IV. CALCIUM CONTENT OF THE BLOOD DURING PREGNANCY.

By SIBYL TAITE WIDDOWS.

*From the Chemical Laboratory of the London (Royal Free Hospital)
School of Medicine for Women.*

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THE importance of calcium in growth is well established, and in the human foetus it has been shown that calcium retention varies very greatly at the different stages of development.

Paul Bar [1907] by estimating the amount of calcium found in foetuses of different ages concluded that the absorption varied considerably with the stage of growth and was of the order shown in the following table (calcium expressed as calcium oxide):

0.0048 g.	each day up to 120th day.
0.083	„ „ from 120th–150th day.
0.087	„ „ „ 150th–180th „
0.084	„ „ „ 180th–210th „
0.638	„ „ „ 210th–270th „

From these figures it would appear that at certain periods there is an abrupt alteration in the calcium retention which must mean a sudden change in the demands upon the maternal stores of that element.

The question then arises does the blood of the mother show at these stages an alteration in its calcium content (an increase if the blood carries more calcium to meet the heavier demand made upon it, or a decrease if the demand is not counterbalanced by a greater mobility of the calcium), or is the calcium metabolism so perfectly balanced that the blood maintains a constant value in spite of the varying demands made upon it? In an attempt to answer this question, a number of analyses of the blood of women at different stages of pregnancy have been made, paying particular attention to those periods at which the abrupt changes in the calcium metabolism of the foetus seem to occur. The samples were taken 3rd–4th month, 5th month, 6th–7th month, and 8th–9th month.

METHOD.

The analysis was always made on serum, and not on the whole blood. The calcium is present in very different amounts in the corpuscles and plasma, and unless therefore the proportion of corpuscles to plasma is ascertained, the true value of the calcium content cannot be arrived at. Some of Lyman's varying results may be due to his always making use of whole blood. The method finally adopted was based on that of McCrudden [1912] modified for small quantities.

The working out of this method was started in 1919, and it was found that a similar method was being adopted by other workers for estimating small quantities of calcium. 5 cc. of serum were found to be sufficient and concordant analyses could be made when this amount was available.

Precipitation of the protein.

The protein of the serum is precipitated with 6.5 % trichloroacetic acid, the volume taken being five times that of the serum, a rather larger ratio than that used by Lyman [1917, 1, 2]. The serum is added very slowly drop by drop from a pipette to the trichloroacetic solution in a small flask, rotating the liquid continuously. The flask is then stoppered and the contents thoroughly mixed by shaking. After standing for about half-an-hour, the mixture is filtered through a very small calcium-free filter paper, and an aliquot part of the filtrate is taken for the estimation of the calcium present.

Precipitation and estimation of the calcium.

The aliquot part of the filtrate is made just alkaline with concentrated ammonia, added drop by drop, using methyl red as indicator, then just acid with $N/2$ hydrochloric acid, added drop by drop from a burette or 1 cc. pipette. To this is added 1 cc. of $N/2$ hydrochloric acid, 1 cc. of oxalic acid solution 4 %, and 1 cc. of a 20 % sodium acetate solution, stirring or shaking carefully while adding the latter. The solution is allowed to stand for a day, and the precipitate of calcium oxalate is separated by means of a high speed centrifuge and washed twice with water, centrifuging between each washing. The calcium oxalate so obtained is dissolved in 4 cc. of N sulphuric acid made just pink with $N/100$ potassium permanganate, heated to about 70° on a water-bath, and then titrated with $N/100$ potassium permanganate solution, made according to the direction of Halverson and Bergeim [1917]. In this estimation of course all materials used must be tested for calcium, and only calcium-free materials employed. The water is always twice distilled and every care must be taken throughout the estimation to protect the solutions from contamination with dust or other reducing matter. Kramer and Howland [1920] described a method for estimating calcium in 1 cc. of serum, and on testing this method satisfactory results were obtained. This method was therefore occasionally adopted when only 2 cc. of serum were available, duplicate estimations always being made.

RESULTS.

The first series of analyses were made during 1920 to 1921, and in these there was no attempt to follow the same case throughout the different stages. The figures for this series, given below in Tables I and II are therefore the mean of experiments made on different women at the same period of pregnancy. The specimens of blood were obtained from maternity patients attending at the Royal Free Hospital.

The amount of calcium in the following tables is always given in milligrams of calcium per 10 cc. of serum.

Table I. (1st Series 1920-21.)

Stage month	Number of specimens analysed	Average calcium found	Range of calcium found	
			Highest	Lowest
3-4	12	0.968	1.17	0.763
5-5½	10	1.00	1.29	0.72
6-7	29	1.02	1.2	0.72
8-9	15	0.966	1.25	0.74

The above table shows little variation between the highest and lowest amounts of calcium in 10 cc. of serum at the different stages. Usually the range is from 1.05 to 0.93. These results seem to bear out De Wesselow's conclusion [1922], that during the last months of pregnancy there is a tendency to a fall in the calcium content of the serum. The results above are taken from all the cases examined during the year 1920-21 without separating the normal cases of pregnancy from those who in previous pregnancies had had miscarriages or still-born infants. In Table II this separation has been made.

Table II.

Stage month	Number of specimens analysed	Average calcium found normal	Numbers of specimens analysed	Average calcium found in cases previously abnormal
3-4	10	0.97	2	0.96
5	4	1.13	6	0.916
6-7	25	1.024	4	0.989
8-9	11	0.957	4	0.992

The numbers in Table II show that in the normal cases there is a decided drop in the calcium content during the last month of pregnancy, while in the other cases the difference in the calcium content at the different stages is not so marked, the calcium being slightly higher in the last month than at the beginning. The number of these latter cases is small, and a larger and more complete set is being investigated.

From the wide range of values for any given month in different cases it was clearly seen that for a definite answer to the problem the analyses must be made on the *same* individual at the different periods. A second series of analyses on these lines was carried out during the year 1921-22. In this way compensation was made for any individual idiosyncracies causing a variation

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in results. In the second series in as many cases as possible analyses of the blood calcium have also been made within 14 days after confinement to determine the effect of lactation on the calcium content. Analyses are also now being made of the blood calcium of cases 2-3 months after confinement to investigate the effect of continued lactation. These results are given in Table III.

Table III. (2nd Series 1921-22.)

Stage Month	Number of specimens analysed	Average calcium found	Range of calcium found	
			Highest	Lowest
3-4	22	1.11	1.28	0.898
6-7	24	0.996	1.17	0.848
8-9	24	0.94	1.03	0.82
Post-confinement within 14 days	18	0.994	1.15	0.814

These results are in accord with those of last year and show a decrease in the calcium content of the serum during the last month of pregnancy, and a tendency to rise after confinement and during the early stages of lactation.

As stated earlier in this paper, during this year an endeavour was made to obtain the calcium content of the serum of the *same* woman at the above different stages of pregnancy, and this has been successfully carried out in a number of cases. In some, however, the series are not complete owing to the women failing to come to the hospital at one or other of the periods under investigation. The more complete figures are given in Table IV.

Table IV.

Case	Month 3-4	Month 6-7	Month 8-9	Post-confinement
4	1.23	1.1	—	1.07 (10 days)
7	1.09	0.916	0.775	1.07 (10 days)
8	0.898	0.93	0.92	—
9	—	1.17	1.00	—
12	1.17	0.88	—	1.05 (5 days)
13	0.913	0.898	0.92	1.01 (6 days)
14	1.2	0.948	—	1.04 (10 days)
16	—	0.944	0.96	0.814 (13 days)
17	—	1.15	0.866	—
18	—	0.97	0.82	0.999
20	1.17	0.943	0.91	0.999 (7 days)
22	—	1.03	0.93	1.06 (13 days)
23	1.17	1.11	0.983	—
29	—	0.85	1.03	—
30	—	0.943	0.897	0.889
36	—	0.91	—	0.999 (13 days)
38	—	0.95	—	1.02 (7 days)
41	0.93	1.07	0.88	—
42	—	—	0.984	1.15 (14 days)
44	—	0.856	0.92	—
45	—	—	0.79	0.933 (10 days)

From a study of these numbers it is seen in almost all the cases there is tendency to a decrease of calcium content of the blood toward the last months of pregnancy and a general tendency to rise directly after confinement. In

cases 13, 16, 29, 44 the results do not seem to be in accord with this general tendency, but in 13 and 16, the calcium content of the serum throughout pregnancy varies very little. These variations may be due to some other concurrent condition of the system at the time of observation. In one case of nephritis recently investigated the calcium content of the serum was found as low as 0.32 mg. per 10 cc.

Other workers in this field do not seem to have observed the above regular rise and fall in the calcium content of the blood at the different stages of pregnancy. Jansen [1918, 1, 2] thinks that the calcium content is little altered by pregnancy. He worked however on the whole blood and the disadvantages of this have been already indicated. Lamers [1912] thinks that the calcium content in blood in pregnant women is higher than that of non-pregnant women. Blair Bell [1908] showed in experiments on hens that the calcium content of the blood always rose previous to the laying of an egg and then immediately fell, there being a continuous rise and fall in the case of a hen that laid an egg on alternate days. According to this the calcium content of the blood of a woman ought to rise immediately before the birth of the infant and drop immediately after. The estimates given in the previous tables have not been made later than during the 8th and 9th month—never the day before the confinement. Also the earliest time after confinement that specimens of blood have been obtainable is five days. It would be interesting to examine the blood the day before and the day after confinement in the light of Bell's results.

The fact that the foetus is constantly taking in larger and larger quantities of calcium during growth, especially large quantities during the last two months of pregnancy, and that the maternal blood shows a distinct tendency for its calcium content to decrease during the 8th and 9th months of pregnancy indicates the importance of a sufficiency of calcium in the diet of the mother during this time. Otherwise the calcium needs of the foetus may be supplied by withdrawing too large an amount from the tissues of the mother (*e.g.* bone, teeth), as it is evident from the figures in the above tables that the blood tends to maintain a fairly constant value in spite of the heavy demands made upon it. The serum of the blood of a certain number of cases has been examined for calcium where there have been continuous miscarriages or still-born infants, and where a variation might be expected. So far no very definite relationship seems to be shown. These results are given in Table V.

Number 14 gave a calcium content on one occasion a week after miscarriage of 1.1 mg.

The case of a haemophylliac was interesting. The calcium content of the serum per 10 cc. at the 7th month of pregnancy was 0.66 mg., at the 8th month 1.05 mg., and after being treated with calcium chloride for a month, at the 9th month was 1.12 mg. About nine months later the same case when not pregnant gave 0.83 mg. of calcium per 10 cc. of serum, and after treating with calcium chloride gave 0.94 mg. of calcium.

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Table V.

Case	Characteristics of case	Calcium found	Percentage increase or decrease of calcium compared with average. (Calcium average 0.99 mg. per 10 cc. of serum.)	
1	Sterile	1.14	15.1	+
2	Five miscarriages	1.23	24.2	+
3	Still birth	1.09	10.1	+
4	Three miscarriages	1.08	9.09	+
5	Still birth	1.03	4.04	+
6	Twelve miscarriages	1.00	1.01	+
7	Six still-born	1.00	1.01	+
8	Several miscarriages	0.961	2.92	-
9	Several miscarriages	1.02	3.03	+
10	Several miscarriages	0.98	1.01	-
11	Four miscarriages and one still-born	0.996	0.6	-
12	Six miscarriages	0.918	7.2	-
13	Four premature births	0.88	11.11	-
14	Four miscarriages	0.74	25.2	-
15	Several miscarriages	0.561	43.3	-

Investigations have also been made into the question of the variation of the calcium content of the blood in connection with menstruation. These results have already been published [Widdows, 1922]. Since the calcium content of the blood of pregnant women is at the commencement of pregnancy slightly higher than that of non-pregnant women it was expected that there might be a rise in the calcium content at the beginning of menstruation. Bell [1908] had indicated that there was a rise just before menstruation followed by a very marked drop. Sherman, Gillett and Pope [1918] could show no distinct monthly cycle in the metabolism of nitrogen, phosphorus or calcium in the case of women. Although in two of the cases referred to in the *Lancet* of November 11th, 1922, there was a suggestion of a rhythmic rise and fall in the calcium content of the serum of the blood, the variations shown by the figures as a whole are not greater than those obtained in any normal series of estimations of blood calcium, and do not support the idea of a cyclic variation in the calcium content of the blood. The question of the relationship of the amount of calcium to the phosphorus in the blood has always been kept in view, and it was intended to take up that point this coming year, but de Wesselow [1922] has already examined his cases for this relationship and shows that the amount of phosphorus decreases in the later phases of pregnancy similarly to the calcium, but that during lactation there is a relatively high value for phosphorus in the plasma compared with that of the non-lactating female.

This work emphasises the perfect regulation of the human organism to the many and varied demands made upon it. The very great demand that undoubtedly at certain stages of its development is made by the foetus for calcium, is certainly met by a very perfectly balanced calcium metabolism, as at no time is the variation of the calcium content of the serum very marked. Even so in the later stages of pregnancy there does seem to be a distinct tendency for the calcium content of the blood to decrease. This indicates that at this time attention should be paid to the diet of the mother from the point

of view of its calcium content. During the last two months of pregnancy milk (on account of its high percentage of calcium) should be given the mother in a larger amount than that included in the average adult's diet and should be considered a necessary constituent of her diet.

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