appropriation of the dietary supply of iron serves to emphasize the existence of factors other than total iron intake which govern its utilization. The iron intake was fairly constant for a given subject in the presence of these wide variations in retention, or if dietary fluctuations occurred they were wholly unrelated to the irregular retention rates (Cases II and IV).

The greatest tendency to regular storage of a given proportion of the intake appeared in the case of sodium. There was also a similar tendency for most women to store regularly the same proportions of dietary calcium (Cases I and V). The percentage of total base retained from the diets was expected to conform to the general trend for all the elements. From Table 19 this is seen to be actually the case.

It is noteworthy, however, that there was a tendency to retain approximately half of the excess of base afforded by the diets. The exception to this rule was found only where retentions for all elements were consistently low in a given period (Periods IV-1, V-2 and VI-6) or in one instance where high phosphorus retention was not accomplished by good calcium storage (Period VI-1).

Paths of Excretion for Inorganic Constituents. In Table 20 is shown a summary of the relative amounts of the different minerals occurring in the urine, and Figure VII shows the proportions appearing in the feces. The figures are expressed as percentages of total output rather than of intake, because of wide variations in the amounts stored of some elements.

The physiological behavior of these subjects in regard to the paths of excretion were not markedly unusual. Sodium, potassium, and chlorine, and to a less extent sulfur, appear in the urine chiefly. The average percentages were 92, 86, 97 and 84 respectively. Phosphorus was about equally divided between urine and feces. Only about ⅓ of the magnesium output was found in the urine, whereas calcium excretion by this path varied widely from 6 to 61 percent of the total output. Iron occurred in the urine in only a little more than traces, being almost completely excreted by way of feces.

The small amount of calcium in the urine is a finding in accord with that of a predominance of base-forming elements in the diets (63). The calcium was not needed in the urine in order to maintain normal reaction. The high urinary calcium shown consistently by Case IV was unusual and associated with a low pH. This was not the result of an acid-forming diet nor of a lack of base-forming elements in the urine (Table 16). Since urinary ammonia and titrable acidity were high (Table 26), it has been assumed that unusual amounts of organic acids from some source, probably endogenous, were being eliminated in the urine of this individual. These were referred to in the section on acid-base balance. The urinary magnesium was, like calcium, above the average in this subject. Contradictory

TABLE 20. Excretion of Inorganic Constituents by Way of Urine.
Expressed as Percentage of Total Output

Subject and period	Week of pregnancy	Percentage of output in urine							
		Ca	Mg	Na	K	Cl	S	P	Fe
I-2	27	8	32	90	88	97	84	59	3
I-3	31	7	32	88	90	97	87	63	2
I-4	34	6	34	88	90	97	86	58	2
I-5	38	9	39	90	88	95	87	60	2
II-1	26	25	29	92	92	98	84	58	2
II-2	30	21	29	91	90	98	85	57	1
II-3	34	24	29	93	92	98	85	61	1
IV-1	31	61	29	95	80	98	86	68	2
IV-2	35	55	36	93	88	99	86	70	2
IV-3	39	50	36	90	86	97	84	76	3
V-1	28	28	33	98	92	99	88	64	2
V-2	33	24	21	96	81	98	84	52	2
V-3	38	14	19	95	86	98	83	58	2
V-4	39	14	27	96	85	99	84	61	3
VI-1	18	13	24	93	82	97	81	54	1
VI-2	23	12	23	93	85	96	83	50	2
VI-3	27	21	33	91	84	96	85	61	2
VI-4	31	13	20	82	78	93	69	50	2
VI-5	35	14	30	85	83	95	78	55	4
VI-6	38	10	15	92	86	98	81	53	1
Range									
Minimum		6	15	82	78	93	69	50	1
Maximum		61	39	98	92	99	88	76	3
Averages									
Urine		21	28	92	86	97	84	59	2
Feces		79	72	8	14	3	16	41	98

to the above explanations are the facts that there was a tendency for more than the usual amounts of phosphorus and of sulfur to be excreted by way of urine in this case. Such greater renal excretion of all elements seemed an individual characteristic. It was not associated with tendencies to constipations, as will be seen from moisture content of the feces (Table 25) and as shown in original records by the frequency of stools.

Attention is directed to the rather constant percentage of the magnesium output found in the urine, particularly for Case I, II and IV. Only chlorides and iron of the remaining inorganic constituents show such regular tendencies in paths of excretion. Sulfur, potassium and sodium show about the same degree of irregularity.

These facts are illustrated in Figure VII from the viewpoint of fecal excretions. Thus the path of excretion for calcium is seen to be the most irregular from individual to individual of any of the series, while that for magnesium was similar to that for phosphorus. Wide variations

FIGURE VII

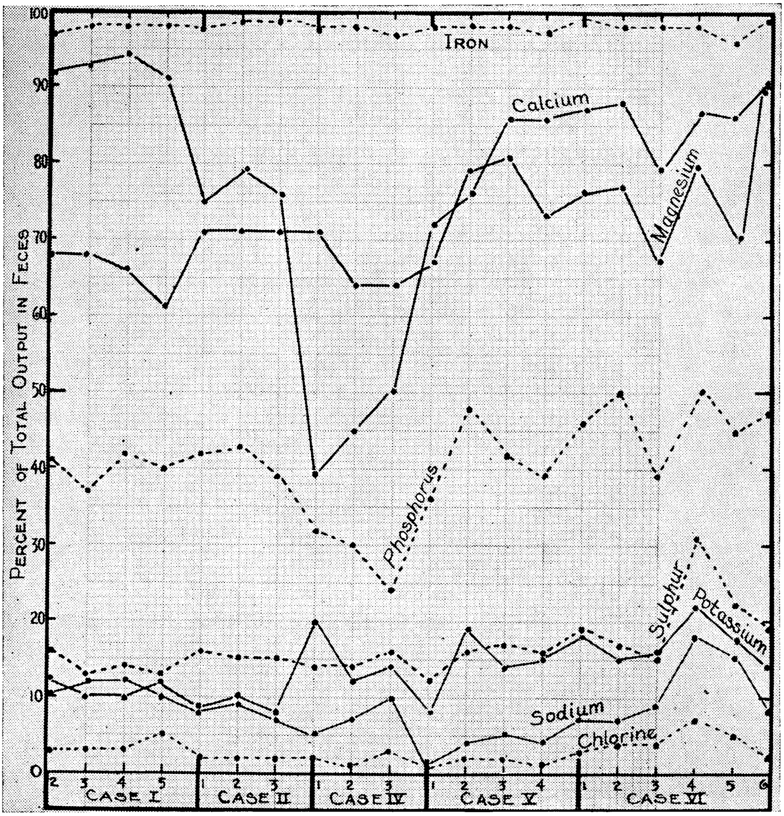


Fig. VII.—The role of the feces as a path for the excretion of certain inorganic substances.

in paths of excretion seem characteristic of certain subjects, Case II as compared to Case VI. It has been shown that varied and larger fecal excretions for Case VI were attributable to the diet changes and to frequent stools. From this chart it is evident that phosphorus occupies a half-way position on the percentage scale, approximately 50 percent being excreted by way of feces.

TABLE 21. Paths of Excretion of Minerals by Other Adults.
(Cited by Hawk and Bergeim)

No. of cases	Moisture content of feces	Fecal output in percent of total output							
		H_2O	N	S	Cl	P	Ca	Mg	K
5	76 (formed stools)	6	10	10	3	36	90	72	18
9	84 (diarrhoeal stools)	16	15	10	9	33	89	68	27

These findings for the paths of excretion are only in general accord with those reported for normal adults. Meyers and Fine (90), comparing the kidney and intestine as excretory routes for various inorganic constituents, report the data summarized in Table 21 as presented by Hawk and Bergeim. Magnesium and chloride excretion in these women followed much the same tendencies as in the normal group. More sulfur and phosphorus was excreted through feces, less potassium and calcium.

The amounts of sodium and potassium in the urine were smaller than that reported by Salkowski (181), who found 95 to 99 percent of the sodium and 89 to 94 percent of the potassium in the urine. Only Case V had excretions approaching these figures for sodium, Cases I and II for potassium, while the average for the group were below the minimum found by Salkowski. Both sodium and potassium were higher in feces when stools were frequent as in Case VI. Figures for fecal sulfur and nitrogen in this subject also approached those for diarrhoeal stools.

NITROGEN

Literature. Nitrogen is the element which has been studied earliest and most extensively in the metabolism of pregnancy. The extent of these investigations may be seen from Table 22, where all of the balances except those of Sillevis (202), some by Bar (10) and those of a very recent paper have been summarized according to investigator (43). The data have been summarized by workers because each group reported represents an era with its peculiar diet trends, or individuals with common physiological characteristics, or subjects living under common dietary regimen.

The higher intakes as well as retentions in early investigations are at once evident. Excepting for Hunscher's three women (106) accustomed to liberal diets, the intakes in later studies average around 11 to 13 grams of nitrogen daily. Retentions in later studies show a wider and more irregular range, with some negative balances but with a consistently lower average storage. Doubtless this trend is the result of the tendency to lower nitrogen in present-day dietaries (44). The low urinary nitrogen, 8.22 grams daily, found in the pregnancies reported by Rowe and associates (177) also supports this hypothesis.

Since a preponderance of the high balances published by early workers were for late gestation, it follows that figures for average storage, obtained from a compilation of all balances with reference to the stage of gestation, would show greater retentions in late pregnancy. Such a compilation has

TABLE 22. Summary of Nitrogen Balance Studies.

Daily averages

Investigator	Date	No. of subjects	No. of balances	Total days	Nitrogen intake			Nitrogen retention			Comment
					Max'm	Min'm	Mean	Max'm	Min'm	Mean	
					gm.	gm.	gm.	gm.	gm.	gm.	
Zacharjewsky	1893	6	6	60	11.55	24.08	18.54	0.95	5.37	3.05	6-18 da. antepartum; limited diet.
Schrader	1900	2	2	11	22.20	22.54	22.37	5.97	6.75	6.36	4-7 da. antepartum; computed diet.
Slemmons	1904	4	4	39	13.80	16.77	14.89	0.42	4.72	2.39	2 sets twins; computed diet.
Hahl	1905	2	7	41	15.89	20.79	18.00	0.60	4.11	2.04	17-24 da. antepartum.
Bar	1905	3	3	34	13.71	21.66	17.43	5.10	10.07	6.68	Low Fecal N.
Hoffstrœm	1909	1	23	161	7.15	17.46	12.90	—1.21	3.53	1.84	Continuous observations.
Landsberg	1912	6	13	53	12.03	18.90	16.10	1.38	3.94	2.50	
Landsberg	1914	9	14	70	12.21	16.83	16.10	0.05	2.98	1.23	
Wilson	1916	3	37	259	7.88	19.72	13.14	0.46	6.37	2.84	Continuous observations; Computed diet.
Coons-Blunt	1930	9	23	116	7.74	14.31	11.03	—0.43	8.45	2.15	Home diets.
Sandiford et al.	1930	1	5	50	11.20	14.92	13.88	—1.90	1.10	0.30	Computed diet; feces not analyzed.
Macy et al.	1931	1	2	15	11.50	12.20	11.80	2.20	2.90	2.55	Specified diet.
Hunscher et al.	1933	3	12	49	11.58	23.58	17.58	—0.67	6.25	1.90	Pregnancy subsequent to heavy lactation.
This study	1932	6	23	92	8.08	14.62	11.58	0.49	5.20	1.97	Home diets.

been made by Hunscher and associates (106). They found that the total 954 daily nitrogen balance studies available in the literature were distributed from the 3rd to the 10th lunar months as follows: 30, 29, 71, 81, 131, 132, 134, and 365 days of balances respectively. Mean retentions for the months amounted correspondingly to 2.84, —0.15, 1.99, 2.59, 2.18, 2.18, 3.23, and 3.46 grams of nitrogen daily. The average daily retention throughout gestation was 2.28 grams, and 48 percent of the 954 balances fell within the range of 1 to 3 grams of nitrogen stored daily. Although there were marked variations in the retentions on the same level of intake, it was evident that negative balances predominated when the intake was below 10 grams daily but were entirely lacking on intakes above 18 grams of nitrogen daily.

Causes for irregularities in storage found in more recent studies have been discussed in a previous publication (43). The low calorie as well as low protein intake in some cases, the poor quality of protein coming from an excess of fruits and vegetables, and a minimum of animal proteins (29) (Table 27) probably account for many instances of low storage. History of undernutrition in the mother, particularly the primipara, periods of nausea or semi-starvation prior to balance experiments carried out in early gestation, impulses to replenish depleted body reserves and supply growth material to a rapidly developing fetus, may all be factors concurring in unusually high nitrogen retention, especially in early pregnancy. The absence of regularity marks the influence of factors extraneous to fetal demand which tends to be regular in its progression (Figure II). Thus for nitrogen, as for calcium and iron, there seems to be a tendency to store plentifully whenever during the pregnancy all factors are conducive to retention. In this way adequate maternal reserves are built up in advance of the heaviest needs of fetal development and lactation.

Hoffstrœm's continuous study of one case enabled him to compute a total nitrogen retention of 310 grams for all of gestation. An average storage of 2 grams daily from the 3rd to the 10th month would amount to approximately 400 grams of nitrogen retained. However, only Wilson's (247) cases studied over long period of time show indications of such continuous high storage.

As was shown in the discussion of fetal composition, a storage of 1 gram of nitrogen daily is ample to meet fetal needs even at the time of highest demand. Hunscher et al. (106) estimated a total of 145 grams of nitrogen was consumed by the fetus, fetal structures, hypertrophied tissues and developing breasts in the gravid mother. Thus it is concluded that actual storage may be considerably in excess of the needs of embryonic development.

Harding (86) has reviewed the significance of the various phases of nitrogen metabolism during pregnancy. Sandiford, Wheeler and Boothby (182) presented urinalysis along with the balances on their subject. They found creatine output increased slightly, but creatinine excretion quite constant until the last month when there was a sudden decrease. Ammonia nitrogen was also slightly increased, as was that in the cases studied by Rowe and coworkers (177). Otherwise the nitrogen partition in the urine of pregnant women differed little from the normal.

Nitrogen Balances in This Study. Table 23 shows the individual nitrogen balances found for this series of 6 women, a total of 24 periods of four days each. In addition to the daily intake and outgo this table shows the nitrogen per kilo in intake and the amounts retained.

The intake varied from 8.08 to 19.13 grams daily, with a mean of 11.58 grams. This was lower than the intake for most other studies shown in Table 22. On the whole there was a tendency to lower nitrogen retention by these women than that recorded for other groups on similar intake (41, 142, 247). Although the retention varied somewhat, in 58 percent of the periods it was between 1 and 2 grams daily, with an average of 1.97 for the entire group. Thus, the storage was not only lower but more constant from woman to woman than those of previous studies in which 48 percent of the balances were between 1 and 3 grams daily (106). Two-thirds of the balances gave a storage equivalent to that expected for only the 4th and 5th months of gestation, according to the norm constructed by Hunscher, while in only four periods was the expected rate of storage for the 9th and 10th months approached.

In this group, differences in retention rates were apparently unrelated to any period of gestation. Tendencies to high storage were individualistic, exhibited only by Cases II and VI. From period to period, however, the retention was related to the intake (VI-2, VI-3, V and III). The dietary nitrogen in Periods I-0 and V-2 doubtless represented a near-maintenance quota for these individuals. Further, when high storage paralleled high intake, doubtless the quantity consumed represented a measure of the appetite which in turn had been prompted by the metabolic demand and impulse to store. It seems safe to postulate that better quality of protein

TABLE 23. Nitrogen Retention by Oklahoma Women during Pregnancy.
Daily averages

Subject and period	Week of pregnancy	Nitrogen intake	Nitrogen outgo			Nitrogen balance	Nitrogen per kilo	
			Urine	Feces	Total		Intake	Retained
		gm.	gm.	gm.	gm.	gm.	gm.	gm.
I-0	—4	8.16	6.82	0.85	7.67	0.49	0.17	0.010
I-1	2	9.37	6.79	0.88	7.67	1.70	0.19	0.030
I-2	27	9.61	7.40	1.04	8.44	1.17	0.17	0.021
I-3	31	9.89	7.74	1.00	8.74	1.15	0.17	0.019
I-4	34	9.88	7.83	1.04	8.88	1.00	0.16	0.016
I-5	38	8.80	5.98	0.91	6.89	1.91	0.14	0.030
II-1	26	11.84	8.13	1.26	9.39	2.44	0.22	0.045
II-2	30	14.26	9.13	1.35	10.48	3.78	0.25	0.067
II-3	34	14.62	9.92	1.60	11.52	3.10	0.25	0.052
III-1	33	11.56	8.57	1.58	10.15	1.40	0.16	0.020
III-2	37	12.86	8.85	1.75	10.60	2.26	0.18	0.031
IV-1	31	11.84	9.18	1.22	10.40	1.44	0.14	0.017
IV-2	35	9.74	7.18	1.27	8.45	1.29	0.11	0.015
IV-3	39	11.64	8.64	1.45	10.09	1.56	0.13	0.017
V-1	28	10.08	7.38	1.02	8.40	1.68	0.16	0.026
V-2	33	8.08	6.02	1.33	7.35	0.73	0.12	0.011
V-3	38	10.01	5.85	1.34	7.19	2.82	0.15	0.043
V-4	39	8.97	6.61	1.25	7.86	1.11	0.14	0.017
VI-1	18	17.70	12.46	2.07	14.53	3.17	0.32	0.058
VI-2	23	19.13	11.99	1.94	13.93	5.20	0.32	0.087
VI-3	27	13.54	10.59	1.50	12.09	1.45	0.22	0.023
VI-4	31	12.01	8.12	2.04	10.16	1.85	0.18	0.028
VI-5	35	11.96	7.64	1.61	9.25	2.71	0.18	0.041
VI-6	38	9.90	7.31	1.57	8.85	1.02	0.15	0.015

in the diets of Cases II and VI were largely responsible for the better utilization of nitrogen. Table 27 in the section on dietaries shows a higher proportion of meat, milk, eggs and liver in these diets.

The higher nitrogen intake for certain individuals was more evident when the quantities are compared with reference to body weight. Thus a range of 0.14 to 0.19 grams of nitrogen per kilo included 15 of the 24 periods, while Cases II and VI showed intakes as high as 0.25 to 0.32 grams per kilo. The storage per kilo was irregular with respect to the stage of pregnancy, but high on the better diets (II and VI), lowest on the poorest intakes (V-2).

The question naturally arises as to what extent the average figures for nitrogen intake represent habitual food nitrogen levels, and how great is the variation from day to day. The facts involved apply to the entire metabolism study since the 4-day periods of self-chosen diets have been assumed to be fairly typical for each individual and for the period of pregnancy in question. A measure of the fluctuations in nitrogen intake would be reflected in the daily urinary nitrogen. Such data are presented in Table 24, in connection with the average found for the com-

TABLE 24. Daily Variations in Total Urinary Nitrogen.

Subject and period	Week of pregnancy	Daily urinary nitrogen				Average for period	Analyses of composite sample
		First day	Second day	Third day	Fourth day		
		gm.	gm.	gm.	gm.	gm.	gm.
I-2	27	7.28	7.05	7.98	6.71	7.25	7.40
I-3	31	8.35	7.53	7.86	7.04	7.69	7.74
I-4	34	7.74	8.44	7.93	7.22	7.83	7.95
I-5	38	5.94	5.71	6.39	6.04	6.02	5.98
II-1	26	7.25	8.62	7.94	8.28	8.02	8.13
II-2	30	9.80	9.38	8.49	8.69	9.09	9.13
II-3	34	8.66	9.85	10.69	9.97	9.77	9.92
III-1	33	8.12	9.60	8.52	8.53	8.69	8.57
	37	9.34	9.10	8.14	9.08	8.91	8.85
IV-1	31	8.09	7.48	11.87	9.42	9.21	9.18
IV-2	35	6.24	7.45	6.99	8.04	7.21	7.18
IV-3	39	10.82	8.12	7.95	7.82	8.68	8.64
V-1	28	6.96	7.61	8.19	6.70	7.45	7.38
V-2	33	5.64	5.39	5.71	7.36	6.02	6.02
V-3	38	5.70	5.76	6.00	5.94	5.85	5.85
V-4	39	7.50	5.73	6.32	6.96	6.63	6.61
VI-1	18	11.02	12.11	12.24	13.93	12.33	12.45
VI-2	23	12.51	10.48	12.36	12.81	12.04	11.99
VI-3	27	10.63	12.20	10.20	9.58	10.65	10.59
VI-4	31	7.70	9.36	9.00	6.30	8.09	8.12
VI-5	35	6.48	8.05	8.17	7.70	7.50	7.64
VI-6	38	7.91	6.85	6.92	7.57	7.31	7.38

posite. The table shows that in most periods the quantities of nitrogen-containing foods probably varied little from day to day. The widest range in daily urinary nitrogen was 4 grams (Period IV-1), and in two periods there was as much as 3 grams difference between the lowest and highest figures. In the majority of periods, however, the range of variation was one gram or less. The last two columns of this table show something of the accuracy to be expected in making urine composites from daily aliquots with one set of chemical determinations, as compared to making daily determinations. Usually the difference in the two methods was within the range of experimental error.

Rates of Digestion and Absorption. Various ratios have been used to express degrees of digestion and absorption. Some of these for the subjects of this experiment are shown in Table 25. In few instances did the coefficient of digestibility for nitrogen equal or exceed the commonly used 90 percent. The range was only 83 to 91, with many around 85, and an average of only 88. However, the entire group showed better absorp-

TABLE 25. Measures of Rates of Digestion and Absorption.

Case-Period	Solids			Nitrogen	Sulfur in feces	Moisture content of feces	Iron in feces
	Food daily (dry)	Feces daily (dry)	Ratio Food: Feces	Coeff. of digestibility	Percent of intake		(Percent intake)
	gm.	gm.	gm.	percent	percent	percent	
I-0	355	17	20	90	....	....	....
I-1	433	17	25	91	....	....	....
I-2	433	24	18	89	17	80	73
I-3	461	21	22	90	13	81	63
I-4	439	23	19	90	16	79	79
I-5	482	21	23	90	14	84	72
II-1	538	26	20	89	14	73	64
II-2	560	26	21	91	14	76	96
II-3	560	31	18	89	15	69	91
III-1	518	36	14	86	....	85	89
III-2	627	28	22	86	....	84	81
IV-1	575	27	21	90	14	88	99
IV-2	541	25	22	86	15	81	92
IV-3	705	31	23	88	16	87	62
V-1	499	18	28	90	12	71	68
V-2	454	27	17	84	17	70	97
V-3	519	26	20	87	17	80	89
V-4	501	25	20	86	17	72	69
VI-1	577	41	14	88	19	82	79
VI-2	617	33	18	90	15	83	82
VI-3	542	26	21	89	15	83	80
VI-4	553	39	14	83	28	87	93
VI-5	492	33	15	87	19	86	88
VI-6	465	29	16	84	19	80	88
Average	530	28	20	88	16	79	81
Chicago series	464	34	14	83	....	....	....

tion of nitrogen than did one previously reported by Coons and Blunt (41). For that group the average coefficient was 83, equal to the lowest in the present series. Likewise, the average for this series was equal to the maximum, 88, for the former series. Doubtless this better digestion aided in the increased utilization of all elements, especially calcium. Case VI presented the poorest record for coefficients of digestibility and her good storage must be attributed to the large total intake of high quality protein.

Ratio of food solids to feces solids is another way of expressing completeness of digestion and absorption. The food solids in the daily diets of these women were surprisingly constant, around 500 grams daily and averaging 530 grams. Feces solids showed more variations, 18 to 41, average 28 grams daily. Accordingly, ratios of food solids to feces solids varied from 14 to 38, with an average of 20. By this criterion also, digestion and absorption was poorer in the Chicago group previously reported. The more frequent use of laxatives and the greater prevalence of constipation in the southern subjects is explained by these tendencies to lower fecal residues.

Meyers and Fine (Table 21), cited by Hawk and Bergeim, found 74 to 79, average 76, percent moisture in well formed stools, while diarrhoeal stools contained 79 to 89, average 84, percent moisture. The moisture content of the feces in this series ranged from 69 to 88 percent. Six periods would classify as constipated and 16 as diarrhoeal by the above criteria, the latter including 6 periods for Case VI. On the other hand the patients themselves complained of constipation in periods represented by stools with moisture content less than 75, and undigested food particles representing diarrhoeal tendencies appeared in the stools only when the moisture content exceeded 85 percent. Further, stools with moisture content of 69 to 74 percent frequently represented gastro-intestinal rates of 48 to 72 hours as measured by the carmine markers used at the beginning and end of the balance experiments. The shortest rate measured by the markers was 12 hours for Case VI-1, and for that day the average moisture content was 88 percent.

Sulfur metabolism is closely related to that of nitrogen. The percent of sulfur intake in the feces was remarkably constant except for three instances. The normal range was 14 to 17 percent except for Case VI. In Period VI-4 the fecal sulfur amounted to 28 percent, which corresponds to the period of lowest coefficient of digestibility for nitrogen, of highest moisture content of feces, and of largest amount of feces solids. Retention of most inorganic constituents of the diet was correspondingly poor in this period. Fecal sulfur as low as 12 percent of the intake was associated with definite constipation (Period V-1) and high ratios of food to feces solids. Thus the sulfur content of the feces appeared to be a fairly reliable index to the efficiency of utilization and absorption, correlating closely with indices based on fecal nitrogen, moisture and solids.

Iron in the feces is sometimes used as a measure of digestion or utilization of a diet or ration (21), and diarrhoeas frequently result in negative iron balances (40, 193). However, when there is occasion for iron storage, fecal iron is no measure of digestion or absorption. This is because most of the iron is excreted by way of the feces (Figure VII) and wide fluctuations in storage tend to destroy any regularity in iron content of feces (Cases IV and V). From Table 25 it may be seen that fecal iron was 93 percent of the intake in Period VI-4, already referred to as characterized by frequent stools. However, fecal iron was even higher in Period II-2 where excessive dietary iron failed to induce good retention.

TABLE 26. Constituents Characterizing Urine.

Daily averages

Case-Period	Wk. of pregnancy	Body weight	Urine volume	Specific gravity	Nitrogen		Creatinine		Ammonia N		Urine acidity		
					Total	Per kilo	Total	Per kilo	Weight	Percent of total	pH	Titrable acidity	Excess base
		kilo	cc.		gm.	gm.	gm.	mgm.	gm.	%		cc.	0.1 N.
I-2	27	56.7	1637	1.012	7.40	0.13	1.00	18	0.19	2.5	6.6	196	21
I-3	31	59.0	1655	1.013	7.74	0.13	0.90	15	0.20	2.5	6.6	283	—5
I-4	34	61.2	1914	1.011	7.83	0.13	0.95	15	0.22	2.8	6.7	197	89
I-5	38	63.5	2045	1.010	5.98	0.09	0.96	15	0.37	6.2	6.8	259	282
II-1	26	54.4	1900	1.014	8.13	0.15	0.88	16	0.25	3.1	6.8	187	196
II-2	30	56.2	1902	1.013	9.13	0.16	0.94	17	0.32	3.4	7.1	209	61
II-3	34	59.4	1950	1.013	9.92	0.17	0.93	16	0.37	3.8	7.0	271	468
III-1	33	71.2	2370	1.012	8.57	0.12	1.10	15	0.20	2.3	7.9*	124	*
III-2	37	72.5	2792	1.011	8.85	0.12	1.14	16	0.14	1.6	8.0	131	
IV-1	31	86.6	1975	1.012	9.18	0.11	1.23	14	0.38	4.1	5.9	444	319
IV-2	35	88.4	1980	1.010	7.18	0.08	1.20	14	0.33	4.6	6.3	261	632
IV-3	39	90.7	1942	1.012	8.64	0.09	1.40	15	0.52	6.0	5.8	364	102
V-1	28	63.5	1775	1.012	7.38	0.12	0.96	15	0.26	3.5	7.4	109	314
V-2	33	64.4	1450	1.011	6.02	0.09	0.80	12	0.40	6.6	7.6	86	243
V-3	38	65.3	1265	1.011	5.85	0.09	1.07	16	0.36	6.2	7.2	122	49
V-4	39	65.8	1875	1.009	6.61	0.10	1.10	16	0.36	5.5	7.0	146	27
VI-1	18	54.9	2292	1.013	12.46	0.23	0.93	17	0.38	3.1	7.0	273	77
VI-2	23	60.0	2110	1.013	11.99	0.20	0.93	16	0.35	2.9	6.8	297	11
VI-3	27	62.6	1530	1.014	10.59	0.17	0.90	14	0.39	3.6	6.1	349	—54
VI-4	31	65.3	1727	1.011	8.12	0.12	0.92	14	0.33	4.0	6.2	265	—41
VI-5	35	65.8	1582	1.012	7.64	0.12	1.00	15	0.32	4.2	5.6	323	—51
VI-6	38	66.2	1992	1.009	7.31	0.11	1.07	16	0.36	5.0	5.8	284	101

*$NaHCO_3$, 12-20 grams ingested daily. Total base undetermined.

Some Properties of Urine Excreted Daily. Table 26 shows the data resulting from certain daily routine analyses of the urine. No effort was made to determine completely the nitrogen partition in the urine. Urine volume was of interest in relation to specific gravity because it showed habitual tendencies of the subjects in the way of water excretion through the kidneys, yet enough variation in both volume and specific gravity to indicate that kidney function was normal (154). There was a distinct tendency to a more dilute urine as gestation progressed. This was due to increase in urine volume and to retention of electrolytes.

The nitrogen content of the urine, when referred to body weight, was fairly constant through the series. On low diets it probably expressed an approximate minimum of protein intake needed for such individuals. This approached 0.09 gram per kilo in 8 periods. On liberal diets, excretion of nitrogen amounted to as much as 0.23 gram per kilo.

Creatinine was determined daily as a measure of completeness in urine collections. The amounts were fairly constant, 14 to 17, except one low of 12 mgm. per kilo, over a four-day period. Since total urinary nitrogen was also low in this period, as was storage and intake, it is doubtful whether the figures refer to incomplete collections.

Other facts in Table 26 pertain to regulation of acidity in metabolism and its correlation with the acid-base findings.

When the excess base in the urine was low the titrable acidity was high (Periods I-3, VI-3 and VI-6) and frequently the ammonia nitrogen was above normal (Periods V-3 and V-4). Such relations were to be expected (163, 177, 182). Ammonia nitrogen output showed considerable variation from time to time in the same subject (Cases I and V). In 6 periods the amounts were above the 4.6 percent of total urinary nitrogen given as normal by Rowe and associates (177). Likewise, ammonia nitrogen was low and the pH was high for Case III, who ingested much sodium bicarbonate. Acidification of this urine showed excessive amounts of carbonates eliminated by this route, another indication of the presence of an excess of base-forming elements. Other low figures for ammonia nitrogen were attributed to excess base in the diets (Cases I and VI) (cf. also Table 16).

Attention is directed to the acidity findings for the urine of Case IV. A fairly high excess base in the urine was associated with a high ammonia and titrable acidity as well as low pH. These facts suggest the presence of an abnormal amount of organic acid and phosphates in the urine. Since the proportion of phosphorus excreted by way of urine was not usually high and large amounts of calcium were also present, the question of the role of organic acids becomes paramount. Either an unusual amount of organic acids of the diet escaped normal oxidation in the body of this subject or there was a tendency to endogenous production of such acids (189, 190). Varying states of acidosis seem common to pregnancy (178, 186). Obviously, the problem deserves further investigation.

SUMMARY ON NITROGEN METABOLISM

1. No negative balances occurred. Usually the storage was low, amounting in almost two-thirds of the periods to 1 to 2 grams daily.

2. When high storage occurred it was associated with diets superior in respect to minerals and vitamins as well as protein.

3. Digestion and absorption by this group of women seemed to proceed on a high plane, whether described by the proportion of solids in the feces, or by the percentage of nitrogen, sulfur or moisture in the

stools. Iron in the feces proved unreliable as a criterion for judging absorption.

4. Urinary acidity did not always reflect the acid-base values found by analyses of the diets. The role of organic acids in this connection is discussed.

SELF-SELECTED DIETARIES OF WOMEN DURING PREGNANCY

Literature. Few even partially complete dietary studies on women during pregnancy are recorded in the literature (39, 97, 182, 200). Balance experiments constituted the primary aim in these few observations. However, parallel metabolic studies have aided materially in evaluating the adequacy of the diets which have been reported. Furthermore, the accuracy necessitated by balance experiments has resulted in chemically

TABLE 27. Some Qualitative Aspects of Diets Chosen by Women; Average Daily Intake of Foodstuffs by Periods.[1]

Case-period	Wk. of pregnancy	Meat	Liver	Eggs	Milk	Butter	Green vegetables	Other vegetables [2]	Total fruits	Cereal [3] (dry)
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
I-2	27	4	9	36	697	24	30	528	388	75
I-3	31	31	0	39	536	17	36	445	275	88
I-4	34	30	0	30	600	21	19	417	285	111
I-5	38	21	0	22	527	19	42	326	305	88
II-1	26	34	17	56	593	44	16	443	156	55
II-2	30	78	12	68	835	32	38	487	312	66
II-3	34	62	19	61	726	31	95	554	307	95
III-1	33	144	0	42	178	38	54	559	260	104
III-2	37	43	0	38	459	30	56	524	272	152
IV-1	31	115	0	11	329	28	114	347	260	141
IV-2	35	20	0	11	465	54	263	618	405	104
IV-3	39	46	0		649	59	418	739	499	127
V-1	28	30	0	60	209	22	94	358	97	110
V-2	33	11	0	58	195	47	47	248	145	122
V-3	38	57	0	19	249	48	115	425	262	143
V-4	39	24	0	22	485	31	69	377	182	107
VI-1	18	30	48	60	1162	24	47	225	98	138
VI-2	23	36	21	50	1285	42	104	490	389	130
VI-3	27	31	0	44	796	37	58	381	163	74
VI-4	31	10	0	31	1008	32	55	564	367	108
VI-5	35	0	0	10	887	19	57	595	214	82
VI-6	38	0	0	0	957	27	112	304	51	116
Average		39	21	35	628	32	88	433	247	101

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[1] Does not include concentrated sweets, fat and constituents of complicated mixtures as croquettes, puddings, sauces, etc.

[2] All vegetables including potatoes, but excluding green vegetables listed separately.

[3] Cereals, dry basis, reckoned as 1, 1/5, ½ and 1/3 of actual weight respectively, for precooked cereals, hot cooked cereals, cold yeast bread and hot quick breads.

analyzed diets for the most part instead of those computed from existing data on food composition.

With our changing dietary habits there is need for more complete information concerning the food actually consumed by the normal present-day American woman during pregnancy when she is living under the freedom of ordinary home conditions with unrestricted choice of food. In view of the excellent metabolic performance of the women of this study, it seemed worthwhile to study their self-selected diets further as to fat, carbohydrate, protein and calorie content, and to consider these as a whole along with the data obtained in balance experiments showing the intake of inorganic constituents. All data presented herein represent actual chemical analyses. It is hoped that eventually the total vitamin content of the diets can be estimated, but this phase of the study has not proceeded far enough to be included in the present report.

This Study. Table 27 describes in a roughly quantitative manner some of the foodstuff entering into the diets chosen by these women. Figures in this table represent averages for the four-day period, hence may seem low in instances where an item was chosen only once. For example, if liver was served only once, in a 60-gram portion, the table shows an average of 15 grams daily.

From this table it is evident that meat was little used and liver appeared in only six periods. Good retentions of minerals were recorded in all six, however. Eggs averaged less than one or 50 grams per day. Except for Cases III and V, milk consumption ranged from a pint to more than a quart daily. Case VI chose most of this food. These figures do not include milk concealed in cooking, such as puddings, sauces, ice creams and baked products. They do include cocoa or other milk beverages. Choices of cereals and green vegetables express individual habits with tendencies for variations in amounts of the two to run parallel (Cases IV and VI). Approximately a pound of vegetables other than green leafy was consumed daily. The quantities of both vegetables and fruits used by Case IV exceeded those of any other individual. This fact has been referred to in connection with her apparent state of continued acidosis. Butter was used least by Case I, but raw fruits appeared most in her diet and in the diet of Case IV.

In general Case IV consumed diets most abundant in every respect except milk. However, her retentions were poor except for the last period. This behavior was believed to be due to plentiful reserves carried by her at the beginning of pregnancy and accounting for some of her overweight condition.

Diets of Cases II and VI ranked next best from present-day criteria with respect to the sources of vitamins, minerals and proteins. These two women also tended to show the best metabolic performance, and that in spite of the frequent stools and greater fecal losses for Case VI.

The poorest diet, as regards quantities of milk, vegetables, raw fruits and total food intake, was found for Case V, who also recorded lowest retention of minerals and of nitrogen as well as complete failure in the performance of lactation.

With this general summary of the character of the diets in mind, attention is called to Table 28 showing the total calories, protein, fat and carbohydrate per 24 hours for each period. In addition is shown the distribution of calories between protein, fat and carbohydrate, and the nutritive ratios for the individual periods. By nutritive ratio is meant the number of calories from digestible protein in proportion to those from fat and carbohydrate.

Narrow ratios signify much protein, wide ratios little protein, in comparison to the amounts of fat and carbohydrate.

The average 24-hour intake fluctuated considerably from period to period and seemed little related to any phase of gestation. The highest food consumption tended to come early in pregnancy for those women with low reserves (Case VI). The increase in food intake due to pregnancy cannot be estimated from this series, since only for Case I was there a pre-gestation study, and altogether too few records are for early gestation, while none are continuous for all of pregnancy.

The protein intake was low, both by weight and by percentage of total calories. In only two instances was it over 100 grams daily, while 5 of the 25 diets contained less than 60 grams daily. These figures obtain in spite of the fact that the method used for computing crude protein leads to a positive error more often than to a negative one, the 6.25 factor being ac-

TABLE 28. Proximate Composition of Diets Chosen by Women.

Case-period	Wk. of pregnancy	Average daily consumption				Distribution of calories[1]	Nutritive[2] ratio. 1:
		Calories	Protein	Fat	Carbohydrate		
			gm.	gm.	gm.	percent	
I-0	—4	1724	57	....[3]	....[3]	12: ....[3]	7.4
I-1	2	2115	59	....	....	11:	7.9
I-2	27	2164	60	87	286	11:36:53	8.0
I-3	31	2249	62	81	318	11:32:57	8.1
I-4	34	2136	62	76	301	12:32:56	7.6
I-5	38	2299	55	74	353	10:29:61	9.4
II-1	26	2752	74	120	344	11:39:50	8.3
II-2	30	2880	89	128	343	12:40:48	7.1
II-3	34	2870	91	126	343	12:40:48	6.9
III-1	33	2702	72	126	320	11:42:47	8.4
III-2	37	3093	80	117	430	10:34:56	8.7
IV-1	31	2980	74	136	365	10:41:49	9.1
IV-2	35	2744	61	116	364	9:38:53	10.2
IV-3	39	3585	73	153	479	8:39:53	11.3
V-1	28	2461	63	93	343	10:34:56	8.8
V-2	33	2293	50	95	309	9:37:54	10.4
V-3	38	2601	63	105	351	10:36:54	9.3
V-4	39	2459	56	91	354	9:33:58	10.0
VI-1	18	2982	111	134	333	15:40:45	5.7
VI-2	23	3043	120	115	382	16:34:50	5.3
VI-3	27	2674	85	101	356	13:34:53	6.9
VI-4	31	2592	75	76	402	12:26:62	7.6
VI-5	35	2378	75	82	335	13:31:56	6.9
VI-6	38	2170	62	62	341	11:26:63	7.7

[1] Protein :Fat :Carbohylrate.

[2] $$\text{Nutritive ratio} = \frac{\text{Calories from digestible protein}}{\text{Calories from carbohydrate and fat}}.$$

[3] Undetermined.

curate for the proteins in animal foods and legumes, but too high for the proteins in cereals and vegetables (108), for which 5.8 is a more accurate factor.

The distribution of calories allotted a minimum, 8 to 12 percent, to protein in most cases. Only Case VI chose liberally, 15 to 16 percent, a part of the time. There was a corresponding tendency for the calories from carbohydrate to be high if measured by commonly used standards: 10 to 15 percent from protein, 35 to 38 percent from fat, and 47 to 55 percent from carbohydrate (172). The means for percentage distribution of calories in the diets of these women were 11, 34 and 54, respectively, compared to 15, 37 and 48 for the Detroit women (200) with superior lactation capacities and diets liberal in every respect.

There was considerable range in the variation of the nutritive ratio of these diets, 1:5.3 to 1:11.3, with a mean of 1:7.9. Hoobler (99) found a ratio of 1:6 or less best adapted to the needs of the nursing mother and Adair (2) recommended ratios narrower than 1:8 for lactation. It is noteworthy that the women least successful in lactation showed widest ratios in their diets (Cases I and V) and also that the Detroit women (200) who were capable of heavy milk production chose during pregnancy diets characterized by a mean ratio 1:5.2, which was less than the narrowest of this series.

All such data must be evaluated, of course, with a knowledge of the quality of the protein, whether of animal or vegetable origin and of the chief amino acids present, as well as the adequacy of other dietary factors. They do emphasize, however, the probability that, in the enthusiasm for including plenty of fruits and vegetables in the diet, there may be a tendency to slight the quantity and quality of protein. Lane (118) has described an apparently successful human reproductive cycle in one mother on a diet very low in animal protein, but no figures were presented for biochemical analyses to indicate some of the metabolic changes taking place during pregnancy as well as during the growth of the infants.

Table 29, giving 24-hour intakes of the various diet constituents, shows individual tendencies of each woman in the matter of food habits. At the end of the table is given the weighted mean for this group as well as for others reported in the literature. Many are incomplete as to content of minerals in the diets. Final comparison may be made with Sherman's figures compiled from adult dietaries and computed on the basis of 3000 calories per man per day, and also with Macy's and Hunscher's proposed standards for pregnancy.

The calcium content of the diets ranged from less than a gram to almost 2 grams daily. Most of the values were around 1.2 grams. Toverud and Toverud, like Macy and Hunscher, concluded (231) that the daily calcium intake must not fall below 1.6 to 1.7 grams daily, the phosphorus 2.0 grams. However, the accessory factors concerned with calcium utilization must be considered because women of this series with intakes as low as 0.9 grams daily showed good retention, and in no instance was the retention poor on intakes of 1.2 grams or above. Similar observations were true for phosphorus, 1.5 being adequate for good retention, with two exceptions. Only the two women with superior diets measured up to Toverud's standards, yet all stored calcium well, phosphorus fairly well.

The amounts of dietary magnesium were more constant, 0.3 to 0.4 grams daily. They were lowest in the diets of Cases I and V, and these diets were also lowest in fruits, vegetables and milk. Retention of this element was fair, although one intake of 0.29 resulted in equilibrium. Furthermore, the average intakes for these women were somewhat above those reported

TABLE 29. Probable Adequacy of the Self-Selected Diets; Average 24-hour Intake for all Periods According to Subject.

SUBJECT	Wt.[1]	Days observed	Total Calories	Pro-tein	Fat	Carbo-hydrate	Cal-cium	Phos-phorus	Mag-nesium	Iron	Sodium	Potas-sium	Chlor-ine	Sulfur	Excess base	Total water
	kgm.			gm.	gm.	gm.	gm.	gm.	gm.	mgm.	gm.	gm.	gm.	gm.	cc. 0.1 N	cc.
I	49.0	22	2212	60	79	314	1.136	1.408	0.324	10.7	3.784	3.360	3.979	0.604	1120	2554
II	49.9	14	2834	85	125	343	1.827	1.972	0.500	25.5	4.838	4.385	5.301	0.847	1316	2538
III	59.4	8	2897	76	121	375	1.171	1.581	0.340	13.7	.......[2]	....	....	....	....	3953
IV	84.8	12	3103	69	135	403	1.273	1.485	0.422	13.9	5.932	4.025	7.515	0.703	1169	3419
V	54.4	16	2453	58	96	339	0.950	1.191	0.337	12.9	4.757	2.997	6.099	0.602	802	2400
VI	50.3	24	2640	88	95	358	1.846	1.976	0.413	17.7	5.216	3.672	6.277	0.942	942	2881
Weighted Mean			2618	73	105	352	1.418	1.632	0.393	15.7	4.907	3.630	5.822	0.757	1040	2748
Chicago group			2476	69	101	321	1.096	1.431	0.375	14.7	.......[2]	....	....	....	....	....
Hoffstrœm case			2283	81	123	196	1.712	1.952	0.282	.......[2]	....	....	....	1.101	....	....
Sandiford case			2111	87	108	184	1.100	1.440	0.260	.......[2]	3.970	3.700	6.090	1.030	....	....
Macy's 3 cases			2930	113	126	329	1.920	2.247	.......[2]	....	....	....	....	....	....	....
Sherman's Dietaries			3000	75	....	....	0.73	1.59	0.34	17.3	1.95	3.40	2.88	1.30	....	....
Macy-Hunscher Standards			....	70 to 100	....	....	1.6	2.0	0.3	20.0	....	....	....	....	....	....

[1] Pre-gravid weight.
[2] Undetermined.
[3] Average amounts in 150 American dietaries.

in the literature, although very close to those of the Chicago group who exhibited frequent negative balances. The average intake is well above the 0.3 grams daily recommended by Macy and Hunscher for pregnancy. On the other hand these higher intakes of magnesium seemed desirable in view of the fact that the Oklahoma women retained more on the more liberal diets and that the retention did not represent a substitution of this element for calcium or an antagonizing of calcium retention for which the rates were excellent.

The iron intakes showed a wide range, 11 to 25 milligrams per day on the average per individual. However, as has been pointed out already in the section on iron metabolism, the total intake was not the controlling factor in the matter of retention. Since all studies reported averaged an intake of about 15 mgm. (39) and the Chicago women made good retentions on this amount, it seems probable that this quantity may suffice as a minimum if other dietary and physiological conditions are favorable to good utilization. Otherwise, 20 mgm. as recommended by Rose (173) and by Macy (143) is a safer figure.

There seemed to be no marked deficiencies in the retention of sodium, potassium and chlorine. Negative balances which occurred were explained by metabolic upsets, such as dehydration, rather than by low total intake. Sodium intakes ranged from 4 to 6 grams daily per person, potassium lower, 3 to 4.5 grams daily, and chlorine, the highest of the three, 4.0 to 7.5 grams daily.

In the matter of sulfur, all women showed poor metabolism. Case II ranked best, all periods considered. Hence the intakes chosen by this group were inadequate or there was a deficiency of factors promoting utilization. Best retention occurred on the higher diets, and these approached 1 gram daily. The range was 0.60 to 0.94, with an average of 0.76. The higher quantity corresponded closely to the average intakes for Hoffstrœm's woman and that for Sandiford's case, although the diet of the latter was computed from published tables of food composition. The intakes for Oklahoma women were significantly below the average found in Sherman's adult dietaries (192).

Excess base ranged lowest in the diets of Case V, and this was expected from the recorded scarcity of fruits and vegetables used by her. The high cereal intake of Case VI also left relatively less excess base in her intake. The average 1040 cc. of 0.1 N excess base daily seems a fair amount for a proposed standard, since better calcium retentions occurred on diets containing this amount or more.

All women showed a habit of taking 2.5 to 3 liters of liquids daily, except Cases II and IV, whose intakes were higher, approximately 3.5 liters daily. The amount consumed corresponded closely to the caloric intake in the proportion of 1 gram of water per calorie of diet.

These figures for accustomed intakes are interesting in comparison with commonly used standards for adults, namely 1.0 gram of protein per kilo, 1.0 grams of calcium, 1.32 grams of phosphorus and 15 mgm. of iron daily. It seems doubtful if this amount of protein per kilo is adequate under any conditions during pregnancy. Except in a few instances the same may be said for calcium and phosphorus. The quantity of iron may be adequate under favorable conditions, but such at present are ill-defined.

Most writers have hesitated to set up even tentative quantitative standards for diet during pregnancy, since so few experiments showed that the requirements had been met adequately. Consequently the minimum standards lately proposed by Macy and Hunscher (143) based on accumulated experimental data are of more than usual interest here. For the

TABLE 30. Average Daily Food Intake Per Kilogram of Body Weight.[2]

Case	Weight		No. days observed	Calories	Protein	Fat	Carbohydrates	Calcium	Phosphorus	Magnesium	Iron	Sodium	Potassium	Sulfur	Chlorine	Excess base	Water[3]
	Actual[1]	Predicated[2]															
	kgm.	kgm.			gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	cc. N/10	cc.
I	49.0	59.4	22	38	1.0	1.3	5.3	19	24	5.5	0.18	65	56	1.0	67	19	43
II	49.9	54.0	14	52	1.6	2.3	6.4	34	36	9.3	0.47	89	81	1.6	98	24	47
III	59.4	61.2	8	47	1.2	2.0	6.1	19	26	5.5	0.22						64
IV	84.8	66.7	12	46	1.0	2.0	6.0	19	22	6.3	0.21	89	60	1.1	112	17	51
V	54.4	58.1	16	42	1.0	1.6	5.8	16	21	5.8	0.22	82	52	1.0	105	14	41
VI	50.3	60.3	24	44	1.4	1.6	5.9	31	33	6.8	0.29	86	61	1.6	104	16	48
Average	58.0	60.0		45	1.2	1.8	5.9	23	27	6.5	0.26	82	62	1.3	97	18	46

[1] Actual weight at beginning of gestation.
[2] Predicted from statistical averages for height and age.
[3] Water representing total fluid intake.

normal woman, these were 70 to 100 grams of protein daily or 1.3 to 1.5 grams per kilo of pregravid body weight. For minerals, a minimum of 1.6 grams of calcium, 2.0 grams of phosphorus, 0.3 gram of magnesium and 20 mgm. of iron, is recommended. Ten and 25 percent increases in energy are proposed for the second and third trimesters of pregnancy. The findings described in the present paper conform rather closely to the standard for protein and iron; but, as shown above, more than 0.3 gram of magnesium seems desirable and advantageous. In contrast, the quantities of calcium and of phosphorus proposed are more than generous minimums for women of the southern United States. This study would indicate that 1.3 grams of calcium, 1.5 grams of phosphorus, 0.4 grams of magnesium and 0.20 mgm. of iron in the diet could take care of the gestational needs of the women of this section. Actually, 1.5 to 1.8 grams of calcium daily provided optimum storage in most instances, although nearly as good retention was obtained on intakes ranging 1.0 to 1.4 grams daily.

However, error on the side of plenty carries with it none of the ill effects likely to accompany deficient intakes. Moreover, none of these standards will be adequate if the diet is grossly deficient in other respects, particularly accessory factors. And finally, more of the nutrition standards must in the future be differentiated with due regard to sections and environmental conditions of the peoples as well as by the nutritional level of the tribe. Such differentiations have already been applied to the standards for basal metabolism and energy requirement.

Table 30 refers the facts concerning the habitual food intake of these women to a basis of body weight as predicted for average from height and age. On the per kilo basis, there was little variation in the quantities of the different constituents chosen by the women, except for potassium, chlorine and phosphorus. Slight variations were found for the iron and excess base per kilo. The averages shown in this table represent amounts which were probably fairly adequate or minimum, except in the case of sulfur, for the women living under these conditions of activity, outdoor exercise, and exposure to sunshine. They might or might not be adequate in other situations. Only two women chose amounts of protein which conformed to the standard of 1.3 to 1.5 grams per kilo as proposed by Macy and Hunscher (143).

Relation of Food Intake to Gains in Weight. Since the question of controlling weight gains during pregnancy frequently causes concern (87, 179, 201), it seemed pertinent in connection with this summary of dietary habits. In Figures VIII and IX are shown the weight curves of these six women insofar as the records are complete, and corresponding curves for such items of diet or balance experiments as are often related to weight gains. These latter include calorie intakes, retentions of nitrogen and of total electrolytes as well as the unaccounted water balance at the time of the experimental period.

A lack of constant relation between food intake and gain in weight is evident. Water balance more frequently showed the same direction of change as the weight curve when the former was not correlated with the changes in the other factors charted (Cases IV and VI). However, in all except two cases the water balance tended to parallel the caloric intake. These graphs show a surprisingly constant relation between caloric intake and nitrogen storage in the diets of some individuals (Cases I, II, V and VI). They suggest that inadequate caloric intake may have been a limiting factor in nitrogen retention.

Weight gains were greatest in the most underweight subjects (Cases I and VI) (cf. Table 3), and least in the overweight individual (Case IV).

FIGURE VIII

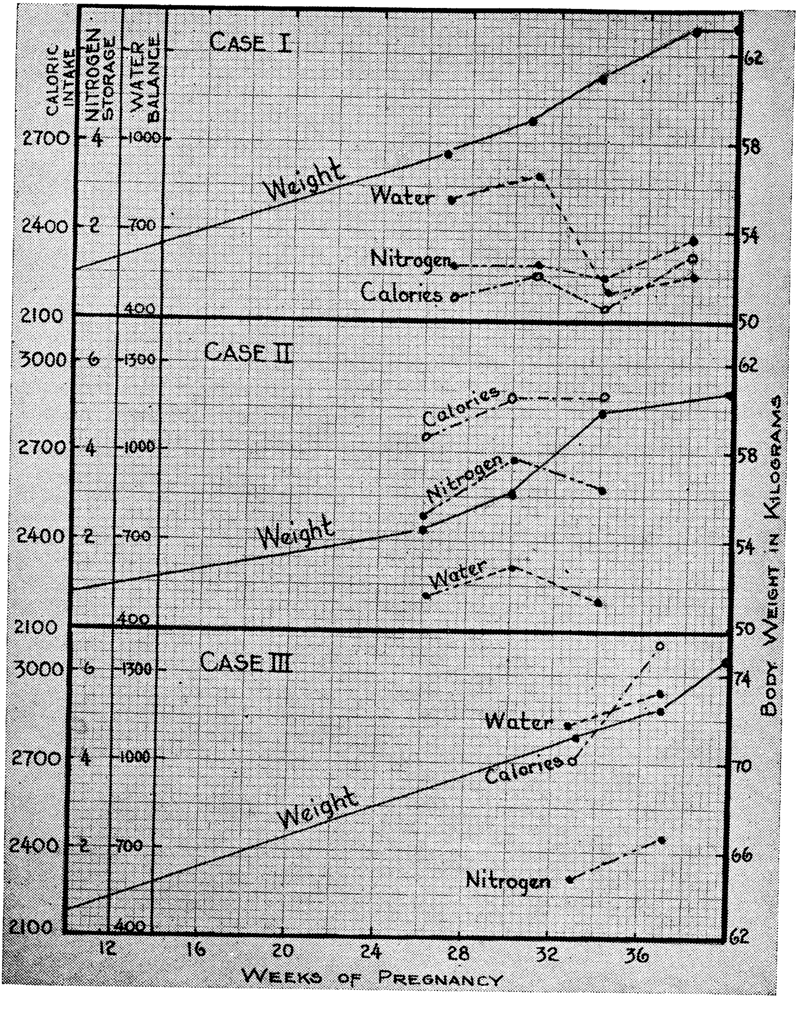

Fig. VIII.—Weight changes during pregnancy as affected by intake of calories and retention of water and nitrogen. Cases I, II and III.

FIGURE IX

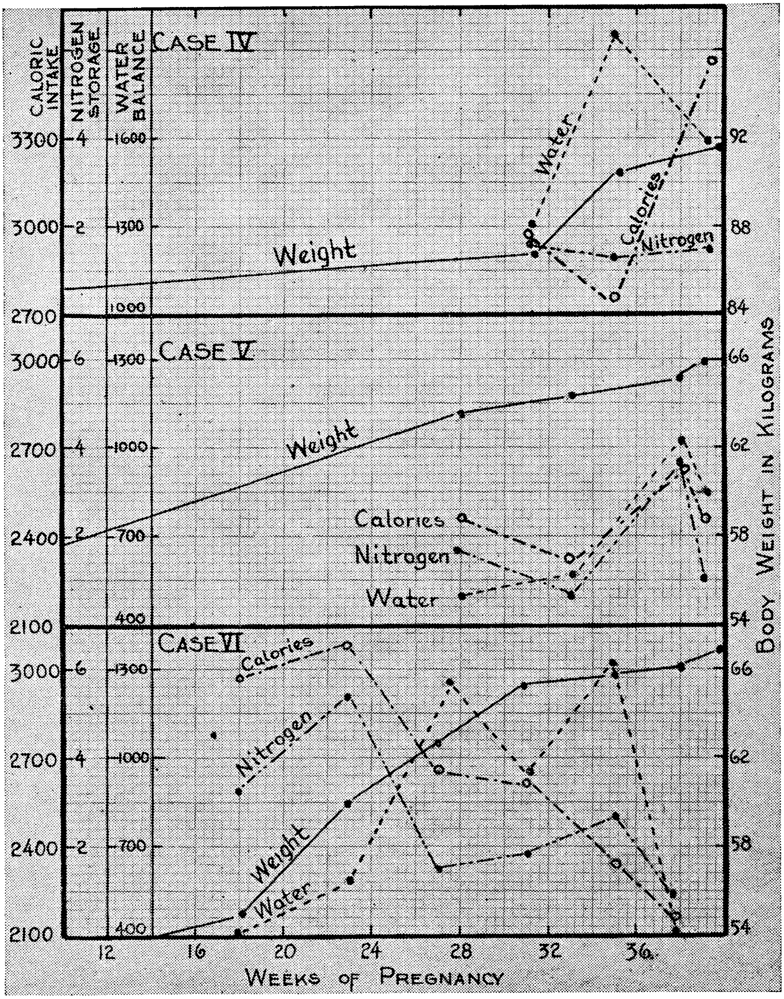

Fig. IX.—Weight changes during pregnancy as affected by intake of calories and retention of water and nitrogen. Cases IV, V, and VI.

For example, Case IV was some 30 pounds above average weight for her height at the beginning of gestation and gained only 15 pounds during the entire period, most of that during the last trimester. Case VI, some 20 pounds below average at the beginning, gained a total of 35 pounds, and 30 of it before the last quarter of pregnancy. Thus her gain was only about 15 pounds in excess of that needed for good nutrition. Likewise the other primiparae gained 15 to 20 pounds above that needed for normal nutrition (Table 3). Harding and Van Wyck believe that gains up to 5 pounds per month may be considered normal. Cummings (46) found that the average monthly graphs for 1000 cases were 0-1-1-4-4-5-5-3-3 pounds. The weight curve of Case IV least resembles this norm; that of Case VI is most like it, and the metabolic exchanges of the latter were typical of the group as a whole.

The amount and composition of maternal accretions, as measured by weight gains, would be influenced obviously by the quality of the diets chosen. During the time covered by these balance experiments the gains in weight were similar, about 2 kilograms per month for each of the different subjects. Cases III and V made smaller gains. Rowe (179) concluded from his studies of 77 individuals that average gains during the last 5 months of pregnancy approximate 1.2 to 2.4 kilograms per lunar month. There was an average loss of 9 kilos at parturition or soon thereafter, of which the weight of the child was about one-third. In view of the probable reserves retained for lactation, this suggests further how small a proportion of the total gestational needs is represented by the fetus.

Success in Lactation. Other facts to be considered in the evaluation of the adequacy of these diets were those pertaining to the physiological maternal behavior of the women post-partum and of the infants in post-natal life. Lactation, although unsatisfactory in most cases, was a complete failure in only one, Case V. Her diet also presented the most deficiencies. This was a better nursing record than was found for the 9 Chicago women and suggests that calcium, the retention of which element was most deficient, may have been an important limiting factor in their diets antepartum.

Nitrogen intake and storage is believed to have been a limiting factor common to the diets of both groups. Goss and Schmidt reported a failure to lactate in rats receiving diets containing excess base in the form of sodium bicarbonate (79). This factor in present-day diets in relation to lactation deserves further study.

Dentition in the Infants. From Table 3 it will be observed that dentition occurred rather early in these babies. All except one had one or more teeth at six months of age, and most of them had four by eight months. Baby IV had eight by 8 months. In the Chicago group the average age for the appearance of the first tooth was 7 months. Likewise, muscular development as evidenced by sitting and walking tended to progress more rapidly in the southern group. One interesting item not recorded in Table 3 was the age at which the infant was able to elevate the head when placed face downward on a firm surface. Such ability was observed within the first week after birth in most of those of the present series as well as in a group of newborn observed at the Oklahoma University Hospital. In the Chicago group this ability was not evident until near the beginning of the second month. More studies are needed before definite conclusions are possible.

It has been implied (83) that the newborn carries reserves of calcium as well as of iron. Fetal analyses tend to support this hypothesis, as do the findings of Booher and Hansmann (26). This could easily explain the more rapid physical development during early life of babies born of mothers properly nourished during pregnancy. Babies slightly heavier at birth were noted also in the present group. The average weight approximated 8.5 pounds, and in the Chicago group it was 7.5 pounds.

SUMMARY OF FACTS CONCERNING DIETARIES CHOSEN BY THE WOMEN

1. The amounts of milk, meats, liver, green vegetables and fruits chosen by the women varied widely. Milk intake varied from ⅓ cup to over a quart daily, green vegetables from ½ ounce to almost a pound daily. Butter, cereals and other vegetables were less variable in amounts.

2. Caloric intake ranged from 236 to 3585 calories daily, of which usually 12 percent or less came from proteins. Nutritive ratios were wider than commonly recommended for such diets.

3. Mineral intakes tended to range well above those found for average adult dietaries, but the amounts for calcium, phosphorus and iron were less than has been proposed for diets during pregnancy. Sulfur intake was low compared to that reported from other studies.

4. The amounts of excess base were unexpectedly high in the diets but had less alkalinizing effect on metabolism than their potential base-forming properties indicated.

5. Approximately 2.5 to 3.0 liters of liquid daily, slightly less than 1 cc. of water per calorie of intake, was consumed habitually by the subjects.

6. Weight gains tended to be most rapid in early pregnancy if the woman was underweight at the beginning.

FINAL SUMMARY AND CONCLUSIONS

1. Intake-outgo studies for 24 experimental periods on six women during pregnancy show balances for calcium, phosphorus, sodium, potassium, sulfur, chlorine, iron and nitrogen. Further analyses of the diets selected were made and certain additional urine determinations were carried out daily.

2. In marked contrast to similar studies reported in the literature, high retentions were found for calcium, and to a less extent for phosphorus and magnesium. Storage tended to be low for nitrogen, definitely inadequate for iron, and mostly negative for sulfur. Of the total 159 balances reported for the various elements, only 16 were negative and 11 of these occurred in the sulfur series, while sodium and magnesium furnished 1 each, chlorine 3. Probable causes for each are discussed in the text.

3. High storage of calcium, nitrogen and iron in early pregnancy proceeded independently of and far in excess of fetal needs when conditions were favorable for storage. In such cases there was no evidence of increased retentions, such as has been found for women receiving deficient diets, occurring in late pregnancy and corresponding to the period of high fetal demand.

6. Average daily intakes and retentions, expressed as grams daily for the different elements were as follows: calcium, intake 1.418, storage 0.279; phosphorus, 1.632, and 0.299 respectively; magnesium, 0.394 and 0.063; sulfur, 0.757 and 0.004; chlorine, 5.822 and 0.877; sodium, 4.907 and 1.265; potassium, 3.630 and 0.508; nitrogen, 11.58 and 1.97; iron in milligrams, 15.74 and 2.46.

7. Results which were of unusual interest have been discussed in relation to clinical problems and obstacles complicating the formulation of even tentative dietary standards for pregnancy. Questions urgently in

need of further investigations have been pointed out, particularly with reference to iron and sulfur.

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