STUDIES ON CALCIUM METABOLISM

I. CALCIUM METABOLISM IN THE PREGNANT AND LACTATING RABBIT.

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(With Four Graphs.)

Since the appearance of the works of Weiske, Voit, Forster, and others of later years, numerous attempts have been made to determine the various factors involved in calcium metabolism. Patterson carried out feeding experiments on rabbits and on himself and found that even a dietary deficient in calcium induced no loss of calcium in the blood. Moreover, with a fixed diet the urinary calcium varied but slightly, the variations being parallel with total amounts of urine excreted. One of his conclusions was that the greater the volume of urine excreted the greater was the mass of salts eliminated.

About the same time Blair Bell discovered a method of ascertaining the amount of calcium in small samples of blood. He precipitated the calcium as calcium oxalate and made a microscopic count of the crystals, so obtaining an “index.” This cannot be as accurate as finding quantitatively the amount of calcium in a given volume of blood or blood serum. The work, however, was one of relative accuracy, for in obtaining “indices” at various periods a comparison of the amount of calcium in the blood at these times was made.

Following upon this, Bell and Hick, in their observations on the physiology of the female genital organs, came to the conclusion that calcium played a very important part in the causation of parturition. Bell’s previous findings suggested that just before birth the calcium of the mother’s blood increased, but fell immediately after confinement. Bell and Hick accordingly promulgated the hypothesis that the increase in blood calcium in the later months of pregnancy is the normal stimulus for the induction of labour.

A few years after this Kastle and Healy seemed to confirm this hypothesis in that they almost invariably got pregnant guinea-pigs to abort following upon an introduction of calcium into the peritonium. More recently Widdows, also working on the calcium content of the blood during pregnancy, obtained results which fully supported Bell’s hypothesis, the calcium of the mother’s blood rising just before birth and falling immediately after confinement.

Underhill and Dimick, on the other hand, working on the inorganic salts
content of the blood, during pregnancy, failed to find evidence in support of Bell's hypothesis, since their figures either fell within normal limits or else blood calcium was lower rather than higher during the later months of pregnancy. They worked with whole blood and their results led them to conclude that estimated upon whole blood average figures for calcium content of the blood of pregnant women are somewhat higher than non-pregnant ones. With the course of pregnancy they observed little change and therefore they could not support Bell and Hick's contention that an increase in calcium of the blood at term was of direct significance in the induction of labour. They maintain that in general the inorganic constituents of the blood show little change during pregnancy.

According to Harding, in his review of the subject of metabolism in pregnancy, so far as blood calcium is concerned, only Bell and Hick and Lamars had found an increase in calcium of whole blood, but some had found a slight decrease in serum in the later months of pregnancy.

An examination of the literature shows that little work has been done on blood calcium during pregnancy. Comparatively little has been done with regard to serum-calcium of the rabbit to verify or contradict the theory of Bell and Hick. Various investigators had preferred to work on whole blood, accepting the view that the corpuscles held an appreciable amount of calcium. Others, perhaps more recent workers, had found that the corpuscles held a negligible amount of calcium and therefore worked with serum.

The theory of Bell and Hick seemed so interesting that the writer decided to follow up the serum-calcium content of the blood of the pregnant rabbit and so endeavour to verify Bell's hypothesis that parturition is brought about by an increase of blood calcium.

The rabbits used in this study were three Himalayan and five $F_1$ Belgian Hare × Flemish Giant. It was found possible to obtain the necessary samples of blood from the ear-veins as frequently as the experiment required them. Four c.c. blood was usually withdrawn every third day and this gave the necessary 2 c.c. serum for analysis.

The method adopted for the whole series of analyses was that of Kramer and Tisdall with slight modifications. The calcium in the 2 c.c. serum was precipitated as calcium oxalate which was separated from the liquid by centrifuging and decanting off the supernatant fluid. The precipitate was then washed in 2 per cent. ammonium hydroxide to free from magnesium. The method of Kramer and Tisdall demands that the precipitate should be washed three times, but a publication of Clark and Collip and personal experience showed that one washing with 2 per cent. ammonium hydroxide was sufficient. The precipitate was then dissolved in 2 c.c. approx. $N\ H_2SO_4$ by heating in a water bath. This solution, when titrated against a weak solution of KMnO₄, gave the amount of calcium present according to the formula:

\[ \text{mg. Ca per 100 c.c. serum,} \]

where

\[ x = \text{volume of KMnO}_4 \text{ used} \]

and

\[ y = \text{normality of the KMnO}_4. \]
Studies on Calcium Metabolism

\[ y \] was found by titrating 10 c.c. \( N/100 \) \( \text{Na}_2\text{C}_2\text{O}_4 \) (Sörensen) against the \( \text{KMnO}_4 \) solution, according to the formula:

\[ \frac{1}{\text{c.c. \( \text{KMnO}_4 \) \times 10}} = y. \]

The permanganate solution was made up according to Halversen and Bergeim, but the \( 0.0133^N \) \( \text{KMnO}_4 \) was diluted still further to make the solution in the neighbourhood of \( 0.0117^N \).

The study was started with three virginal Himalayan females, six months old. Blood was taken from each at three-day intervals for a month, then they were mated. During the pre-coital period the calcium in their blood serum varied from 13 mg. to 15 mg. per 100 c.c. with suggestion of a rhythmic rise and fall. As time passed, it became manifest that of the three only one was really pregnant, and only in the case of this one was there any noticeable peculiarity in the swing of the calcium content, for on the 26th day of pregnancy she gave a remarkably low reading, 11.26 mg. On the 29th day blood was withdrawn again and the calcium content was found to be higher. She had her litter on the 32nd day. On the following day the Ca content was 14.11 mg. and remained at this level until the 19th day of lactation, when again there was a fall to 11.9 mg.; three days later it had fallen to 10.5 mg., but at the time of the next examination it rose to 18.18 mg., thereafter dropping to 13.76 mg. and remaining at the 13-15 mg. level (Graph I).
From this preliminary study the suggestion emerged that there was a significant drop in calcium content just before parturition and again about the 19th day of lactation, and that this later fall was immediately followed by a sharp rise to a maximum. The study was therefore continued with selected material, rabbits with long ears and prominent ear-veins which would allow blood to be withdrawn at frequent intervals and over a long period of time. One of the five $F_1$ Belgian Hare × Flemish Giant females was kept unmated to serve as a control.

Graph II shows the results obtained from two females $\varphi \, D$ (mated) and $\varphi \, H$ (the control). It is here seen that until just prior to parturition there is no significant difference between the pregnant and the virgin female in the matter of serum-calcium content, save that there were indications of a rise in the case of the pregnant rabbit about the 20th day of pregnancy. Two days before parturition, however, the graph of the pregnant female shows a sudden drop. Immediately after littering, this doe was again mated and two days before parturition there was again the significant drop. Immediately after parturition the calcium content becomes the same as that of the virgin, but about the 19th day of lactation there is the second steep fall.

Graph III gives the figures for females $E$, $F$, and $G$. In early pregnancy there is some slight variation for the same animal, but there is not much difference between one animal and another. Soon after mid-pregnancy is reached there seems to be a rapid rise in serum-calcium, shown by all three animals. The maximum seems to be reached by the 10th day before parturition, after which there is a rapid fall to a minimum (in the case of $\varphi \, F$ this fall was exceptionally big). About the 10th day
before parturition the figures for $\varphi E$, $F$, and $G$ were 15.91 mg., 15.25 mg., and 14.92 mg. respectively. Two days before parturition $\varphi G$ had fallen to 10.67 mg., $\varphi F$ to 8.74 mg., and a day before parturition $\varphi E$ fell to 11.08 mg. It is interesting to note that the graphs of all the animals that were truly pregnant show often a marked increase in serum-calcium towards the close of pregnancy but always a sudden fall (1, 2, or 3 days) before parturition.

Graph IV shows the serum-calcium of $\varphi H$, but this time in a pregnant condition. There are slight variations in early pregnancy, but as the mid-pregnant period passes, there seems to be a fall followed by a rapid rise to over 15 mg. Following this there are slight variations, but a day before parturition there is the usual sudden fall to the pregnancy minimum of 11.17 mg. To complete the similarity with the figures for the other rabbits, 2 days after parturition the serum-calcium soared to 14.65 mg. and 6 days after this to 16.15 mg., the lactation maximum.

An examination of the graphs show that in every case of true pregnancy, followed by a period of lactation, there is a sudden rise to normal and above in serum-calcium. In the case of $\varphi A$ during the first 2 weeks of lactation the serum-calcium
remains fairly high in the neighbourhood of 14 mg. Soon after this there seems to be again a sudden fall, the minimum reached being much lower than that before parturition, e.g. pre-parturition minimum was 11.26 mg., lactation minimum was 10.5 mg. Three days after this fall there was a very sudden rise to the maximum of 18.18 mg. This maximum was indeed very peculiar, for 3 days after the serum-calcium fell to normal. D showed a sudden rise after parturition, rising to 14.28 mg., but in 6 days during lactation there was a sudden fall to 10.75 mg. In 6 days the figure rose to 12.31 mg., but at the end of 3 days there was a still bigger drop to the minimum of 10.27 mg. From this minimum till the end of the lactation period the serum-calcium showed a tendency to rise gradually, arriving at the normal of 14.17 mg., when the young were separated. The graph of E shows that soon after parturition the serum-calcium soars to above normal, reaching the high figure of 15.55 mg. in the middle of the second week of lactation. Thereafter there is a rise and fall, but the latter is never in the neighbourhood of the pre-parturition minimum. In the case of G the pre-parturition minimum was 2 days before parturition and was 10.67 mg. On the day after parturition the figure had soared to 13.88 mg., but thereafter the figure seemed to remain normal though there were slight variations during lactation. The graph of F shows a greater fall during lactation. Two days before parturition the minimum of 8.74 mg. was recorded, but a day after parturition the figure soared to 13.13 mg., and 3 days later to 14.5 mg. As lactation progressed
Studies on Calcium Metabolism

the serum-calcium seemed to fall, a minimum of 12.09 mg. being reached at the beginning of the third week of lactation. Thereafter the serum-calcium seemed to rise to the neighbourhood of 14 mg. and remain there with slight variations till the litter was weaned. The graph of \( \varphi H \) seems to be the most remarkable during the lactation period. The pre-parturition figure (reached a day before parturition) was low, 11.17 mg., but 3 days later the figure rose suddenly to 14.65 mg. Then there was a slight fall, but 8 days after parturition the abnormal maximum of 16.15 mg. was reached (cf. graph of \( \varphi A \)). Six days after this the serum-calcium seems to fall very suddenly to the lactation minimum of 9.71 mg. The rise to normal is as sudden, for 3 days after this the figure is at 14.74 mg. The serum-calcium thereafter seems to remain normal in the neighbourhood of 14 mg. None of the Belgian Hare cross does show the most abnormal maximum that the Himalayan \( \varphi A \) showed at the close of the third week of lactation.

From the work of previous investigators it would seem that the food of the rabbits would not make differences in their calcium metabolism. Patterson points out that rabbits getting food deficient in calcium lose no blood calcium. The rabbits used in the above experiments were fed and attended to by the writer. The foods given were the ordinary rabbit foods and these consequently had little effect on the calcium metabolism. It seems justifiable to assume, therefore, that the food and everyday care and attention given to the rabbits had made no variations in the calcium findings. Experience, however, showed that the animals were not to be handled in a forceful manner or excited unnecessarily, as this assuredly led to an unnecessary fall in the serum-calcium. The graphs of the rabbits \( \varphi A-\varphi H \) show that under normal conditions there is little or no difference between the early pregnant and the non-pregnant animals, the serum-calcium being in the region of 14 mg. per 100 c.c. serum. There are slight variations from day to day, but it is only in the last week or two of pregnancy that it is possible to differentiate between a pregnant and a non-pregnant rabbit. From 7-10 days before parturition the serum-calcium, which is then normal or slightly above normal, commences to fall and although 4 days before parturition the figure may be above normal, a day before parturition it declines suddenly to the minimum of the pregnant period. The figures for the non-pregnant, on the other hand, remain more or less normal. This sudden fall in the serum-calcium shortly before parturition is remarkable and seems to hold good in all cases of true pregnancy in the rabbit. One is accordingly led to ask if there is a similar fall in other metallic radicals besides calcium.

According to Dixon and Marshall (1924) there is an ovario-pituitary endocrine mechanism which comes into operation at the close of pregnancy "when the corpora lutea are in an advanced stage of involution, the normal secretory activity is once more produced and the pituitary is excited to secrete in greater quantity," so resulting in parturition. If such is the case, the above results show that at this special time not only is the pituitary excited to secrete but a state of acidosis in the blood suddenly sets in. The excitement of the pituitary not only causes that endocrine to secrete, but also brings about a very sudden depression in the serum-calcium, perhaps through the pituitary secretion. This state of acidosis in the
blood, in all probability, renders the uterine muscles more responsive to excitation by the pituitary secretion.

Stewart and Percival point out that extensive haemorrhage causes a lowering of the serum-calcium in rabbits. This lowering can be understood in the case of a rabbit undergoing an operation, but the above results have no bearing on any operation, and the pre-parturition fall in the serum-calcium was not caused by any visible haemorrhage. This sudden fall may or may not be caused by extensive internal haemorrhage. Whether there is or not internal haemorrhage in the rabbit before parturition remains to be investigated.

As pointed out by Culhane there seems to be an immediate rise in the serum-calcium of rabbits on feeding with cabbage. The rabbits under the writer’s observation were fed on cabbage when available, failing which they were given other green stuff or a drink of water. That feeding with cabbage causes a rise in the serum-calcium may be the reason why the results on the rabbits were not the same on two succeeding estimations.

The caging of rabbits raises the blood calcium as shown by Grant and Gates. The rabbits under discussion were encaged throughout the experiment so that the caging factor would be a constant. The variations in the above results, however, may be due to seasonal variations to a small extent. The graphs show the months during which the experiments were carried out, but it is hardly possible that seasonal variations would result in so sudden and great a fall in serum-calcium prior to parturition. The fall is due to a more deep-seated cause than seasonal variation.

In conclusion, it may be stated that the results obtained from the pregnant rabbits fail to support the theory of Bell and Hick that an increase in blood calcium at the close of pregnancy is the normal stimulus for the induction of labour. The results show, rather, that at the close of pregnancy there is a sudden fall in blood calcium and it is very probable that a state of acidosis in the blood is the normal stimulus for parturition and not an increase in blood calcium.

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**SUMMARY.**

1. Seven to ten days before parturition in the rabbit there is a fall in the serum-calcium content and one day before parturition there is a further and sudden fall to a minimum.

2. After parturition the calcium content is restored to normality but on the 19th day or thereabout of the lactation period there is a second sudden fall.
REFERENCES.