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STUDIES ON PARTURIENT PARESIS IN DAIRY COWS

IV. CALCIUM AND PROTEINS IN BOVINE SERUM NORMALLY, AFTER PARTURITION, AND IN PARTURIENT PARESIS

By

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From the viewpoint of blood-chemistry the most distinctive feature of parturient paresis is that the calcium concentration of the blood is reduced below the physiological threshold value. The pathogenic importance of this reduction is shown by, for instance, the good effect of parenteral calcium therapy in the said disease. In this connection, however, the blood is only a transport medium conveying calcium from the supply area, the intestine, and the storehouse, the bones, to other tissues and organs of the body (during lactation the udder, in particular), where calcium exerts its biochemical activity or is secreted or excreted.

Parturient paresis is a pathological condition which can be controlled by calcium therapy, and it may therefore be inferred that the supply of calcium to the body's organs is inadequate in this disease. Such an inadequate supply can have three causes: 1) The demand for calcium is so great that it cannot be satisfied by mobilization of available reserves; 2) The mobilizable reserves are abnormally small or the mobilization occurs abnormally slowly; 3) The ability of the blood as a transport medium is reduced.

The present work is part of an investigation into the ability of the blood to transport calcium in parturient paresis.

It has long been known that the blood proteins bind calcium, so that only part of it is available in the form that is most impor-

tant for its diffusion from the blood, namely, as free calcium ions. At parturition and beginning lactation it is to be expected that the proteins of the blood undergo quantitative changes which, possibly, also involve their calcium-binding capacity and, thus, the ability of the blood to transport calcium. It is possible that in parturient paresis further changes of a similar kind occur.

To investigate this problem a number of blood specimens were taken from healthy cows in the period 1 month after the last calving to 1 month before the next calving (the non-calving period), from healthy cows immediately after calving, and from cows with parturient paresis. These specimens were examined by paper-electrophoretic fractionation of the serum proteins. Determination of total calcium and of free calcium ions in blood serum was also done.

MATERIAL

The material consisted of blood specimens collected over the period January—April 1959. All the cows from which the blood samples were taken belonged to the Swedish red and white breed and had before the collection of samples had a long period of stable feeding. The specimens were divided into 3 groups.

Group 1 consisted of 60 specimens from 60 healthy cows, taken in the non-calving period. The specimens were collected on farms registered with the Skaraborg Artificial Breeders Co-operative and situated within the town of Skara in the west of Sweden and in an area west of Skara at a distance not exceeding 20 kilometres from the town. The age-distribution of the cows approximated that reported by *Jönsson* (5) for cases of parturient paresis in the veterinary districts of Lidköping and Järpås, which border on the area in which the specimens were collected. In this group "age" refers to the parturition ordinal of the calving following upon the collection of specimens.

Group 2 consisted of 60 specimens from 60 healthy cows, taken within 72 hours after calving, that is, during the period when parturient paresis usually develops. The area in which the specimens were collected and the age-distribution were the same as in Group 1 ("age" is, of course, here the ordinal of the calving at which the specimen was taken).

Group 3 consisted of 47 specimens from 47 cows with parturient paresis, all collected before the treatment. In taking these

specimens we were assisted by the veterinarians called in at the time of illness. The specimens were collected from a larger area than those in Groups 1 and 2, but in no case did the distance from Skara exceed 35 kilometres. The age-distribution in this group was not exactly the same as that in Groups 1 and 2, but since the mean age, as expressed in parturition ordinals, is 6.1 in Groups 1 and 2, and 6.4 in Group 3, and since there was no abnormally large accumulation of cases with the same parturition ordinal in Group 3 as compared with Groups 1 and 2, the three groups would be comparable in this respect.

In no group were more than four specimens collected from the same farm. In a few cases blood specimens were taken from the same cow both in the calving and the non-calving period.

METHODS

The blood samples were collected as described in a previous report in this series (4), two samples being taken in every case, one under liquid paraffin for determination of pH and free calcium ions, and one without liquid paraffin for other determinations. The specimens were transported to the laboratory by car, since it was found that samples collected under liquid paraffin do not stand conveyance by post.

Unless taken for immediate analysis, the serum samples were for all the analyses kept in the refrigerator for a day or two, with the exception of those for electrophoresis, which were stored by deep-freezing.

The methods for determination of calcium ions have been described in previous reports in this series (1, 4). Total protein was determined by a biuret method devised by *Kingsley* (6, 7), *Weichselbaum* (11) and *Gornall et al.* (3), using the technique described by *Reiner* in Standard Methods of Clinical Chemistry, Vol. I (10). At checking against van Slyke's copper-sulphate method, which was used in a previous work (4), both methods gave the same result within the limits of the experimental errors.

The fractionation of protein was done in a Spinco electrophoresis cell, model R. Veronal buffers were used, with a pH of 8.6 and ionic strength 0.075, consisting of 2.75 g. of diethyl barbituric acid and 15.40 g. of sodium diethyl barbiturate, dissolved in 1 litre of distilled water. Each buffer solution was used only for two electrophoretic determinations (16 strips), and between

these two procedures the cell was emptied and re-filled with the same solution, so that the composition of the solutions in the two chambers was always the same at the start. The strips used were Beckman no. 300-846 (S & S 2043 A); amount of serum 0.008 ml. per strip; time 16 hours; constant strength of current 2.5 mA.

After electrophoresis the papers were dried at about 120°C for half an hour. They were then stained with Amidoschwarz 10 B. The following technique was used for staining and washing: The staining solution consisted of 2 g. of Amidoschwarz 10 B, 100 ml. of acetic acid and methanol to 1000 ml. This solution was used until it no longer covered the papers (about 5 changes or 40 strips). Four solutions for washing were used, each containing 30 g. of phenol, 100 ml. of acetic acid and water to 1000 ml. Each solution was used four times, on each occasion the vessel with the most used solution being emptied and re-filled with freshly prepared solution and thereafter placed as the last vessel. The paper strips were put in the staining solution for 15 minutes and then in each of the washing solutions for 15 minutes. The papers were dried at room temperature or slightly higher temperature (on top of a thermostat).

After drying the staining of the paper strips was measured in a Spinco Analytrol registering and integrating photometer for paper electrophoresis. This is on delivery adjusted for and provided with a filter suitable for measuring of strips stained with bromophenol blue, the adjustment and the filter also being suitable for Amidoschwarz 10 B. (The reason for using Amidoschwarz 10 B instead of bromophenol blue was that, in addition to the good properties of bromophenol blue—good stain intensity on the protein compound, staining in proportion to the protein content fairly independent of the type of protein, low staining of the paper, capable of being washed off—Amidoschwarz 10 B also gives a stain that lasts for at least five years, while bromophenol blue fades fairly soon.)

In the afore-mentioned study (4) the accuracy of the analytical methods for the investigations concerned was assessed by calculating the error of the method from a series of 50 duplicate determinations. Since the same methods were used for analysis of calcium in the present work, this calculation would also be valid here. Determination of the error of the method as regards total protein using the same technique gave a percentage error

Table 1.
Errors of the methods used in protein determination and fractionation, calculated by means of 50 duplicate determinations.

Analysis	Mean $\bar{x}$	Range of variation	Standard error of single determination s	Error of method v
Serum protein g./100 ml.	8.01	6.3—10.0	0.10	1.22
Albumin g./100 g. protein	53.35	42.6—64.2	1.67	3.12
Albumin g./100 ml. serum	4.25	3.43—5.42	0.14	3.18
α-globulin g./100 g. protein	11.93	8.7—15.2	0.86	7.17
α-globulin g./100 ml. serum	0.95	0.70—1.23	0.07	7.04
β-globulin g./100 g. protein	9.63	6.0—14.8	0.50	5.24
β-globulin g./100 ml. serum	0.77	0.46—1.17	0.04	5.33
γ-globulin g./100 g. protein	25.10	12.2—37.7	1.44	5.75
γ-globulin g./100 ml. serum	2.04	0.84—3.48	0.12	5.81

of 1.22, the range of variation for the analytical results being 6.3 %—10. 0 % of protein.

The error of the method was also calculated for the determination of the protein fractions. Since in the following the results of this determination will be reported both as a percentage of total protein (the composition of the protein) and as the content in serum of each fraction, the methodical error was calculated for these two series of results. In the latter case the total-protein content, too, influences the result and, hence, these methodical errors are not consistent. The results will be seen in Table 1.

STATISTICAL TREATMENT OF THE ANALYTICAL RESULTS

As has been said earlier, the analytical results for the different calcium and protein fractions were divided into three groups and each of these was divided into age-classes according to parturition

ordinal. In each group analysis of variance was done by current methods. The results of these analyses are found in Table 3. For want of space, only those values for which in any group a significant variance was obtained between age-classes are included in the table.

In estimating differences between the groups a point to be considered is that any age-variation present will cause a difference between the groups, which is ascribable only to the age-distribution of the material. To eliminate such a difference a comparison between the groups was made by means of analysis of variance with multi-stage grouping. For each substance a comparison was first made between the two groups whose mean values deviated most markedly from each other. If a significant difference ($P < 0.05$) was obtained, other comparisons were also made, as will be seen from Table 2 in which mean values and standard errors for the groups will also be found.

For want of space the individual analytical results are not reported in this paper, and of the variance analyses the results only (F and probability) are given. The complete material of figures can be obtained from the author.

RESULTS AND DISCUSSION

It will be seen from Table 2 that after normal calving the total blood-calcium content is lower than it is in a non-calving cow, this decrease being noted for both the organically bound and the free fraction, and that a further lowering of both fractions occurs in the clinical condition of parturient paresis. All the differences mentioned here are significant ($P < 0.001$).

The total protein content is found to be lower after normal calving than in healthy cows in the non-calving period. The difference is probably significant ($P < 0.05$). The group with parturient paresis shows a value which lies between the two normal groups and does not differ significantly from either of these.

As regards the contents of the individual fractions it is at once found that the albumin concentration is virtually the same in the three groups and that, accordingly, the lowering of total protein that occurs after calving is wholly concentrated to the globulins. It is further found that the difference in other fractions, too, between normally calving cows and cows with parturient paresis is fairly slight and in no fraction significant. The lowering

Table 2.

Analytical results in groups, and F-values at comparison between group values, all according to the text.

Comparison between groups no.	Group	Number of cases	Total Ca Mean ± ε mg. per 100 ml.	Ca^{++} Mean ± ε mg. per 100 ml.	Bound Ca (calculated) Mean ± ε mg. per 100 ml.
	1	60	10.47±0.08	4.37±0.07	6.10±0.08
	2	60	9.16±0.13	3.94±0.08	5.23±0.10
	3	47	6.00±0.26	2.31±0.14	3.69±0.14
1 and 2; F		120	75.63***	16.35***	33.69***
2 and 3; F		107	123.23***	112.72***	57.71***
1 and 3; F		107	300.22***	183.33***	231.75***

Comparison between groups no.	Group	Number of cases	Serum protein Mean ± ε g. per 100 ml.	Albumin Mean ± ε g. per 100 ml.	α-globulin Mean ± ε g. per 100 ml.
	1	60	8.31±0.09	4.21±0.05	1.02±0.02
	2	60	7.79±0.09	4.15±0.05	0.95±0.02
	3	47	8.04±0.12	4.24±0.06	0.95±0.02
1 and 2; F		120	8.21*		7.48*
2 and 3; F		107	1.60	0.90	
1 and 3; F		107	2.46		6.05*

Comparison between groups no.	Group	Number of cases	β-globulin Mean ± ε g. per 100 ml.	γ-globulin Mean ± ε g. per 100 ml.	Albumin as a percentage of serum protein Mean ± ε
	1	60	0.78±0.02	2.31±0.07	50.79±0.59
	2	60	0.68±0.02	2.01±0.08	53.54±0.68
	3	47	0.70±0.02	2.15±0.09	53.17±0.89
1 and 2; F		120	13.45***	2.71	3.47
2 and 3; F		107	0.31		
1 and 3; F		107	6.93**		

Comparison between groups no.	Group	Number of cases	α-globulin as a percentage of serum protein Mean ± ε	β-globulin as a percentage of serum protein Mean ± ε	γ-globulin as a percentage of serum protein Mean ± ε
	1	60	12.28±0.19	9.44±0.25	27.58±0.64
	2	60	12.28±0.20	8.80±0.19	25.46±0.71
	3	47	11.87±0.20	8.67±0.22	26.36±0.82
1 and 2; F		120			1.47
2 and 3; F		107	1.60		
1 and 3; F		107	1.60	4.38	

at calving is noted for all the globulin fractions. Both groups of newly calved cows show a probably significant lowering of α-globulin as compared with healthy cows in the non-calving period ($P < 0.05$), and a significant lowering of β-globulin ($P < 0.01$ and $P < 0.001$, respectively), while for the γ-globulin a numerical but not statistically significant lowering is noted. This decrease in globulin after calving is at least partly attributable to the transmission (via the colostrum) of immune-globulins from the mother to the calf (see *Larsson* (8)). Further, *Witschi* (12), among others, has found that newly born calves have a high α-globulin content.

If we consider only the distribution of protein among the different fractions, we will of course find that the proportion of albumin rises and that of globulins falls after calving, both normally and in parturient paresis. The decrease is noted for all the globulins, though the proportion of α-globulin in total protein does not fall in healthy calving cows. The said differences are not statistically significant.

A point that is often overlooked in studies of this and a similar nature is the importance of considering not only the composition of the protein but also its relation to the total protein content. Accordingly, the afore-said increase in the proportion of albumin in the total protein content at calving is not paralleled by an increase of serum albumin but is only a result of the decrease in serum globulin.

In establishing a correlation between the protein content and the content of free and bound calcium in blood serum, it would be justifiable to proceed from *McLean* and *Hastings'* (9) presumption that Guldberg-Waage's law would be valid for this correlation. These authors showed that the law is valid for human serum, and by making certain assumptions concerning the nature of the protein they were able to find a formula by which, knowing the content of total serum calcium and total serum protein, the content of free serum-calcium ions could be calculated. By using an independent method for determining the content of free calcium ions they were able to show that this formula is valid for human serum.

In a previous study of bovine serum (2) we had been able to show that McLean and Hastings' original equation is not applicable to this medium. We assumed then that the presuppositions concerning the nature of protein, on which this equation

was based, would not be valid for the proteins of bovine serum. The presuppositions were that 1 g. of serum protein would be equivalent to 0.243 mEq of base-combining calcium-binding proteinate groups (on the assumption, borne out by McLean and Hastings, that in relation to calcium the protein acts like a divalent anion) and that the albumin-globulin ratio is 1.8. We found that if these presuppositions are not correct as far as bovine serum is concerned, but if the number of milliequivalents of calcium-binding proteinate groups that equal 1 g. of protein, as well as the albumin-globulin ratio, are constant in bovine serum, too, the conditions would be as follows:

If in a co-ordinate system the content of free calcium ions is plotted as the ordinate against $\frac{Ca^{2+} \cdot Prot}{Ca - Ca^{2+}}$ as the abscissa, the points thus obtained should form a straight line. (Ca is the total calcium content and Ca^{2+} the content of free calcium ions, both expressed in mg. per 100 ml., and Prot is the total protein content expressed in g. per 100 ml.) We found then that this was not the case. Now, it seems hardly probable that the number of calcium-binding proteinate groups per g. of protein would vary quite irregularly, and this investigation shows that the albumin-globulin ratio varies but hardly so much that this could explain the complete failure of obtaining a straight line. It is therefore probable that in certain conditions the proteins undergo changes of such a nature that their calcium-binding capacity is affected, that is, the number of milliequivalents of calcium-binding proteinate groups per g. of protein is altered. Each one of these conditions should then, with the above-mentioned method, give points arranged in groups around a straight line (a fairly great scatter is to be expected, since the changes are of course likely to be of different magnitude in different cases).

The above-mentioned study (2), in which a straight line was not obtained, comprised a material of blood sera from diseased as well as from healthy cows. Since the purpose was to find out whether any generally applicable equation similar to that found by McLean and Hastings could be used on bovine sera, and of course both in diseased and healthy cows, no division into groups was done. The material presented here was divided, however, and exactly into 3 groups that differ from one another, for instance, with respect to the serum-calcium content, and of which each one represents a clearly defined condition, namely, healthy

Tables 3 (a—f).

Analytical results in age classes (parturition ordinals) according to text, and F-values at comparison between age classes.

Table 3 a.

Bound Ca, mg. per 100 ml.

Parturition ordinal	Group 1		Group 2		Group 3	
	Number of cases	Mean ± ε	Number of cases	Mean ± ε	Number of cases	Mean ± ε
2	1	6.55	1	5.20	1	5.05
3	3	6.27±0.22	3	5.07±0.22	5	4.38±0.48
4	9	5.99±0.16	9	5.38±0.24	5	3.65±0.46
5	12	6.09±0.26	12	5.81±0.29	5	3.41±0.26
6	12	6.22±0.14	12	4.75±0.19	5	3.34±0.61
7	9	6.07±0.26	9	4.95±0.17	9	4.01±0.30
8	6	5.71±0.31	6	5.44±0.33	9	3.65±0.28
9	5	6.46±0.22	5	4.90±0.12	7	3.27±0.24
10 and higher	3	6.03±0.34	3	5.47±0.37	1	2.65

Comparison	Number of cases	F	Number of cases	F	Number of cases	F
	60	0.59	60	2.13*	47	1.25

Table 3 b.

Serum Protein, g. per 100 ml.

Parturition ordinal	Group 1		Group 2		Group 3	
	Number of cases	Mean ± ε	Number of cases	Mean ± ε	Number of cases	Mean ± ε
2	1	7.8	1	8.6	1	7.2
3	3	8.13±0.39	3	7.10±0.26	5	7.30±0.41
4	9	7.83±0.24	9	7.27±0.14	5	7.72±0.37
5	12	8.15±0.15	12	7.69±0.14	5	7.94±0.45
6	12	8.25±0.17	12	7.68±0.13	5	7.92±0.20
7	9	8.63±0.24	9	8.34±0.34	9	8.40±0.30
8	6	8.50±0.30	6	8.05±0.18	9	8.33±0.25
9	5	8.64±0.29	5	7.78±0.21	7	8.27±0.29
10 and higher	3	9.10±0.15	3	8.50±0.51	1	7.7

Comparison	Number of cases	F	Number of cases	F	Number of cases	F
	60	1.97	60	3.26**	47	1.21

Table 3c.
Albumin, g. per 100 ml.

Parturition ordinal	Group 1		Group 2		Group 3	
	Number of cases	Mean ± ε	Number of cases	Mean ± ε	Number of cases	Mean ± ε
2	1	4.55	1	5.43	1	3.94
3	3	4.36±0.13	3	3.87±0.16	5	4.22±0.10
4	9	4.12±0.15	9	4.25±0.12	5	4.21±0.12
5	12	4.13±0.14	12	4.18±0.07	5	4.29±0.12
6	12	4.29±0.10	12	4.22±0.10	5	4.31±0.29
7	9	4.27±0.13	9	4.24±0.13	9	4.09±0.21
8	6	4.15±0.10	6	4.03±0.17	9	4.45±0.17
9	5	4.06±0.10	5	3.87±0.09	7	4.19±0.12
10 and higher	3	4.33±0.25	3	3.78±0.16	1	4.01

Comparison	Number of cases	F	Number of cases	F	Number of cases	F
	60	0.50	60	3.45**	47	0.54

Table 3d.
γ-globulin, g. per 100 ml.

Parturition ordinal	Group 1		Group 2		Group 3	
	Number of cases	Mean ± ε	Number of cases	Mean ± ε	Number of cases	Mean ± ε
2	1	1.54	1	1.38	1	1.65
3	3	2.01±0.14	3	1.55±0.07	5	1.53±0.24
4	9	1.99±0.18	9	1.46±0.10	5	1.87±0.28
5	12	2.13±0.12	12	1.94±0.10	5	1.92±0.26
6	12	2.14±0.12	12	1.79±0.09	5	2.07±0.16
7	9	2.57±0.16	9	2.49±0.27	9	2.55±0.26
8	6	2.65±0.22	6	2.33±0.18	9	2.31±0.14
9	5	2.85±0.24	5	2.31±0.10	7	2.40±0.26
10 and higher	3	2.91±0.22	3	2.90±0.33	1	1.96

Comparison	Number of cases	F	Number of cases	F	Number of cases	F
	60	3.66**	60	6.15***	47	1.78

Table 3e.
Albumin as a percentage of serum protein.

Parturition ordinal	Group 1		Group 2		Group 3	
	Number of cases	Mean ± ε	Number of cases	Mean ± ε	Number of cases	Mean ± ε
2	1	58.3	1	63.2	1	54.7
3	3	53.77±1.33	3	54.43±0.82	5	58.34±2.33
4	9	52.67±1.32	9	58.47±1.32	5	54.94±2.29
5	12	50.75±1.59	12	54.54±1.09	5	54.46±2.34
6	12	52.14±1.36	12	55.01±1.08	5	54.58±2.04
7	9	49.57±1.21	9	51.23±1.94	9	49.12±2.64
8	6	49.05±1.51	6	50.08±1.84	9	53.38±1.40
9	5	47.14±1.65	5	49.86±1.18	7	51.16±2.68
10 and higher	3	47.60±2.36	3	44.63±1.06	1	52.1

Comparison	Number of cases	F	Number of cases	F	Number of cases	F
	60	1.74	60	5.84***	47	1.22

Table 3f.
γ-globulin as a percentage of serum protein.

Parturition ordinal	Group 1		Group 2		Group 3	
	Number of cases	Mean ± ε	Number of cases	Mean ± ε	Number of cases	Mean ± ε
2	1	19.7	1	16.0	1	22.9
3	3	24.47±1.46	3	21.83±0.43	5	20.52±2.12
4	9	25.22±1.79	9	19.97±1.14	5	23.90±2.29
5	12	26.04±1.35	12	25.13±1.01	5	23.90±2.05
6	12	25.81±1.02	12	23.25±0.96	5	26.00±1.54
7	9	29.62±1.42	9	29.19±2.13	9	29.94±2.28
8	6	30.95±1.73	6	28.88±1.84	9	27.63±1.29
9	5	32.82±1.77	5	29.70±0.62	7	28.70±2.26
10 and higher	3	32.00±2.45	3	33.80±2.01	1	25.5

Comparison	Number of cases	F	Number of cases	F	Number of cases	F
	60	3.39**	60	7.50***	47	1.90

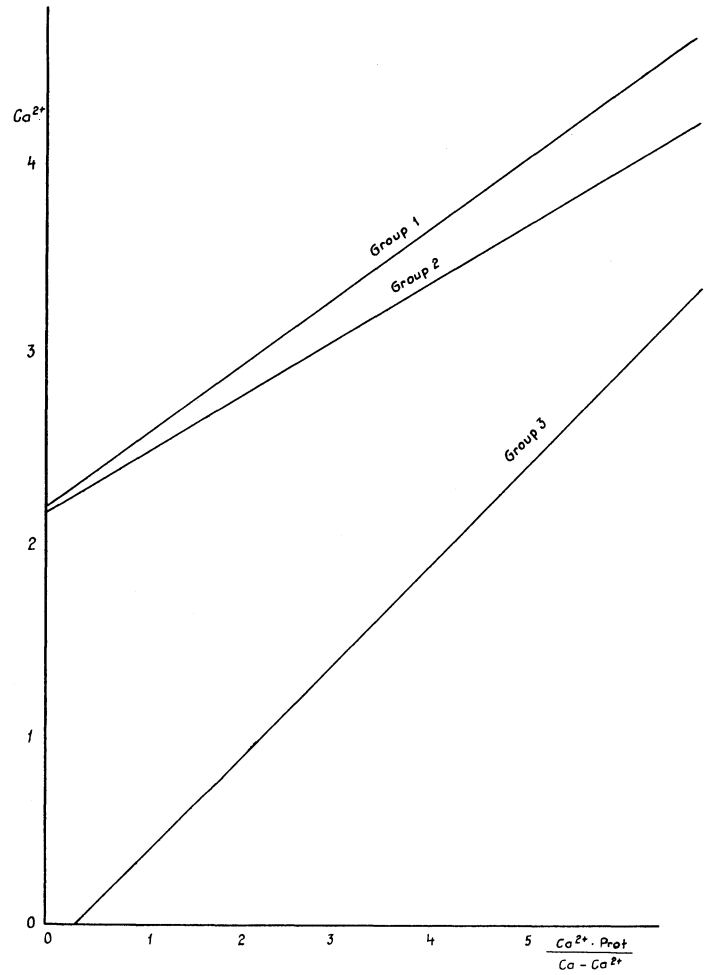

Fig. 1. The line $Ca^{2+} = a + b \cdot \frac{Ca^{2+} \cdot Prot}{Ca - Ca^{2+}}$

cows in the non-calving period (Group 1), healthy cows in the calving period (Group 2), and cows with parturient paresis (Group 3).

If for each of these groups the values for a and b in the complete formula for such a straight line are calculated by means of the least square method:

$$Ca^{2+} = a + b \cdot \frac{Ca^{2+} \cdot Prot}{Ca - Ca^{2+}}$$

the results given in Table 4 a will be found. The uncertainty in a and b, s_a and s_b, and the correlation coefficient r of the line, its t-value and its probability value P are also given in the table. The course of the three lines is seen in Fig. 1. It is now evident that the probability for the existence of the three lines is very high ($P < 0.001^{***}$), and that the slope b of all the three lines is different, while the intersection on the ordinate a is, within the experimental errors, the same for the two normal groups 1 and 2, but quite different for the paresis group 3. For this reason it seems probable that at calving there occurs a change in the calcium-binding capacity of the proteins, and that in parturient paresis further changes in this respect, possibly of a different nature, would occur.

Table 4 a.
Statistical results of the estimation of the line

$$Ca^{2+} = a + b \cdot \frac{Ca^{2+} \cdot Prot}{Ca - Ca^{2+}} \quad \text{(see text)}$$

	a	sa	b	sb	r	t	P
Group 1	2.19	0.19	0.36	0.03	0.84	11.805	$<0.001^{***}$
Group 2	2.16	0.25	0.30	0.04	0.69	7.191	$<0.001^{***}$
Group 3	—0.15	0.33	0.51	0.65	0.77	7.843	$<0.001^{***}$

Table 4 b.
Statistical results of the estimation of the line

$$Ca^{2+} = a + b \cdot \frac{Ca^{2+} \cdot alb}{Ca - Ca^{2+}} \quad \text{(see text)}$$

	a	sa	b	sb	r	t	P
Group 1	2.10	0.19	0.74	0.06	0.85	12.106	$<0.001^{***}$
Group 2	2.09	0.24	0.58	0.07	0.71	7.785	$<0.001^{***}$
Group 3	—0.46	0.39	1.10	0.15	0.75	7.399	$<0.001^{***}$

The above-said changes occuring in the proteins at calving and parturient paresis could possibly be due to disturbances in the albumin-globulin ratio. We have not been able to find any great disturbances of this kind. If the fairly common presumption is correct, albumin being the principal calcium-binding substance, we can in the equation replace the total protein content by the albumin content, thereby completely eliminating any disturbances in the albumin-globulin ratio. Now, if these alone were the cause

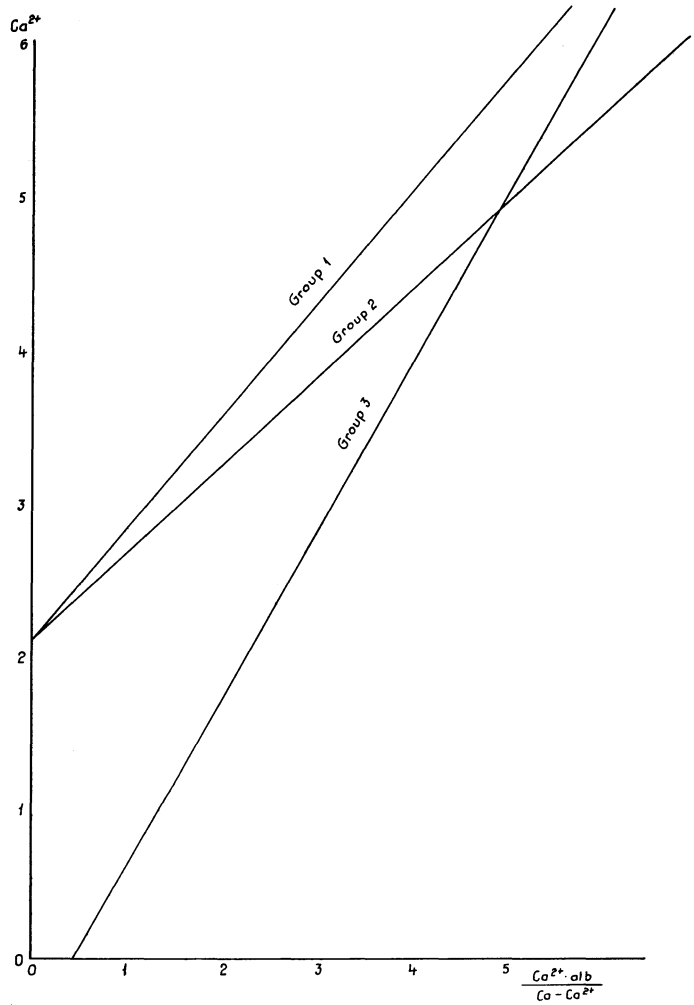

Fig. 2. The line $Ca^{2+} = a + b \cdot \frac{Ca^{2+} \cdot alb}{Ca - Ca^{2+}}$

of the three different lines, it would be found that with this procedure the three lines then obtained would coincide, or at least nearly coincide. It will be seen in Table 4 b and Fig. 2 that this is not the case, but, as before, b is different in the three groups, while a is the same in normal cows in the calving and in the non-calving period but quite different for cows with parturient paresis. Therefore, it can be concluded that it is not probable that a disturbance in the albumin-globulin ratio would be

the cause of the changes in the calcium-binding capacity of protein.

As regards the nature of the changes in protein discussed here, it is not possible at present to give a definite answer. It would be most readily presumed that a change occurs in the structure of the protein molecules, but two other possibilities exist. Firstly, it is conceivable that some other substance competes with calcium for the positions on the protein, and that an alteration in the content of such a substance would be the cause of the changed calcium-binding capacity. Secondly, it should be borne in mind that the calculations done here are based on the assumption that calcium is bound by protein alone. If another substance binds calcium to a high degree, it would in these calculations be included in the "protein", and a change in the content of this substance could then be at least a part of the change in "protein".

VARIATIONS WITH AGE IN SERUM CALCIUM AND SERUM PROTEIN

In all the cases in this study the age of the cow was known, and therefore the variation in serum calcium and serum protein occurring with age was also investigated. The material is fairly small for such an investigation, as there are only a few values in each age-group, and the interpretation of the results is therefore to be regarded as somewhat uncertain. The results are shown in Table 3.

For the content of total calcium and the content of free calcium ions no variation with age is seen. For bound calcium, on the other hand, there is a probably significant variation in the group of normal calving cows. The trend seems to be towards lower values at higher age. A corresponding, though less distinct and not significant, variation possibly occurs in paretic cows but not in normal cows in the non-calving period.

In healthy calving cows there is a tendency to rising protein content with increasing age. The variation with age is significant. The same tendency can be seen both in normal cows in the non-calving period and in paretic cows, but in these groups the variation is not significant.

The albumin content shows a variation with age in healthy calving cows, the variation is significant but the tendency is

difficult to interpret. Possibly, the content of albumin rises at a low age, reaches a maximum value at the fourth or the fifth calving, after which it falls again; but this is extremely uncertain, and the other two groups, in which there is no significant variation with age, show no similar tendency. The α- and β-globulins show no, at least no statistically demonstrable, variation with age in any of the groups, but the γ-globulins in healthy cows, both in the non-calving and the calving period, show distinctly and significantly an increase with age, and in the group of paretic cows, too, the same tendency can be noted, although there the variation is not significant. The material is centainly too small to allow a definite statement, but there seems to be some difference between healthy cows in the non-calving period and calving cows, normal as well as paretic ones, in that the increase of γ-globulins with age in cows in the non-calving period seems to occur throughout, while in calving cows it appears to cease at the seventh parturition, after which it remains relatively constant.

The cause of the rise in γ-globulins with age may be supposed to be that with increasing age, and thus an increasing number of infections, the antibody titre rises.

As regards the composition of serum protein, the result of the increase in γ-globulin with age, other fractions remaining relatively constant, will of course be that the proportion of γ-globulin in the total protein content rises, while the proportion of other fractions falls. This decrease is, however, significant only in the case of albumin in healthy calving cows, but can also be noted in healthy cows in the non-calving period and in paretic cows. In the case of α- and β-globulin no such decrease can be demonstrated statistically.

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SUMMARY

Determination of total calcium and free calcium ions, determination of total protein and paper-electrophoretic fractionation of the protein were done on blood serum from cows, in the non-calving period, a few days after calving, and in parturient paresis. The following results were obtained:

1. Both the content of free calcium ions and the content of bound calcium is normally lower immediately after calving than it is in the non-calving period, and if parturient paresis develops, the content of both calcium fractions is much lower than normally after calving.

2. The protein content is lower after calving than in the non-calving period. This is attributable to a decrease of globulin, while the albumin concentration remains relatively constant. No difference in total protein content or in the contents of the protein fractions between paretic cows and normal calving cows could be demonstrated. It could be demonstrated, however, that it is probable that the calcium-binding capacity of the protein is changed at calving, and that in parturient paresis there occur further changes in this capacity, which are of a different nature from those occurring normally at calving. In neither case does this change seem to be caused by a disturbance in the albumin-globulin ratio.

3. The content of serum protein increases with age, which is attributable mainly to a rise in γ-globulin.

ZUSAMMENFASSUNG

Studien über Paresis puerperalis bei Milchkühen. IV. Kalzium- und Proteingehalt im Rinderserum unter normalen Verhältnissen, nach dem Partus und bei Paresis puerperalis.

Der totale Kalziumgehalt, der Gehalt an freien Kalziumionen und der totale Proteingehalt wurden im Blutserum von Kühen ausserhalb der Kalbezeit, einige Tage nach dem Partus und bei Paresis puerperalis bestimmt. Die Serumproteinfraktionierung geschah papierelektrophoretisch. Folgende Ergebnisse wurden erzielt:

1. Der Gehalt freier Kalziumionen wie auch der Gehalt gebundenen Kalziums ist normal unmittelbar nach dem Partus niedriger als ausserhalb der Kalbezeit, und falls sich Paresis puerperalis entwickelt, liegt der Gehalt an beiden Kalziumfraktionen viel niedriger als normal nach dem Partus.

2. Der Proteingehalt ist nach dem Partus niedriger als ausserhalb der Kalbezeit, was auf einer Erniedrigung des Globulingehaltes beruht, während der Albumingehalt ziemlich konstant verbleibt. Ein Unterschied im totalen Proteingehalt oder im Gehalt der verschiedenen Proteinfraktionen zwischen normal kalbenden Kühen und den von Paresis puerperalis betroffenen Kühen konnte nicht nachgewiesen werden. Es wurde jedoch festgestellt, dass sich das Kalziumbindungsvermögen des Proteins beim Partus wahrscheinlich ändert, und dass bei Paresis puerperalis diese Kapazität weitere Veränderungen erleidet, und zwar anderer Art als der bei normalen Partus auftretenden. In keinem Falle scheint diese Änderung durch Verschiebung der Albumin-Globulinquote verursacht worden zu sein.

3. Der Serumproteingehalt steigt mit zunehmendem Alter, was hauptsächlich auf einer Erhöhung des γ-Globulingehaltes beruht.

SAMMANFATTNING

Studier över paresis puerperalis hos mjölkkor. IV. Kalcium- og proteinhalt i blodserum från nötkreatur under normala förhållanden, efter kalvning och vid paresis puerperalis.

Bestämning av totala kalciumhalten , halten fria kalciumjoner och totalproteinhalten samt papperselektroforetisk proteinfraktionering har utförts på blodserum från kor utanför kalvningstiden, någon dag efter kalvning samt vid paresis puerperalis. Följande resultat erhölls:

1. Såväl halten fria kalciumjoner som halten bundet kalcium är normalt lägre omedelbart efter kalvning än utanför kalvningstiden, och om paresis puerperalis uppkommer, är halten av båda kalciumfraktionerna mycket lägre än normalt efter kalvning.

2. Proteinhalten är lägre efter kalvning än utanför kalvningstiden. Detta beror på en sänkning av globulinhalten, medan albuminhalten förblir rätt konstant. Ingen skillnad i proteinhalt eller i halten av de olika proteinfraktionerna mellan paretiska kor och normalt kalvande

kor kunde påvisas. Emellertid kunde det visas, att det är troligt, att proteinets kapacitet att binda kalcium ändras vid kalvning, och att vid paresis puerperalis uppträder ytterligare förändringar i denna kapacitet, av annan natur än de som uppträder normalt vid kalvning. I intetdera fallet tycks denna ändring vara orsakad av en rubbning av albumin-globulinkvoten.

3. Halten serumprotein stiger med åldern, vilket huvudsakligen beror på en höjning av γ-globulinhalten.

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