CALCIUM AND PHOSPHORUS METABOLISM IN RATS DURING PREGNANCY AND LACTATION AND THE INFLUENCE OF THE REACTION OF THE DIET THEREON.

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From a quantitative standpoint calcium and phosphorus occupy a foremost place in the list of mineral elements needed by the vertebrate animal organism. The necessity for an adequate supply of these elements is particularly striking during the period of growth and during the lactation period. In pregnancy, the normal adult female, which, prior to the onset of this condition, may be in calcium and phosphorus equilibrium, is required to build new tissue for the fetus and at parturition must secrete milk for the nourishment of the young. Up to the age of weaning, all the food elements obtained by the offspring are derived from the mother's milk.

Experimental work dealing with calcium and phosphorus metabolism during pregnancy and lactation has been carried out chiefly on cows. A few experiments on humans have also been reported. It is well known that heavy producing cows are generally in negative calcium and phosphorus balance. Thus Forbes and his coworkers (1) found that in forty-nine balance trials during liberal milk production without exception all of his animals were in negative calcium balance. Supplementing the diet with additional calcium did not alter the negative balances, indicating that the ability of the animal to absorb calcium is limited. The calcium balances changed from negative to positive during the period of pregnancy when the cows were dry. The phosphorus balances were in most cases negative but not invariably so, indicating that the conditions for the assimilation of this
element must be more readily attained than in the case of calcium. In general, the magnesium balances paralleled the calcium balances but not consistently so.

Partial studies on the mineral metabolism of pregnant women have also been reported. Landsberg (2) concludes that from the 2nd to the 9th month of pregnancy there was a positive balance of calcium, phosphorus, and magnesium in his subjects. The storage was in excess of that which he calculated for the content of the fetus. Hoffström's (3) experiments carried out over a longer period point in the same direction.

While the data obtained on cows and on women definitely indicate the need for calcium and phosphorus during pregnancy and lactation, they are, however, incomplete, in that the balance experiments were necessarily carried out during intermittent periods over the period of pregnancy and lactation. No analyses of the offspring at the time of weaning were made. The data of Forbes and his coworkers, while covering the entire period of lactation and pregnancy, were obtained over intermittent periods rather than during the entire cycle of one animal.

The difficulties inherent in experimental work of this type on large animals are apparent. We have sought to obtain a complete picture of the metabolism of calcium and phosphorus by carrying out experiments on animals in which it is possible to follow the calcium and phosphorus balances during the entire period of pregnancy and lactation and also to follow the balances during several such periods without intermission. We have found that the rat is well suited for experiments of this type. The period of pregnancy is comparatively short, the onset of pregnancy can be definitely ascertained, the lactation period is likewise of short duration, the calcium and phosphorus content of the young at the time of weaning can be determined, the experimental animals can be kept for long periods of time on a constant diet, and the excreta can be easily collected.

Since the experiments yielded the information that particularly during the lactation period the dietary requirements of the rat for calcium and phosphorus are increased, we have used the opportunity to study the effect of varying the reaction of the diet upon the absorption of these elements by both mother and offspring.

The relation of the reaction of the diet to the assimilation and
excretion of calcium and phosphorus has also received some experimental attention. Givens and Mendel (4) concluded that the addition of acid or base produces no significant effect upon the balance of calcium and phosphorus in dogs but the addition of hydrochloric acid increased the output of urinary calcium. Sato (5) found that the addition of sodium bicarbonate to milk had an unfavorable influence upon the deposition of calcium in the infant while Zucker (6) experimenting on an adult man found that acid causes a shift of both calcium and phosphorus from feces to urine while the reverse effect was obtained with sodium bicarbonate. The total balance was, however, not changed. Bogert and Kirkpatrick (7) believe that calcium is retained more readily on a base-forming diet than on an acid or neutral diet and that the calcium excretion is greater on an acid-forming diet than on a base-forming diet. The experiments were carried out on human subjects. Clark’s (8) experiments on adult men showed that the storage of calcium in his subjects was not dependent upon the reaction of the diet supplied nor was it found possible to increase materially the retention of calcium by supplementing the diet with milk or calcium lactate. The inability of Hart, Steenbock, Teut, and Humphrey (9) to maintain calcium equilibrium in a liberally milking cow by supplemental hay is of interest in this connection.

**Experimental Methods.**

Adult female rats from the Division of Anatomy were used. Full grown animals were chosen since in these calcium and phosphorus storage, as a factor of growth, had ceased. The animals were kept in individual cages and were made thoroughly accustomed to their environment before any experimental work was begun. The composition of the diets is given in Table I. All of the essential ingredients known to be necessary for the nutrition of the rat were included. Diets I and II are normal natural foods to which sodium chloride and calcium carbonate were added. Diet III was made acid in reaction by replacing the calcium carbonate by calcium chloride and adding hydrochloric acid. Diet IV was rendered basic by addition of sodium bicarbonate. The butter fat was added fresh from time to time to a quantity of the stock diet and each new batch was analyzed for calcium and
phosphorus. Diet 228 is a purified diet\textsuperscript{1} free from vitamin E prepared according to Evans and Burr (10).

The animals were kept in galvanized wire cages with removable screen floors. The food was placed in a cup of the side arm which also carried a trap to catch food which might be spilled. The excreta were collected in a porcelain dish placed beneath the bottom of the cage. After the birth of the young the porcelain dish was replaced by a glass crystallizing dish, the wire screen floor was removed and a screen of smaller mesh was placed on a supporting ring near the bottom of the glass dish. In this way the young were unable to gain entrance to the feed cups. All cages were kept in a constant temperature compartment ventilated by a forced draught of fresh air. The excreta were transferred to

\begin{table}
\centering
\caption{Composition of Diets.}
\begin{tabular}{lcccc}
\hline
Diet No. & I & II & III & IV \\
\hline
Ground whole wheat & 67.7 & 51.0 & 52.0 & 53.0 \\
Wheat germ & 10.0 & 10.0 & 10.0 & 10.0 \\
Alfalfa leaf meal powder & 5.0 & 5.0 & 5.0 & 5.0 \\
Whole milk, powder & 10.0 & 10.0 & 10.0 & 10.0 \\
Casein & 14.8 & 14.75 & 14.6 & 12.7 \\
Butter fat & 5.0 & 7.0 & 7.0 & 7.0 \\
\text{CaCO}_3, \text{U.S.P., ppt.} & 1.5 & 1.25 & & 1.3 \\
\text{CaCl}_2, \text{c.p.} & & & & 1.35 \\
\text{HCl} & & & & 0.05 \\
\text{NaCl} & 1.0 & 1.0 & & \\
\text{NaHCO}_3, \text{c.p.} & & & & 1.0 \\
Alkalinity, cc. 0.1 \text{NaOH} per 100 gm & 300\textsuperscript{*} & 306\textsuperscript{†} & & 440\textsuperscript{‡} \\
Acidity, cc. 0.1 \text{HCl} per 100 gm & & & & 206 \\
\text{Ca, mg. per 100 gm} & 820 & 740 & 758 & 752 \\
\text{P, mg. per 100 gm} & 550 & 500 & 530 & 530 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{*} Alkalinity due to 1.5 per cent \text{CaCO}_3 added.
\textsuperscript{†} Alkalinity due to 1.25 per cent \text{CaCO}_3 added = 250 cc. 0.1 \text{NaOH} per 100 gm. of diet.
\textsuperscript{‡} Alkalinity due to 1.0 per cent \text{NaHCO}_3 = 119 cc. 0.1 \text{NaOH} per 100 gm. of diet. Alkalinity due to 1.3 per cent \text{CaCO}_3 = 260 cc. 0.1 \text{NaOH} per 100 gm. of diet.

\textsuperscript{1} Diet 228 consisted of lard 7.7, casein 27, corn-starch 59.3, Salt Mixture 185 4.0, cod liver oil 2.0, and yeast 0.6 gm. daily.
evaporating dishes at the conclusion of each period and were dried on the steam bath after the addition of sodium carbonate to render the ash distinctly basic. No attempt was made to separate the feces for a given period by means of a marker since the periods were long and followed each other without intermission. No attempt was made to segregate the excreta of the young from those of the mother. The excreta of the young constitute a small part of the total collection and moreover in a balance between intake and output such a segregation is not necessary.

The estrous cycle of each rat was followed by daily examination of the vaginal smear according to the technique described by Long and Evans (11). Following the normal or rest period and when it was evident by the appearance of the vaginal smear that the animal would accept the male during the following 24 hours, she was placed in a cage with the male and examined on the following morning for the vaginal plug and the occurrence of spermatozoa. If this sign of copulation was found the female was returned to her cage. A small error is introduced by the intermission of 1 day but since the female is returned to her cage 24 hours after she was taken from it and since her diet was not changed it may be assumed that the excretion will nearly balance the intake during this time and that the error in a 22 day period will be small.

After the animal's return to the metabolism cage the vaginal smear was again examined daily and the characteristic erythrocyte sign of pregnancy (10) looked for on the 13th, 14th, and 15th day of gestation. The lactation period was continued for 18 to 21 days, at which time the young were usually sufficiently active to climb into the food cup. They were then removed, killed, and subjected to analysis. The mother was continued on the diet during a rest period and whenever possible again bred so that the balances could be followed over another cycle.

The food and excreta were ashed according to standard procedure. Calcium was determined by McCrudden's method and phosphorus by the method of Pemberton-Kilgore.

Results.

The results of the balance experiments are shown graphically in Figs. 1 to 6. The most striking observation shown is the fact
that in seventeen lactation periods on the various diets all show that the animals were in negative calcium balance. In sixteen periods the phosphorus balances were also negative and in only one period was the animal in phosphorus equilibrium. All but two changed from negative to positive calcium and phosphorus balances following removal of the young.

A second observation of note is that during twenty-seven pregnancies on the different diets, in only four was there failure to store calcium and in only three was there a negative balance of phosphorus.

Within the range of acidity employed in Diet III, no marked difference in calcium and phosphorus balance over the normal diet is shown. The animals showed a remarkable capacity to thrive and reproduce normal young on this diet. This is likewise true of alkaline Diet IV. It will be noted, however, that both animals
maintained on Diet IV failed to lactate the young after the second pregnancy. The first litter in each instance was reared. The second pregnancy was normal and full weight young were born to each mother. In both cases the young were not nourished and died of starvation. It is not possible at this time to ascribe the phenomenon to the diet since the number of animals is not sufficient for statistical purposes. It is noteworthy, however, that

![Graph](image)

**Fig. 2. Balance chart for Rat BH8307.**

when after a short rest period these two females were changed to the normal diet of the stock colony, they produced and reared normal young. Further experiments to elucidate this question are in progress.

In Fig. 7 are given the results of a growth experiment in which it was desired to determine the rate of assimilation of the calcium

*The stock diet consisted of yellow corn 76, linseed oil meal 16, casein 5, alfalfa meal 2, sodium chloride 0.5, calcium carbonate 0.5.*
and of the phosphorus in young rats maintained on Diet III and Diet IV respectively. Two young from Rat 10 were placed in a single metabolism cage at the conclusion of the lactation period and calcium and phosphorus balances were carried out for 7 or 8 day periods until six such periods were completed. A similar experiment was carried out on two young from Rat W19 with the alkaline diet. The animals which were maintained on the alkaline

![Graph](image-url)

**Fig. 3.** Balance chart for rats on normal diet (Diet II).

...diet grew at a slightly greater rate. The calcium and phosphorus storage is normal in both cases. The figures show the extraordinary ability of young rats to assimilate and retain both calcium and phosphorus irrespective of the reaction (within the limits studied) of the diet.

In Figs. 8 and 9 are given the results of a study of the calcium and phosphorus metabolism of rats maintained on a purified diet...
containing all of the essential factors except vitamin E. Females which had been reared on the Evans and Burr (10) Diet 232 and which had undergone one resorption of the young were used in these experiments. The animals were maintained on Diet 228 to which yeast was added. After a normal period during which no vitamin E was included in the diet, three animals were given

6 drops of wheat germ oil daily while the other three were kept as controls without this vitamin. No effect on the calcium and phosphorus balances was observed following administration of vitamin E. The animals were then mated. Those which received vitamin E gave birth to young while the controls resorbed.

*Diet 232 consisted of casein 32 gm., corn-starch 40 gm., lard 22 gm., cod liver oil 2 gm., Salt Mixture 185 4 gm., and yeast 0.6 gm. daily.*
TABLE II.
Calcium Balance during Pregnancy.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>BH9393</th>
<th>W9337</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight at littering, gm</td>
<td>230</td>
<td>286</td>
</tr>
<tr>
<td>&quot; of fetus, gm</td>
<td>28.1</td>
<td>28.7</td>
</tr>
<tr>
<td>&quot; placenta and uterus, gm</td>
<td>7.7</td>
<td>11.3</td>
</tr>
<tr>
<td>Food consumption, gm</td>
<td>297</td>
<td>343</td>
</tr>
<tr>
<td>Total Ca in food, mg</td>
<td>2432</td>
<td>2812</td>
</tr>
<tr>
<td>&quot; excreted, mg</td>
<td>2085</td>
<td>2425</td>
</tr>
<tr>
<td>Ca balance, mg</td>
<td>+347</td>
<td>+387</td>
</tr>
<tr>
<td>&quot; found in fetus, mg</td>
<td>65</td>
<td>63</td>
</tr>
<tr>
<td>&quot; in uterus and placentas, mg</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Excess Ca stored, mg</td>
<td>279</td>
<td>321</td>
</tr>
</tbody>
</table>

Fig. 5. Balance chart for rats on acid diet (Diet III).
Rat G2967 following mating showed a large retention of both calcium and phosphorus during the first 7 days of gestation. This could not have been stored in the embryos since implantation does not occur until about the 6th day of gestation. In all of the animals including those which resorbed there was a positive balance of both calcium and phosphorus following mating.

Our experiments have indicated a storage of calcium and phosphorus during pregnancy. Table II shows the distribution of
these stored elements. The data were obtained from two female rats which were placed on a balance experiment. Just before the birth of the young the mothers were killed and the uteri, fetuses, and placentae were removed and separated. It will be noted that the storage of calcium is four to five times that which is contained in the fetus plus the placenta and uterus.

Fig. 7. Balance chart for rats on an acid and an alkaline diet. The results shown on the left are those for two young from Rat W19 fed Diet IV (NaHCO₃); on the right, for two young from Rat 10 fed Diet III (HCl).
No direct correlation was found between the size of the litters reared and the magnitude of the negative balance of the mother during the lactation period. In general, as might be expected, the largest losses of calcium were associated with the largest litters. Since every lactation period resulted in a loss of calcium it is of interest to note the extent of these losses from the body of the mother. The total calcium content of an adult female rat is about 3 gm. and the phosphorus content is about 2 gm. We have calculated that during a heavy lactation period the rat may lose 20 per cent of her total supply of calcium and phosphorus.

![Graphical representation of calcium and phosphorus balance](image-url)
only about 1 per cent of the body calcium is not contained in the bones, the loss must be carried by the skeleton. Bauer, Aub, and Albright (12) have concluded that the trabeculae of the long bones serve as a storehouse of conveniently available calcium.
While it appears from these experiments that within the range of reaction used there is no marked effect of acidity upon calcium and phosphorus absorption during pregnancy and lactation in the rat, the statement cannot be taken as absolute, since from a statistical point of view a sufficient number of animals may not have been employed. The inability of the rat to absorb sufficient calcium from the diet to meet its lactation requirements is striking. Whether this is a problem of ultimate permeability of the intestinal wall or whether factors can operate to increase the calcium transport through the intestines are questions which are not answered by these experiments. It is of interest to note the constancy of calcium and phosphorus in the bodies of young rats at birth. In Table III are summarized the results of analyses of 166 newborn rats. They represent twenty-five litters from mothers on various natural and pure food diets. Sherman and MacLeod (13) and Sherman and Quinn (14) give the following figures for newly born rats: calcium 0.25 per cent and phosphorus 0.34 per cent. The magnitude of our values is in approximate agreement with these figures.

The analytical data relating to the experiments described in this paper are on file in the University of California library.

**SUMMARY.**

1. Calcium and phosphorus balances in the rat have been carried out through continuous periods of rest, pregnancy, and lactation. The reaction of the natural food diets was varied from alkaline to acid in different experiments.

2. During twenty-seven completed pregnancy periods, on the various diets, only four failed to show a positive balance of calcium and only three failed to store phosphorus. In most cases the positive balance was in excess of that estimated to be contained in the young at birth.

3. In seventeen lactation periods, all showed a negative balance of calcium and all but one a loss of phosphorus. The losses, for full sized litters were as great as 20 per cent of the entire body content of these elements in the mother.

4. Within the range of acidity employed, no significant difference could be found between the diets, except that the second
litters from the rats on the diet containing sodium bicarbonate were not nourished by the mother. This was due to failure to lactate.

5. Young rats from mothers which had been maintained on acid Diet III and alkaline Diet IV respectively, were kept for some time on these respective diets. No difference in storage of calcium and phosphorus was noted.

6. Females reared on a vitamin E-free diet stored calcium and phosphorus during the period of pregnancy in which resorption of the fetus took place.

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