SOME AETIOLOGICAL AND CLINICAL ASPECTS OF BOVINE PARTURIENT PARESIS (MILK FEVER).

A thesis submitted for the degree of Doctor of Philosophy, University of Edinburgh, October, 1952.

by

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SOME AETIOLOGICAL AND CLINICAL ASPECTS OF BOVINE PARTURIENT PARESIS (MILK FEVER).

INTRODUCTION.

History.

Most authorities are agreed that milk fever has become prominent among the diseases of dairy cattle only within the last century, or century and a half. It may be, however, that the paucity of references to the disease in early literature is more a reflection of the general lack of veterinary literature on dairy cattle at this time than it is an indication of the incidence of the disease.

Hutyra and Marek (1926) state that the time when the disease became known corresponds with the period when it became customary to feed cows more generously with the object of increasing milk production. Numerous workers have been of the opinion that intensive feeding and breeding for increased milk production have resulted in an increased incidence of milk fever. This opinion appears to be borne out by the fact that the disease occurs rarely in beef cattle.

No attempt will be made here to give a comprehensive review of the voluminous literature on this disease. This has been done recently in an excellent review by Hibbs (1950). Instead reference will be made only to the more important landmarks in the advance of our knowledge of the disease, and, in particular, to those aspects which have an immediate bearing on the subject matter of this thesis.

Although milk fever had been recognised at least a century earlier (Hutyra and Marek, 1926), it was not until 1897 that a definite advance was made in our knowledge of the disease. In that year a Danish veterinarian, Jurgens J. Schmidt, made his classical discovery of the udder infusion, later to be changed to udder inflation, treatment of milk fever. Prior to this the treatments recommended were entirely empirical and indeed often contradictory. The theories as to the aetiology were of a like nature, and are now only of academic interest.

Schmidt's treatment was based on his theory that the disease was due to infection of the udder with a "virus". His theory arose from a mistaken observation. In examining colostrum from cases of milk fever, he observed what he believed to be evidence of cellular disintegration, and concluded that some ferment or toxin was causing degeneration of the mammary alveoli. It is probable that what Schmidt actually saw were normal constituents of colostrum, the so-called "corpuscles of Donne" (mono-nuclear cells filled with fat globules). It is obvious, therefore, that if Schmidt had controlled his observations by examining the colostrum of normal cows, his theory would never have been formulated, and in consequence his highly successful treatment would not have been evolved.

In order to destroy the supposed infection Schmidt (1902) infused the udders of cows affected with milk fever with a 1% solution of potassium iodide. The results were remarkably successful and reduced the mortality of the disease from about 70%

to around 15%. It was soon discovered that other concentrations of potassium iodide were equally effective, and indeed that water alone would effect a cure. Another Danish veterinarian, Andersen of Skanderborg (Greig 1930a), discovered that inflation of the udder with air was even more effective, reducing the mortality to about 1%.

It appeared, therefore, that the mechanical distension of the mammae was the curative factor.

The mammary inflation treatment held the field for over a quarter of a century until it was supplanted by the calcium substitution therapy evolved following the demonstration that milk fever was accompanied by a hypocalcaemia.

Schmidt's treatment, while it provided a therapeutic measure which reduced the mortality of the disease from about 70% to 1%, did little to elucidate the cause. In fact, as Greig (1930a) stated, "The very specificity of the cure rendered the nature of the malady all the more obscure; yet the problem was the more attractive by reason of its apparent simplicity".

From 1897 until 1925 little was added to our knowledge of milk fever. In 1925, two theories as to the nature of the disease were advanced, namely, the Hypoglycaemia Theory of Widmark and Carlens, and the Parathyroid Dysfunction Theory of Dryerre and Greig.

<u>The Hypoglycaemia Theory</u>.

This theory has been refuted, but it is of interest as it was the first theory to stimulate rational biochemical investigation of the disease.

Widmark and Carlens (1925a, 1925b, 1925c, and 1925d) postulated that milk fever was a hypoglycaemic coma. They suggested that the beneficial effects of udder inflation were due to its inducing a relative hyperglycaemia. From the results of fermentation tests they concluded that the hyperglycaemia following inflation was the result of an increased glucose content of the blood, although they stated that occasionally resorption of lactose from the mammary gland took place.

The hypoglycaemia theory was supported by Maguire (1926a), who reported successful results (Maguire 1926b) in the treatment of milk fever by glucose injections. Auger (1926a), McLeod (1926) and Edwards (1926) also supported the hypoglycaemia theory. Auger and McLeod regarded the hypoglycaemia as being due to removal of glucose from the blood by the mammary gland. Widmark (1926) agreed with this theory and regarded the disease as a reaction to intensive breeding for milk production.

Doubts were cast on the hypoglycaemia theory by the work of Hayden and Sholl (1924, 1925), Hayden (1927, 1929), and of Fish (1927, 1928). These workers showed that, in the majority of cases of milk fever, instead of there being a hypoglycaemia present, the blood sugar was in fact in excess of normal amounts. Work by Little and Keith (1926) lent support to the finding of a hyperglycaemia. Moussu and Moussu (1926) could not demonstrate any lowering of the blood sugar values; they found that glucose injections were not successful in treatment.

In defence of the hypoglycaemia theory Auger (1926) stated that the increased level of blood sugar was due to the presence in the blood of lactose, which was not available for tissue metabolism, and that the available sugar, in the form of glucose, was lowered. Using the Folin-Svedburg method for the differentiation of lactose and glucose in the blood and urine, Hayden (1927) was able to disprove Auger's contention. He showed that in milk fever there is a true hyperglycaemia, and that lactose appears in the blood in appreciable quantities only after udder inflation. Schlotthauer (1928) also showed that milk fever is accompanied by a hyperglycaemia. Finally, Petersen, Hewitt, Boyd and Brown (1931) showed that the induction of hypoglycaemia in the cow by insulin injections did not produce symptoms of milk fever, and that the bovine can tolerate a much lower level of blood sugar than other species, without exhibiting clinical signs.

Thus the hypoglycaemia theory was disproved.

The Parathyroid Dysfunction Theory.

This theory, without supporting experimental evidence, was advanced early in 1925 by Dryerre and Greig.

Greig (1930a) has stated that Dryerre and he adopted the approach of submitting the problem to a process of reasoning, based on the available knowledge pertaining to the occurrence and clinical manifestations of the disease. As a result they put forward the hypothesis that milk fever was associated with a dysfunction of the parathyroid glands, resulting in an accumulation of protein toxins e.g. guanidine. They postulated a diminution of available calcium and suggested that udder inflation exerted its beneficial effects by reflexly causing an increased secretion of adrenaline, which hastened oxidation of the toxins and raised the blood pressure, thus promoting recovery.

In a paper given to the 11th. International Veterinary Congress in London Greig (1930a) detailed the main points which had guided Dryerre and himself in the formulation of their hypothesis:-

"Commencing with the fact that specific cure resulted, no matter whether antiseptic fluids, sterile water, oxygen or air were injected into the udder, it seemed obvious that the effect, whatever it might be, depended upon the mechanical distension of the mammae.

"We then premised that simple distension of the mammae must act either:-

1) by eliciting some endocrine disturbance, and/or

2) by mechanically retarding or arresting milk secretion, and so preventing the loss in the milk of some substance vital to the organism.

"That the disease was in some way closely associated with milk secretion was suggested by the facts that:-

1) Its appearance as a clinical entity was coincident with the commencement of the development of the modern heavy milking strains.

2) It was more prevalent in dairy breeds as distinct from beef breeds.

3) It very commonly attacked those individuals which specially possessed deep milking qualities.

4) The period of greatest susceptibility in the milking life of an individual cow corresponded to the period of greatest milk secretion. Primiparae were very rarely affected.

5) The rapid emptying of the udder by hand might precipitate the onset, while the practice of repeatedly removing small quantities of milk, or, alternatively, of permitting the calf to suck the cow for the first few days after calving, was recognised as a valuable preventive procedure.

"The milk cow has been bred to produce an enormous quantity of milk; indeed, her milk secretion may now be regarded as almost pathological.

"The colostrum of the cow is rich in calcium, and it was considered likely that the onset of a profuse lactation might occasion a rapid reduction in the concentration of the blood calcium. This idea seemed to be supported by our further observation that the spastic seizures which often characterise the early stages of milk fever were tetanic in character.

"We believed that the mere mechanical withdrawal of calcium from the blood as the result of the onset of a profuse secretion of milk could not in itself be regarded as the cause of milk fever, because if this were so every heavy milking cow would be subject to the disease.

"For that reason we postulated that some other factor, therefore, must act as a predisposing cause, and we suggested that such might be found in parathyroid dysfunction.

"The following, then, were the essential points in our hypothesis:-

1) The nature of milk fever may be understood as a parathyroid deficiency, resulting in the accumulation of toxic substances such as guanidine, and a fall in blood-calcium, the fall in calcium being further accentuated by lactation.

2) The curative effect of mammary inflation is due to

a) the stimulation of adrenal secretion and the consequent oxidation of toxins, and/or

b) the retardation of the formation of milk and the consequent prevention of further free exchange of calcium from the blood to the milk.

3) The preventive value of a restricted withdrawal of milk from the udder after calving is due to this procedure conserving the concentration of calcium in the blood."

This passage has been quoted in full as it summarises many of the more important facts relating to the occurrence of milk fever.

Dryerre and Greig, in their original paper, gave no experimental evidence in support of their theory. Two months after its publication, however, one aspect of their hypothesis was given the support of experimental evidence. In a paper published in May, 1925, Little and Wright (1925) showed that milk fever was accompanied by a hypocalcaemia. In a study of 12 cases of milk fever they found that the blood calcium varied from 3.70 to 6.35 mgm. per 100 ml., compared with the range of 9.00 to 11.00 mgm. for normal cows. They stated that the severity of the disease was paralleled by the extent of the decrease in the calcium level. Cows with mild symptoms showed a drop of 20-30%, while in severe cases the drop was in the region of 60%.

That milk fever was accompanied by a hypocalcaemia was confirmed by Dryerre and Greig (1928), Sjollema (1928), Fish (1929), and subsequently by numerous other workers. Greig (1930 a) reported maximal, minimal and average concentrations of 7.76, 3.00 and 5.13 mgm. per cent. respectively. He was unable to demonstrate a lowered serum calcium level in a series of 81 cases of diseases of bovines other than milk fever. Greig (1929) reported that the disease of lambing sickness in ewes was also accompanied by a hypocalcaemia.

Sjollema (1928) and Fish (1929) demonstrated that in milk fever there was a lowering of the blood inorganic phosphate. Fish (1930) drew attention to the fact that in milk fever the product of the blood calcium and phosphorus levels is only one fifth of that of normal cows. He criticised the parathyroid dysfunction theory on account of the fact that in experimental parathyroidectomy there is an increase in the level of blood phosphate accompanying the lowering of blood calcium.

Greig (1930b) demonstrated that udder inflation resulted in a rise in the level of blood calcium. He stated that the rise was at first rapid and then became more gradual, and that signs of recovery became evident when the blood calcium level was in the region of 6-7 mgm. per cent. This was confirmed by Fish (1930), who showed also that udder inflation produced a rise in the blood phosphate level, and that this rise was more rapid than the rise in the blood calcium. As a result of the work of Auger (1926b), who was unable to demonstrate any increase in blood pressure following udder inflation, the view of Dryerre and Greig (1925) that udder inflation exercised its beneficial effects by stimulating the secretion of adrenaline, was abandoned. Hayden (1929) was able to show that guanidine accumulation was not an important factor in the aetiology of milk fever.

As a result of the demonstration by Little and Wright that milk fever is associated with a hypocalcaemia, udder inflation was superseded as a treatment by calcium injection therapy. Calcium chloride was the first salt to be employed, but because of its irritant properties, it was supplanted by the less irritant calcium gluconate. Finally calcium borogluconate, which was more soluble and completely nonirritant, and could therefore be given by subcutaneous injection, came to be employed. The literature on these aspects will be discussed in more detail later in this thesis.

The work of Dryerre and Greig and of Little and Wright gave a great impetus to the investigation of the milk fever problem. In the years immediately following their demonstration that milk fever was associated with a hypocalcaemia a great deal of work was done on the various aspects of the problem.

Harding (1929) regarded milk fever as being a shock syndrome resulting from anhydraemia. He was unable, however, to account for the hypocalcaemia and hypophosphataemia on this basis. From a comparison of the blood protein values of normal cows and cows with milk fever, Wilson and Hart (1932) considered that anhydraemia was not an important factor in milk fever.

In Holland, Sjollema, Seekles and van der Kaay followed up the work of Dryerre and Greig and Little and Wright by an intensive study of various aspects of the milk fever problem. The results of their work were published in a series of papers from 1928 to 1932 which added considerably to our knowledge of milk fever.

Sjollema (1928) showed that in milk fever cases the blood inorganic phosphorus was about half the normal value, and later Sjollema and Seekles (1930) found that the blood magnesium level was about 50% above normal. They also pointed out that the ionised serum calcium was very low- an average of 0.44 mgm.compared with an average of 1.70 mgm. in 100 normal cows investigated. Sjollema (1932) and Sjollema, Seekles and van der Kaay (1931) reported that several hundred cows had been cured by the intravenous injection of a mixture of 40 gm. of crystalline calcium chloride and 15 gm of crystalline magnesium chloride, in 300 cc. water. Seekles, Sjollema and van der Kaay (1932a, 1932b) showed that the use of magnesium chloride along with calcium chloride lessened the risk of heart block following calcium chloride injection. From a study of three normal cows observed for a period of a few months before, and a few weeks after calving Seekles, Sjollema and van der Kaay (1932c) concluded that no significant variations occurred in the mineral content of the blood until a few days before parturition. Serum calcium and inorganic phosphorus decreased about four days before calving, reaching minima shortly before or shortly after calving. Observations on one cow in respect of serum magnesium showed small variations inverse to calcium and phosphorus. The vagus tone of the heart, as judged by the response to test injections of adrenaline, was found to increase as pregnancy advanced, due, it was thought, to a rise in the potassium : calcium ratio.

The Dutch workers stressed that milk fever must be regarded as a complex disturbance in mineral metabolism and postulated interference with the autonomic nervous system and endocrine relationships as contributing to the faulty metabolism in milk fever. Their opinions at this stage were summarised by Sjollema (1932b) in a review article.

Later, from 1937 to 1940, Seekles (1937, 1940a, 1940b) published a series of papers in which he elaborated on the concept of milk fever as an upset in the endocrine relationships, and in which he postulated that milk fever was associated with hyperfunction of the anterior pituitary. He claimed to have demonstrated the presence of ketogenic, glycogenolytic and hyperglycaemic factors in the blood of milk fever cases.

In 1948, in a series of lectures on "The Biochemical Approach to Animal Disease", Seekles (1948), gave his latest ideas on the nature of milk fever. He stated that the results of experiments in his laboratory made it probable that "the forced development of the cow as a milk producer causes a hyperfunction of the pituitary gland, which enlarges to as much as twice its normal size during pregnancy. The result is a flooding of the organism with hormones produced by the pituitary at the time of parturition and afterwards. Owing to a correlative influence of internal secretions of other organs a complete dysfunction of hormonal regulation develops. A single stronger stimulus - parturition in the case of milk fever - makes it possible for the latent state to pass into a condition with a manifest complex of symptoms."

The work of Sjollema and Seekles has the merit of emphasising the complex nature of the disturbance in mineral metabolism in milk fever. A great deal of experimental evidence, however, has yet to be produced before their theories as to the nature of milk fever can win universal acceptance.

Apart from the investigations of Allcroft and Godden (1934), Allcroft and Green (1934), Godden and Allcroft (1932), Godden and Duckworth (1935) in this country, and Wilson and Hart (1932), and Hayden (1938) in America, on the mineral metabolism of normal parturient cows, and the studies of Barker (1939) on the clinical aspects of milk fever, comparatively little work was done in the 1930's. This was probably due to the highly successful results obtained in the treatment of milk fever by calcium injection therapy.

Since the end of the war in 1945 a considerable amount of work has been carried out in America.

Hibbs and his co-workers (Hibbs, Krauss, Monroe & Sutton 1946a, Hibbs, Krauss, Pounden, Monroe & Sutton 1946b, Hibbs, Pounden & Krauss 1947, Hibbs 1950, Hibbs, Pounden & Krauss 1951) have published a series of papers dealing in particular with attempts to prevent milk fever by the administration of Vitamin D. By massive dosage with Vitamin D and by limiting the dosage to a period of a few days prior to parturition, Hibbs (1950) claims to have lessened the fall in serum calcium occurring in normal parturient cows, and to have reduced the incidence of milk fever. He believes that a practical and effective method of prevention of milk fever can be based on his findings.

Much of the recent American work has been directed towards an investigation of the effects of lactation on the level of serum calcium in normal cows.

Johnson, Eaton, Spielman, Matterson & Slate (1948) have studied the effects of prepartum milking on the serum calcium and phosphorus, total haemoglobin, plasma carotene and Vitamin A in parturient cows. Mercer, Eaton, Johnson, Spielman, Plastridge, Matterson & Nesvesky (1949) have studied the effects of interruption of milking during the lactation period on the serum calcium level in normal cows. Niedermeier and Smith (1948) have studied the effects of abstention from milking after calving on the levels of blood calcium and phosphorus in normal cows. Niedermeier, Smith and Whitehair (1949) have studied the levels of blood calcium, magnesium and phosphorus in mastectomised cows at parturition.

The findings of these workers on the effects of lactation on the level of serum calcium will be discussed in detail later in this thesis.

Craige (Craige & Stoll 1947, Craige 1947, Craige, Johnson, Blackburn & Coffin 1949) has claimed that the fall in serum calcium in milk fever is due to a parturient alkalosis. He suggests that milk fever can be explained on the basis of a sudden withdrawal of electrolytes from the blood into the colostrum and the supression of efficient calcium mobilisation as a resultof developing alkalosis. He induced a lowering of blood calcium, and claims to have induced symptoms of milk fever and death by artificial alkalinisation by the oral or intravenous administration of sodium carbonate to non-parturient cows (Craige 1947). Study of Craige's work, however, shows that the lowering of serum calcium produced by the administration of sodium carbonate was slight. Furthermore, the clinical symptoms he describes are not typical of milk fever. His hypothesis cannot be regarded as proven.

In Sweden, Garm (1950) has studied the haemocytological picture in eleven cases of milk fever which did not respond to treatment. He has also made histological and histochemical studies of the thyroid, parathyroid, adrenal and pituitary glands of these cases at autopsy. From the results of these studies Garm has suggested that milk fever may be associated with an increased pituitary-adrenal cortical activity and he believes that it should be incorporated in the group of diseases which Selye (1946) has termed "diseases of adaptation".

Reasons for the Present Investigation.

The present investigation was started in the autumn of 1948.

During the spring and summer of that year a considerable number of cases of milk fever which had not responded typically to calcium therapy had been encountered in the clinical practice of the Royal (Dick) Veterinary College. This had been especially the case on one particular farm, where, in the course of a few weeks, four cows in succession had been lost following failure to respond to calcium administration. On this farm resort to udder inflation therapy had proved successful where calcium therapy had failed. An account of these and other cases has been published by Robertson, Burgess, Marr, and Milne (1948). Biochemical analysis of the blood of the cases reported by Robertson et alia suggested that the beneficial effects of udder inflation might be due to its ability to raise the plasma inorganic phosphate as well as the serum calcium. Fish (1930) in his original work in demonstrating the hypophosphataemia in milk fever, had suggested that the low blood phosphate level might be equally as important as the low blood calcium level. While it was difficult to believe that this was the case, it was thought possible that, in cases which did not respond to calcium therapy, the level of blood inorganic phosphate might be of some significance.

Barker (1948) had suggested that hypophosphataemia was a complicating factor in atypical case of milk fever. He had made it a routine to supplement the use of calcium borogluconate by incorporating one ounce of acid sodium phosphate in the calcium solution and he claimed good results from this therapy.

As a perusal of the literature revealed very few biochemical studies of the effects of calcium treatment, it was decided to make a detailed investigation of the clinical aspects of milk fever, concentrating particularly on the biochemical response to treatment.

A further justification for such a study was the fact that many veterinary surgeons had reported that they were encountering an increased number of cases of milk fever which did not respond to calcium therapy. Garm (1950) has reported that in recent years in Norway and Sweden calcium therapy and air inflation have been far less effective in the treatment of milk fever. McBarron (1952) in Australia has observed an increased number of relapses following treatment and states that these cases constitute a difficulty in further treatment.

As most workers are agreed that the elements primarily concerned in the upset in mineral metabolism in milk fever are calcium, magnesium and phosphorus, it was decided to confine our observations to these blood fractions. Of the various forms of blood phosphorus only the inorganic phosphate fraction has been studied in this investigation. A parallel study of the organic phosphate fractions has been made by Moodie (1952).

It was felt that a detailed study of the changes occurring in the blood calcium, magnesium and inorganic phosphorus in normal parturient cows might throw light on the role of these elements in milk fever. A study of the literature on this aspect showed a certain amount of disagreement, particularly as regards the behaviour of serum magnesium in the parturient cow.

Finally, it became apparent during the course of the investigation that it might be useful to make a study of the effects on normal cows of the treatments generally employed in the therapy of milk fever. Accordingly, an investigation was made of the effects of calcium borogluconate and acid sodium phosphate injections, and of udder inflation on normal parturient and non-parturient cows.

While it was not anticipated that this investigation would throw much light on the fundamental cause of milk fever, it was felt that it might help to elucidate some aspects of the problem, and contribute to a better understanding of the nature of the disease.

General Materials and Methods.

Details of the cows used in this investigation are given in the introductions to the relevant sections.

Blood Sampling. Blood samples were withdrawn from either the jugular or mammary veins, the vast majority from the mammary, as the samples could be collected from this vein with a minimum of help. This was important because many of the samples had to be collected during the night when no help was available. An added advantgge of collection from the mammary vein was that it caused a minimum amount of disturbance to the cow.

About 90 ml. of blood were collected at each sampling of which 60 ml. were oxalated and were used for the inorganic phosphate estimations. The other 30 ml. were allowed to clot and the serum to separate, and were used for the estimation of serum calcium and magnesium.

Apparatus. All apparatus used was washed thoroughly, rinsed three times in distilled water, and dried in a hot air oven before use. All reagents were of A.R. quality.

Analysis. Serum calcium was estimated by the Clark-Collip modification of the Kramer-Tisdall method (1925). The supernatant fluid after precipitation of the calcium was used for the estimation of serum magnesium, by the method of Denis (1922) modified according to Hawk (1947). Plasma inorganic phosphate was estimated by the method of Fiske and SubbaRow (1925). The colorimetric readings for the magnesium and inorganic phosphate estimations were carried out on a "Spekker" absorptiometer.

All analyses were done in duplicate and the results given in this thesis represent the mean of two estimations. Where the results of the two estimations differed by more than 5% the estimation was repeated.

The majority of the phosphate estimations were done within three hours of the collection of the samples. Calcium and magnesium estimations were usually carried out after a day had been allowed for separation of the serum.

SECTION I.

SOME AETIOLOGICAL ASPECTS OF MILK FEVER.

PART I.

Calcium, Magnesium, and Inorganic Phosphorus in the blood of Normal Cows at Parturition.

INTRODUCTION.

Meigs & Blatherwick (1917) were the first to note a reduction in the Blood inorganic phosphate in normal cows at calving. Palmer, Cunningham and Eckles (1930) confirmed this observation. They found a reduction of as much as 3.20 mgm. per 100 ml. in inorganic phosphate at parturition. Wilson and Hart (1932) in a study of 19 cows having normal parturitions, found that there was a tendency for the blood calcium of most of the cows to fall slightly sometime within the first three days after calving. They found the tendency to be more marked in cows that had been through a previous lactation. This is an extremely interesting observation which has never been subsequently confirmed. Wilson and Hart also found that the blood inorganic phosphate tended to fall within the first three days after calving, but they found this tendency not to be so consistent as it was for calcium.

Godden and Allcroft (1932), in a study of 13 cows having their 5th. parturition, observed a definite fall in serum calcium just at, or within 24 hours of, calving, with a return to normal in 4-5 days. They stated that there was a sharp fall in inorganic phosphate just prior to calving, and that this was invariably indicative of the onset of labour.

Later, Allcroft and Godden (1934) confirmed their findings as to the changes in serum calcium and inorganic phosphate at calving, and extended their observations to serum magnesium. They found the behaviour of serum magnesium to be more erratic than that of calcium or inorganic phosphate, but that there was a tendency for the level of serum magnesium to be raised just at, or for 24-48 hours after, calving. In some individual cows this change occurred the day before calving. They found that, on the whole, serum magnesium varied inversely with serum calcium. In the light of their observations, they suggested that the abnormal fall in serum calcium in milk fever might be only an exaggeration of the normal physiological change which occurs in the blood of the cow at calving.

Various workers e.g. Seekles, Sjollema and van der Kaay (1932c), Little and Mattick (1933a), Little and Mattick (1932b), Duckworth and Godden (1940) and Blood and White (1949) have confirmed the fall in blood calcium and inorganic phosphate in normal cows at calving. Hayden (1938), however, found the inorganic, lipide, total acid soluble, and total phosphorus at, or shortly after, parturition showed very little deviation from data obtained from non-pregnant cows.

Allcroft and Godden (1934), Seekles, Sjollema & Van der Kaay (1932c) have reported a hypermagnesaemia in normally calving cows. Hayden (1938) and Blood and White (1949) have failed to find a raised serum magnesium level in parturient cows.

The investigation described here was undertaken because it was felt that a detailed study of the changes in blood calcium, magnesium and inorganic phosphate in normal parturient cows might elucidate some of the points on which the literature is contradictory.

It was also felt that a proper understanding of the changes occurring in these elements in the normal cow was a necessary prerequisite for the understanding of the role of calcium , magnesium and inorganic phosphate in milk fever. In view of the fact that the observation of Wilson and Hart (1932) that the changes were more marked in cows with a previous parturition than in first calf animals, had not been confirmed, and that their own findings on this point were not conclusive, it was decided primarily to investigate this aspect of the ppuoblem. It was thought that the investigation would test the validity of the hypothesis of Godden and Allcroft that milk fever was an exaggeration of the normal physiological changes at parturition.

<u>Materials and Methods.</u>

In this part of the investigation 32 normal cows were used. The cows were on two farms under the same management, which was intensive, and were milked three times daily. The feeding practice was the same on the two farms.

The cows were predominantly pedigree Ayrshire cattle, although a few Shorthorn cross, Red Poll cross, and Jersey cross cows were included.

Of the 32 cows seven were having their first calf, eight their second, nine their third and eight their fourth or subsequent calf.

<u>Frequency of Blood Sampling.</u> Blood samples were taken from 14-7 days prepartum to 14-20 days postpartum. It became clear during the course of the investigation that while daily sampling was sufficient in the prepartum and later postpartum periods, very frequent blood sampling was necessary in the immediate postpartum period, if an adequate picture of the changes occurring was to be obtained.

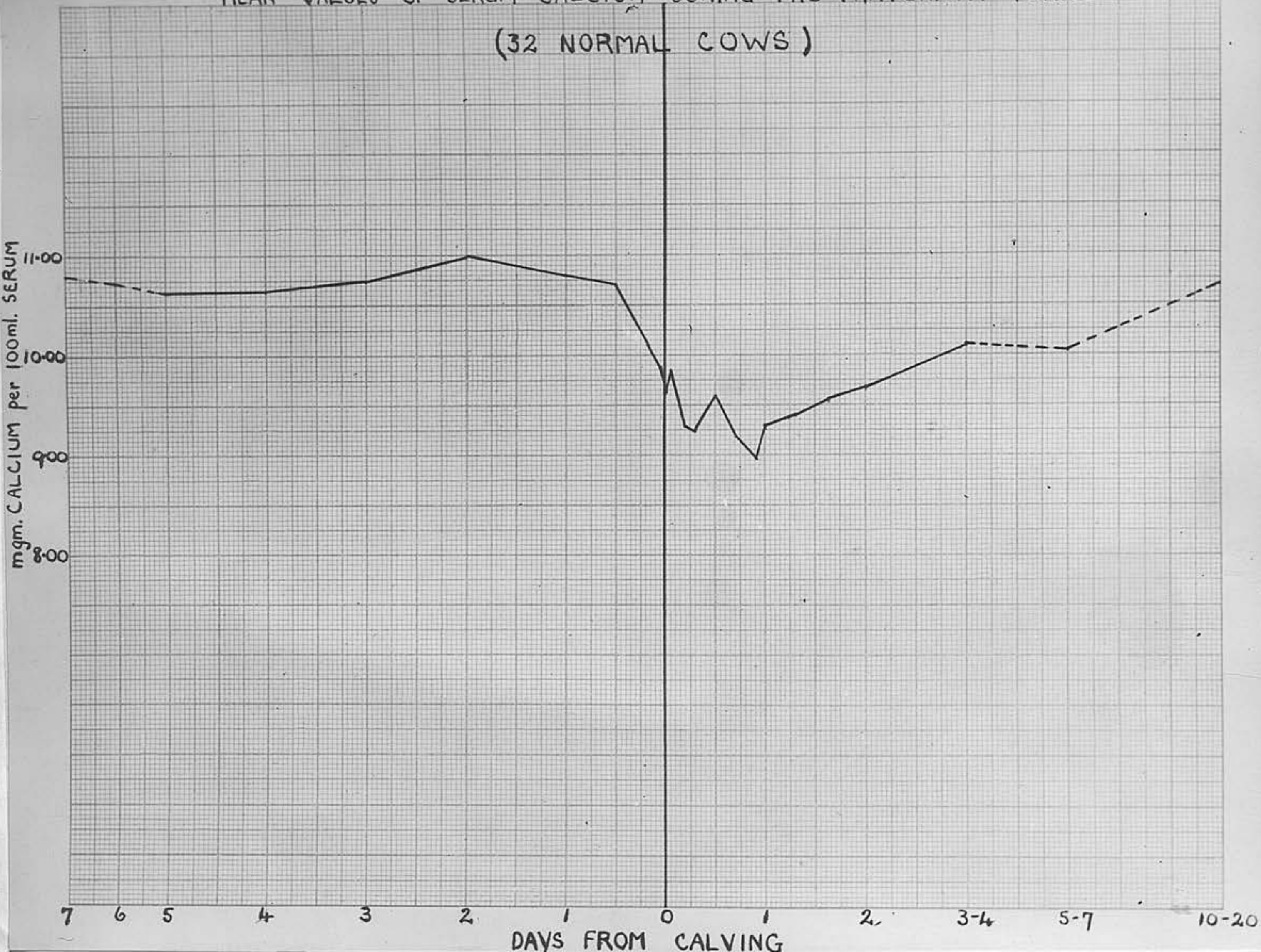
level of serum magnesium by the injection of magnesium salts, and by the lowering of the level of serum calcium by the injection of sodium oxalate. Barker (1939) has laid great stress on the Ca/Mg ratio as a factor in the symptomatology of milk fever. He claims to be able to classify hypocalcaemia into three clinical groups according to whether it is accompanied by a normal, raised, or lowered serum magnesium level. Hypermagnesaemia and hypocalcaemia he associates with reeling, paresis and eventual narcosis; normal serum magnesium and hypocalcaemia with tetany of the hind legs, paresis and eventual coma; and hypomagnesaemia and hypocalcaemia with generalised tetany, hyperaesthesia and convulsions. Robertson (1949) was unable to classify his cases on the basis of the serum magnesium levels as described by Barker.

As a result of the attention focussed on the roles of serum magnesium and plasma phosphate, there has been a tendency in recent years towards the use of complex preparations of calcium, incorporating magnesium and phosphate, in place of the simple calcium solutions previously used in milk fever therapy.

In contrast to the effect of calcium injection therapy, the effect of udder inflation in the treatment of milk fever, has been more extensively studied. Dryerre and Greig (1928) found that udder inflation caused an invariable rise in the blood calcium level. They found that the rise was at first rapid and was followed by a distinct hypercalcaemia, which might persist for several days. Cases showed definite signs of recovery when the serum calcium level was about 6 to 7 mgm. per cent. Fish (1930) confirmed that udder inflation raised the blood calcium level, and extended his observations to the

Accordingly, in the later stages of the investigation, blood samples were taken as nearly as possible every 4 hours in the first day postpartum, every 8 hours in the second day and every 12 hours in the third day. The impossibility of accurately forecasting the time of calving, and the fact that the farms were situated ten miles from the laboratory, made it difficult to adopt a rigid frequency in blood sampling.

FIGURE 1 MEAN VALUES OF SERUM CALCIUM DURING THE PARTURIENT PERIOD



RESULTS.

Serum Calcium.

The complete data for serum calcium estimations for the 32 normal cows are presented in Tables 1, 2, 3 and 4 (Appendix I, pp. i, ii, iii and iv). Figure I shows the mean data for the 32 normal cows in graphic form.

These results show a range for the period 14 days prepartum to 10-20 days postpartum of 12.89 to 5.65 mgm. calcium per 100 ml. serum.

It will be seen from Figure 1 that the mean values for calcium remained fairly constant between 10.50 and 11.00 mgm. per cent. from 14 days prepartum until about 1 day prepartum. The range within this period (Tables 1, 2, 3 and 4; Appendix I, pp. i, ii, iii and iv) was 12.89 to 8.79 mgm. There were no significant changes in the level of serum calcium during this period.

From approximately 12 hours before calving there was a fall in the values of serum calcium which persisted until about 3-7 days postpartum.

Analysis of Results. For the purpose of analysis the levels at 2 days prepartum have been taken as the basis for comparison. This basis has been chosen as samples were available at 2 days prepartum for nearly all of the 32 cows studied, and they represented a fair average of the normal prepartum levels, before the changes associated with parturition became evident. In the few cases where no samples were available for the period 2 days prepartum, the levels at either 1 day or 3 days prepartum have been taken as the basis for comparison.

In the analysis of results the 2 day prepartum levels have been compared with (1) the values nearest calving; (2) the values nearest 8 hours postpartum; (3) the values nearest 2 days postpartum;

(4) the values in the period 3-7 days postpartum; and (5) the values in the period 10-20 days postpartum.

The significance of changes and of differences has been tested by "Student's" t test.

A comparison on this basis of the mean values for the 32 normal cows used in the investigation, is shown in Table 1. Six cows have had to be omitted from the comparison in the period 3-7 days postpartum, and three in the period 10-20 days postpartum, since no samples were available for these animals in the respective periods.

SERUM CALCIUM FIGURE 2 COMPARISON OF DIFFERENT GROUPS DURING THE PARTURIENT PERIOD

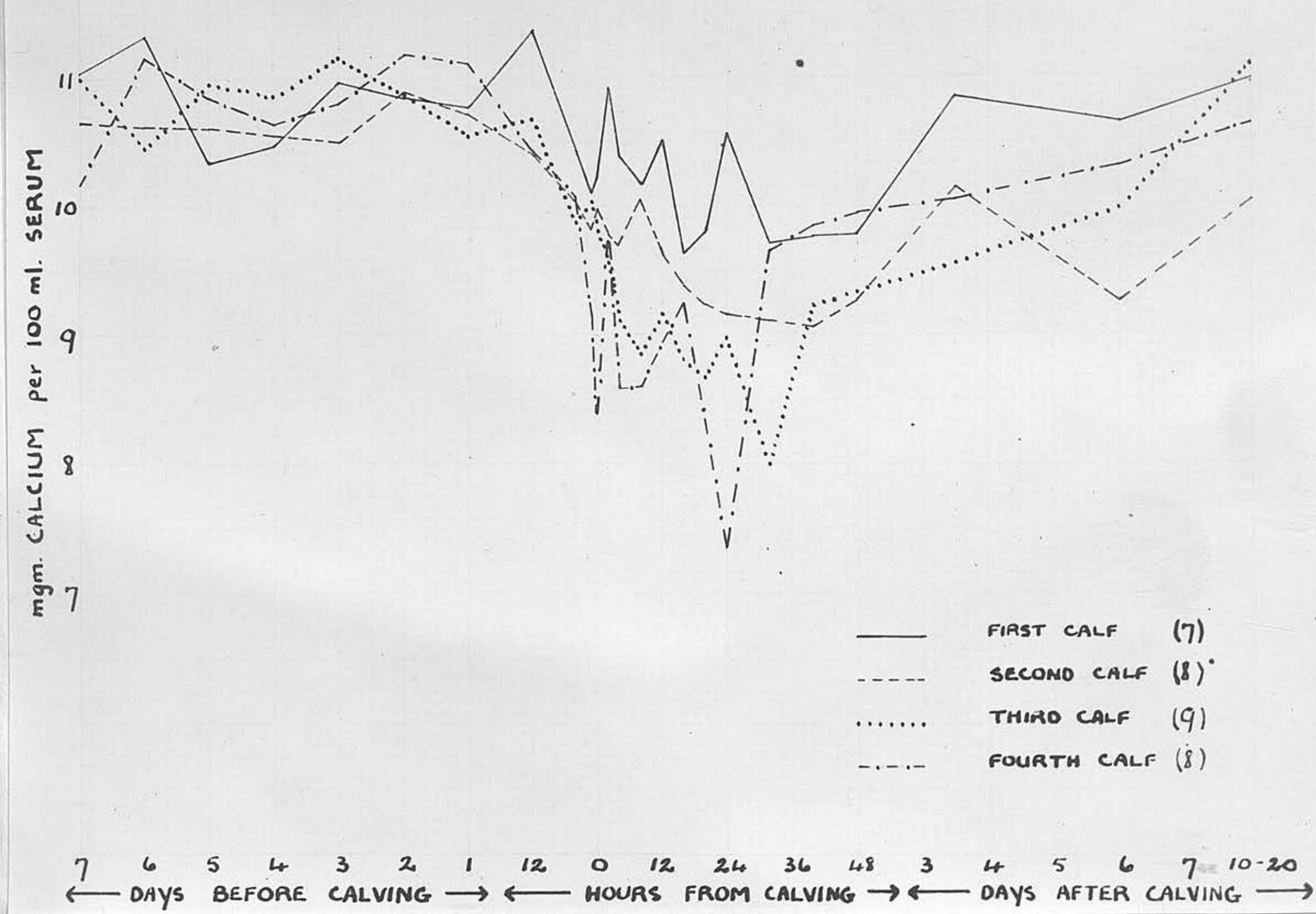


Table 1.

Serum Calcium in Normal Parturient Cows.

Mean Values at 2 days prepartum compared with Mean Values during the postpartum period.

Calcium mgm. per cent.

2 days prepartum	10.92	10.92	10.92	10.80	10.89
Calving (32)	9.53				
8 hours (32) postpartum		9.54			
2 days (32) postpartum			9.72		
3-7 days (26) postpartum				10.18	
10-20 days (29) postpartum					10.68
Mean Change.	-1.39	-1.38	-1.20	-0.62	-0.21
Significance	1%	1%	1%	1%	N.S.

Number of animals in each comparison are shown in parenthesis.)

N.S. = Not significant.

Table 1. shows that there was a highly significant fall in serum calcium at calving, and that it persisted until 3-7 days postpartum. Normal values were regained at 10-20 days postpartum.

Comparison of the Different Groups. The differences in the behaviour of serum calcium in the various groups during the parturient period, is shown in Figure 2.

The analysis which follows was carried out to test the significance of the changes within the groups and to test the significance of the differences between groups.

A comparison of the mean values at 2 days prepartum with the mean values at calving is shown in Table 2.

The numbers of animals in each group are shown in parenthesis.

TABLE 2.

Mgm. calcium per cent.

	First Calf. (7)	Second Calf. (8)	Third Calf. (9)	Fourth Calf. (8)
2 days prepartum.	10.84	10.76	10.89	11.19
Calving	10.24	9.94	9.73	8.26
Mean Change.	-0.60	-0.82	-1.16	-2.93
Significance.	N.S.	1%	1%	1%

It will be seen that, while first calf animals showed no significant change, there was a highly significant fall in the values of serum calcium at calving in 2nd., 3rd., and 4th. calf animals.

Analysis of the differences between groups showed that there was a highly significant difference between 1st. and 4th.; 2nd. and 4th.; and 3rd and 4th. calf animals. The differences between other groups were not significant.

Table 3 shows a comparison of the mean values at 2 days prepartum with the mean values nearest 8 hours postpartum.

TABLE 3.

Mgm. calcium per 100 ml. serum.

	First Calf (7)	Second Calf. (8)	Third Calf. (9)	Fourth Calf. (8)
2 days prepartum.	10.84	10.76	10.89	11.19
8 hours postpartum.	10.78	9.81	9.03	8.76
Mean Change.	-0.06	-0.95	-1.86	-2.43
Significance.	N.S.	5%	1%	1%

At 8 hours postpartum, there was no significant change in the values of serum calcium in first calf animals; second calf animals showed a significant fall; while third and fourth calf animals showed a highly significant fall.

Analysis of the differences between groups showed that there was a highly significant difference between first and fourth, and between second and fourth calf animals; there was a significant difference between first and third calf animals, while the differences between other groups were not significant.

Table 4 shows the comparison between 2 days prepartum and 2 days postpartum.

TABLE 4.

Mgm. calcium per 100 ml. serum.

	First Calf. (7)	Second Calf. (8)	Third Calf. (9)	Fourth Calf (8)
2 days prepartum	10.84	10.76	10.89	11.19
2 days postpartum.	10.29	9.43	9.73	9.50
Mean Change.	-0.55	-1.33	-1.16	-1.69
Significance.	N.S.	1%	5%	1%

At 2 days postpartum there was no significant change in first calf animals, there was a significant fall in third calf animals, and there was a highly significant fall in second and fourth calf animals.

There were no significant differences between groups in this comparison.

Table 5 shows the comparison between 2 days prepartum and 3-7 days postpartum for 26 cows with sampèes available in this period.

TABLE 5.

Mgm. calcium per 100 ml. serum.

	First Calf. (6)	Second Calf. (8)	Third Calf. (6)	Fourth Calf (6)
2 days prepartum.	10.80	10.76	10.68	10.99
3-7 days postpartum.	10.82	10.17	9.65	10.03
Mean Change.	+0.02	-0.59	-1.03	-0.96
Significance.	N.S.	N.S.	N.S.	N.S.

At 3-7 days postpartum none of the individual groups showed a level significantly lower than at 2 days prepartum, although when the 32 animals were considered as a group (Table 1) there was still a highly significant fall in serum calcium.

Analysis of the differences between groups showed that there was a significant difference between first and third calf animals.

Table 6 shows the comparison between 2 days prepartum and 10-20 days postpartum for 29 cows with samples available in this period.

TABLE 6.

Mgm. calcium per 100 ml. serum.

	First Calf (7)	Second Calf (8)	Third Calf. (9)	Fourth Calf. (5)
2 days prepartum	10.84	10.76	10.89	11.18
10-20 days postpartum.	11.00	10.02	11.08	10.60
Mean Change.	+0.16	-0.74	+0.19	-0.58
Significance.	N.S.	5%	N.S.	N.S.

At 10-20 days postpartum only second calf animals showed a level of serum calcium significantly lower than the 2 day prepartum level.

There were no significant differences between groups in this comparison.

Maximum Changes. In order to demonstrate the magnitude of the fall in serum calcium in individual cows and in the various groups, it was decided to compare the 2 day prepartum level with the lowest level reached by serum calcium in the period 12 hours prepartum to three days postpartum. This method of comparison was rendered necessary by the fact that there was considerable variation in the time, relative to calving, when the maximum fall in serum calcium took place; e.g., in some cows serum calcium was at its lowest at calving, while in others it was at its lowest 2-3 days postpartum.

The results of this analysis are shown in Table 5 (Appendix 1, p 5). The means for the various groups are shown in Table 7.

TABLE 7.

Serum Calcium in Normal Parturient Cows.

Mean Values at 2 days Prepartum Compared with Means of the Lowest value reached during the period 12 hours prepartum to 3 days postpartum.

Calcium mgm.per cent.

	First Calf (7)	Second Calf (8)	Third Calf (9)	Fourth Calf (8)
2 days prepartum.	10.84	10.72	10.89	11.18
Lowest value	9.57	8.82	8.40	7.78
Difference	1.27	1.89	2.49	3.41
%age Fall.	11.71	17.63	22.87	30.58

Comparison of the means in Table 7 (t test) showed that there was a highly significant difference between 1st. and 4th., between 1st. and 3rd., and between 2nd. and 4th. calf animals (P less than 0.01). The difference between other groups were not significant.

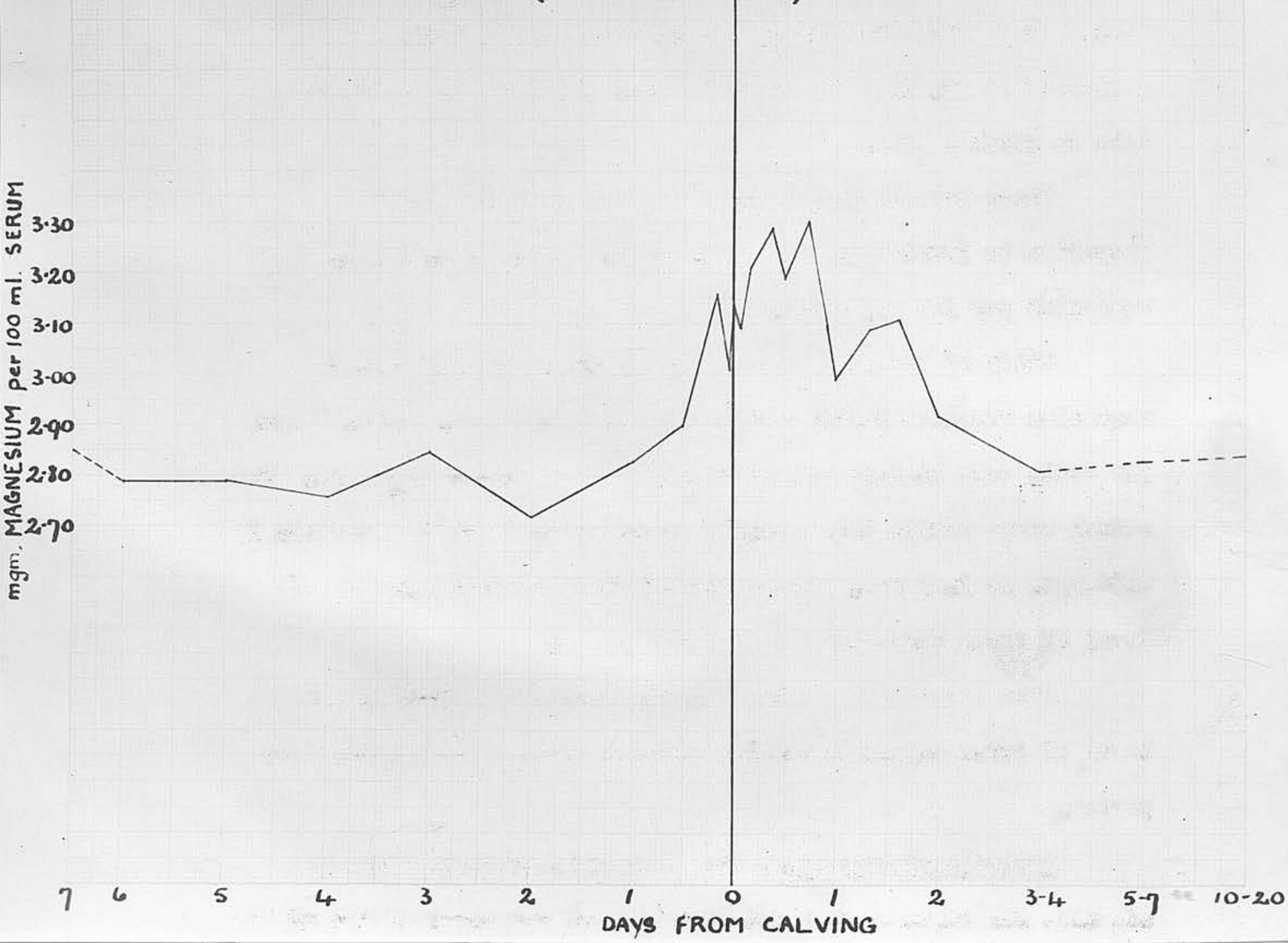
The results for serum calcium show, therefore, that, when the 32 cows were considered as one group, there was a highly significant fall in the level of serum calcium at parturition. The decrease tended to be abrupt commencing 12-24 hours before calving and persisting until about 3-7 days after calving. By 10-20 days postpartum normal prepartum levels had been regained.

Comparison of the different groups showed that the fall in serum calcium was influenced in a significant manner by the number of parturitions which the cow had undergone. Thus, although first calf animals did show a decrease in the level of serum calcium around calving, the fall was not statistically significant. Second, third and fourth calf cows showed a highly significant fall at parturition, and there was a significant tendency for the fall to be greater with each succeeding parturition; e.g. at calving first calf animals showed a mean decrease of 0.60 mgm., second calf animals of 0.82 mgm., third calf animals of 1.16 mgm., and fourth calf animals a decrease of 2.93 mgm.

Study of the data for individual cows shows that, although the fall at parturition was consistent, there was considerable variation in the time, relative to calving, when the lowest level of serum calcium was reached. In some animals the lowest point was reached at, or within 12 hours of calving, with a gradual return to normal levels at about 3 days postpartum; e.g. Cow 26. In others there was an initial drop around parturition, followed by a return towards normal levels, and then a secondary drop 24-48 hours after calving, with a gradual return to prepartum levels; e.g. Cow 55. In some cows this secondary drop was greater than the first.

The fall in blood calcium was often considerable, especially in the older animals. The lowest values recorded were 5.65 mgm. in Cow 30, 6.55 mgm. in Cow 26, 6.94 mgm. in Cow 6, and 7.07 mgm. in Cow 20. These values are as low as those found in some well marked clinical cases of milk fever.

FIGURE 3 MEAN VALUES OF SERUM MAGNESIUM DURING THE PARTURIENT PERIOD (32 NORMAL COWS)



SERUM MAGNESIUM.

The complete data for serum magnesium for the 32 normal cows are presented in tabular form in Tables 6, 7, 8 and 9 (Appendix I, pp. vi, vii, viii and ix). Figure 3 shows the mean data in graphic form.

These results show a range for the period 14 days prepartum to 10-20 days postpartum of 1.88 mgm. to 4.65 mgm. magnesium per 100 ml. serum.

Study of Figure 3 shows that the mean values for serum magnesium remained fairly constant between 2.70 mgm. and 2.90 mgm. per cent. from 14 days prepartum until about 2 days prepartum. The actual range within this period (Tables 6, 7, 8 and 9; Appendix I) 2.08 mgm. to 3.65 mgm. There were no significant changes in the level of serum magnesium during this period.

From about 1 day before calving there was a rise in the level of serum magnesium which persisted until about 2 days postpartum.

Analysis of Results. The same basis of comparison as was used for serum calcium has been adopted for the analysis of the results for serum magnesium. The same number of cows were available for comparison in each period as were available for serum calcium; i.e. 32 cows in the calving, 8 hours postpartum, and 2 days postpartum periods, 26 cows in the 3-7 days postpartum period, and 29 cows in the 10-20 days postpartum period.

Table 8 shows the comparison for these animals.

TABLE 8.

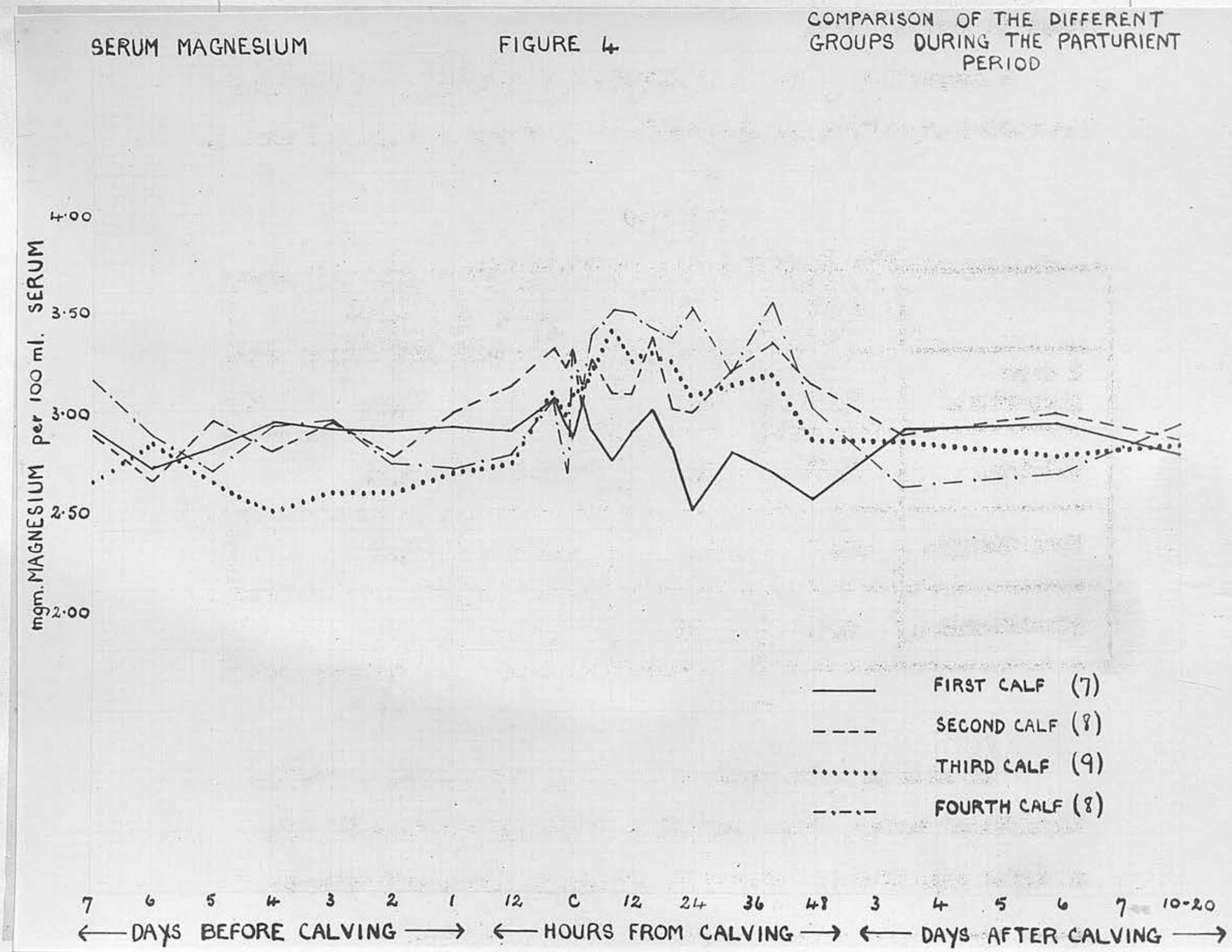
Mean Values of Serum Magnesium.

Mgm. Magnesium per 100 ml. Serum.

2 days prepartum	2.73	2.73	2.73	2.79	2.74
Calving. (32)	3.12				
8 hours postpartum (32)		3.16			
2 days postpartum (32).			2.94		
3-7 days postpartum (26)				2.80	
10-20 days postpartum (29)					2.84
Mean Change.	+0.39	+0.43	+0.21	+0.01	+0.10
Significant.	1%	1%	1%	N.S.	N.S.

Number of animals in each comparison is shown in parenthesis.
N.S. = Not significant.

It will be seen that, when the 32 cows are considered as one group, there was a highly significant rise in the level of serum magnesium at calving, and that it persisted until 2 days postpartum. The rise in magnesium was at its maximum at about 8 hours postpartum. Normal values were regained at about 3-7 days postpartum.


SERUM MAGNESIUM
FIGURE 4
COMPARISON OF THE DIFFERENT GROUPS DURING THE PARTURIENT PERIOD
mgm. MAGNESIUM per 100 ml. SERUM
4·00
3·50
3·00
2·50
2·00
FIRST CALF (7)
SECOND CALF (8)
THIRD CALF (9)
FOURTH CALF (8)
7 6 5 4 3 2 1 12 C 12 24 36 48 3 4 5 6 7 10-20
DAYS BEFORE CALVING
HOURS FROM CALVING
DAYS AFTER CALVING

Comparison of the Different Groups. Figure 4 shows the differences in the behaviour of serum magnesium in the different groups in graphic form.

A comparison of the mean values at 2 days prepartum with the mean values at calving, in the different groups, is shown in Table 9.

TABLE 9.

Mgm. Magnesium per 100 ml. Serum.

	First Calf (7)	Second Calf (8)	Third Calf. (9)	Fourth Calf (8)
2 days prepartum.	2.87	2.78	2.59	2.72
Calving.	2.85	3.30	3.11	3.18
Mean Change.	-0.02	+0.52	+0.52	+0.46
Significance.	N.S.	1%	1%	5%

It will be seen, that, while first calf animals showed no significant change, there was a highly significant rise in the values of serum magnesium at calving in second and third calf animals, while fourth calf animals showed a significant rise.

Analysis of the differences between groups showed that there was a highly significant difference between first and third calf animals, and a significant difference between first and second calf animals. The differences between other groups were not significant.

Table 10 shows a comparison of the mean values at 2 days prepartum with the mean values at 8 hours postpartum.

TABLE 10.

	First Calf (7)	Second Calf (8)	Third Calf. (9)	Fourth Calf (8)
2 days prepartum.	2.87	2.78	2.52	2.72
8 hours postpartum.	2.83	3.08	3.26	3.41
Mean Change.	-0.04	+0.30	+0.74	+0.69
Significance.	N.S.	1%	1%	1%

At 8 hours postpartum there was no significant change in the values of serum magnesium in first calf animals; second, third, and fourth calf animals showed a highly significant rise.

Analysis of the differences between groups showed that there were highly significant differences between first and second, first and third, first and fourth, and between second and fourth calf animals. There was a significant difference between second and third calf animals. The differences between other groups were not significant.

Table 11 shows the comparison between 2 days prepartum and 2 days postpartum.

TABLE 11.

Mgm. Magnesium per 100 ml. serum

	First Calf. (7)	Second Calf. (8)	Third Calf. (9)	Fourth Calf. (8)
2 days prepartum.	2.87	2.78	2.59	2.72
2 days postpartum.	2.65	3.14	2.87	3.09
Mean Change.	-0.22	+0.36	+0.28	+0.37
Significance.	N.S.	N.S.	5%	N.S.

At 2 days postpartum there was a significant rise in third calf animals, but no significant change in the other groups.

Analysis of the differences between groups showed that there was a highly significant difference between first and third calf animals, and significant differences between first and second, and between first and fourth calf animals. The differences between other groups were not significant.

Table 12 shows the comparison between 2 days prepartum and 3-7 days postpartum for 26 cows with samples available in this period.

TABLE 12.

Mgm. Magnesium per 100 ml. Serum.

	First Calf. (6)	Second Calf. (8)	Third Calf. (6)	Fourth Calf. (6)
2 days prepartum	3.01	2.78	2.68	2.75
3-7 days prepartum.	2.85	2.87	2.82	2.62
Mean Change.	-0.16	+0.09	+0.14	-0.13
Significance.	N.S.	N.S.	N.S.	N.S.

At 3-7 days postpartum, therefore, none of the groups showed a level significantly higher than at 2 days prepartum.

Analysis of the differences between groups showed that there were no significant differences between groups in this comparison.

Table 13 shows the comparison between 2 days prepartum and 10-20 days postpartum for 29 cows with samples available in this period.

TABLE 13.

Mgm. Magnesium per 100 ml.serum.

	First Calf. (7)	Second Calf. (8)	Third Calf. (9)	Fourth Calf. (6)
2 days prepartum	2.87	2.78	2.59	2.76
10-20 days postpartum	2.77	2.87	2.82	2.92
Mean Change	-0.10	+0.09	+0.23	+0.16
Significance.	N.S.	N.S.	N.S.	N.S.

At 10-20 days postpartum none of the groups showed a level significantly higher than at 2 days prepartum.

There were no significant differences between groups in this comparison.

Maximum Changes. In order to demonstrate the magnitude of the changes in serum magnesium the same method was adopted as was used for serum calcium i.e., the 2 day prepartum level was compared with the highest level attained by serum magnesium in the period 12 hours prepartum to three days postpartum. The results of this analysis are shown in Table 10 (Appendix I, p x.)

The means for the various groups in this comparison are shown in Table 14.

TABLE 14.

Serum Magnesium in Normal Parturient Cows.

Mean Values at 2 days Prepartum Compared with Means of the Highest value reached during the period 12 hours prepartum to 3 days postpartum.

Magnesium mgm. per cent.

	First Calf. (7)	Second Calf (8)	Third Calf (9)	Fourth Calf (8)
2 days prepartum	2.87	2.78	2.59	2.73
Highest value	3.09	3.45	3.48	3.71
Difference	0.22	0.67	0.89	0.98
%age Rise	7.67	24.10	34.36	35.90

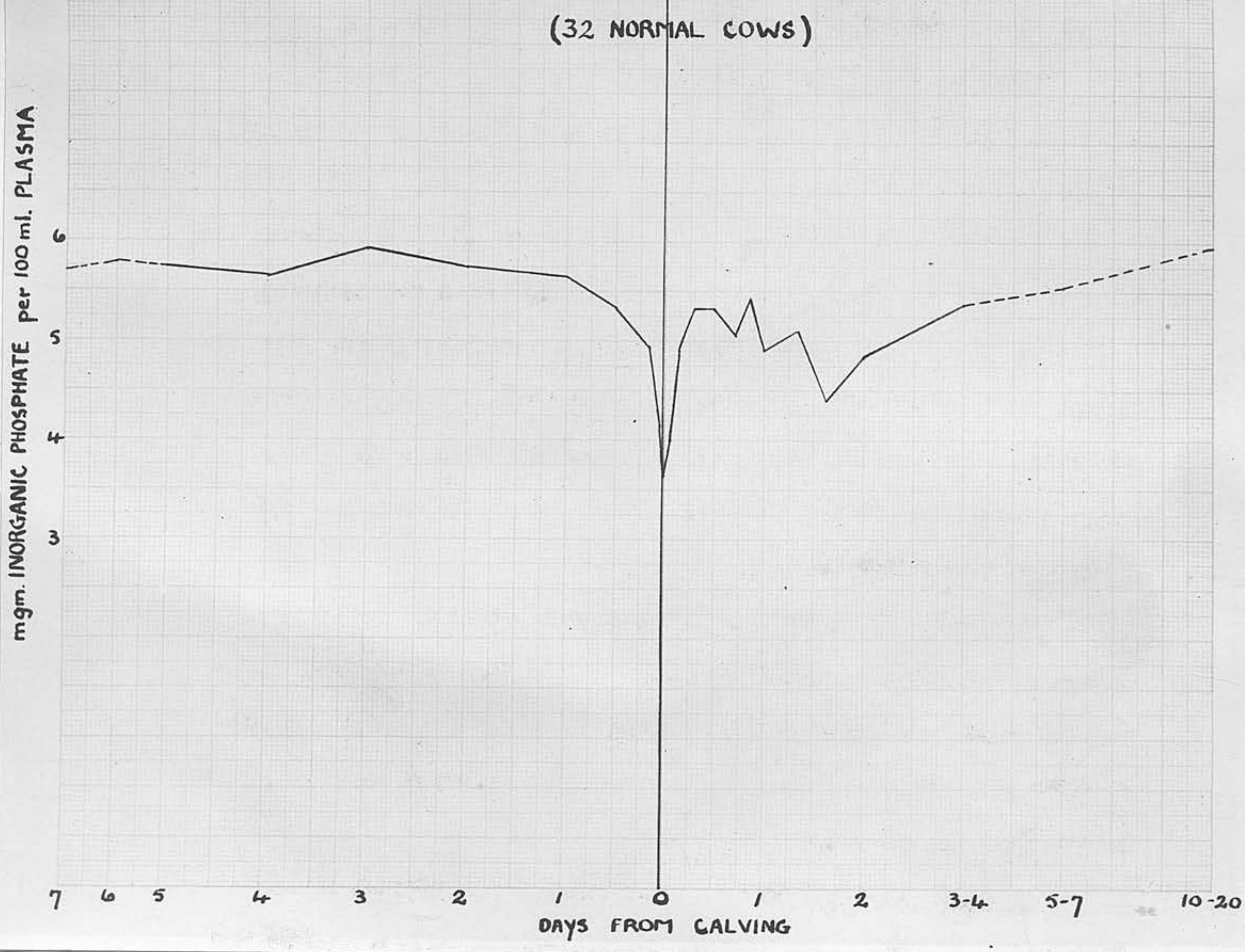
Comparison of these means in Table 14 (t test) shows that there were highly significant (at 1%) differences between first and third, and between first and fourth calf animals. There was a significant difference (at 5%) between first and second calf animals. The differences between other groups were not significant.

The results of the investigation show that when all of the 32 cows were considered as one group, there was a highly significant rise in the level of serum magnesium at parturition. The rise commenced about a day before calving and persisted at a significantly higher level until about 2 days postpartum. Normal levels were regained about 3-7 days postpartum.

Comparison of the different groups showed that there was no significant increase in serum magnesium at calving in first calf animals. Each of the other groups showed a highly significant rise. There was the same tendency for the change to become significantly greater with each succeeding parturition as was evident with serum calcium.

The highest values recorded in serum magnesium in this part of the investigation, have been 4.65 mgm. in a normal parturient 3rd calf cow, and 4.34 mgm. in a fourth calf cow.

FIGURE 5 MEAN VALUES OF PLASMA INORGANIC PHOSPHATE DURING THE PARTURIENT PERIOD



PLASMA INORGANIC PHOSPHATE.

The complete data for plasma inorganic phosphate is presented in tabular form in Tables 11, 12, 13 and 14, (Appendix I pp. xi, xii, xiii and xiv), and in graphic form in Figure 5.

These results show a range for the period 14 days prepartum to 10-20 days postpartum of 1.55 mgm. to 9.40 mgm. phasma inorganic phosphate per 100 ml.

Study of Figure 5 shows that the mean values for inorganic phosphate remained fairly constant between 5.50 mgm. and 6.00 mgm. per cent. from 7 days prepartum until about 1 day prepartum. The actual range within the period 14 days prepartum to 1 day prepartum (Appendix I Tables, 11, 12, 13 and 14) was 3.56 mgm. to 9.40 mgm. There were no significant changes in the level of plasma inorganic phosphate during this period.

From about 1 day prepartum there was a fall in the level of inorganic phosphate to reach a minimum at the time of calving. This was followed by a sharp rise to a maximum at 8 hours postpartum and a further fall at about 2 days postpartum, with a return to normal levels at 3-7 days postpartum.

Analysis of Results. The results have been analysed by the same method as was used for serum calcium and magnesium. All of the 32 cows were available for comparison in the calving, 8 hours postpartum, and 2 days postpartum periods, 27 cows were available in the 3-7 days postpartum period, and 29 cows in the 10-20 days postpartum period.

Table 15 shows the comparison for these animals.

TABLE 15.

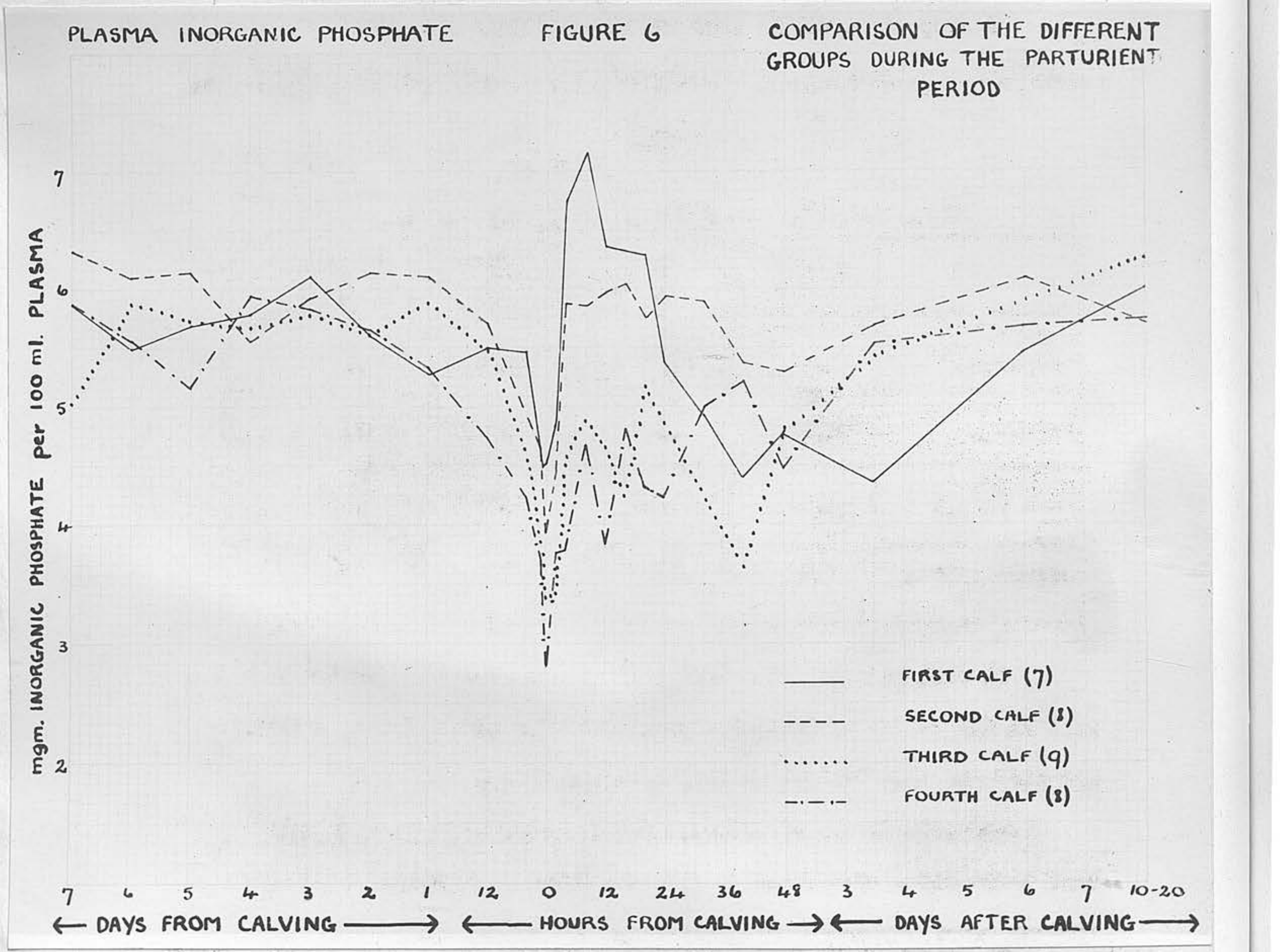
Mean Values of Plasma Inorganic Phosphate.

Phosphate Mgm. per 100 ml.

2 days prepartum	5.79	5.79	5.79	5.72	5.68
Calving (32)	3.73				
8 hours postpartum (32)		5.39			
2 days postpartum (32)			4.89		
3-7 days postpartum (27)				5.44	
10-20 days postpartum (29)					5.94
Mean Change.	-2.06	-0.40	-0.90	-0.28	+0.26
Significance.	1%	N.S.	1%	N.S.	N.S.

N.S. = Not Significant.
Nos. of cows in each comparison are shown in parenthesis.

It will be seen that in this comparison there was a highly significant fall in the level of plasma inorganic phosphate at calving, and at 2 days postpartum. The level at 8 hours postpartum was not significantly below the 2 day prepartum level. This is due to the fact that there was a marked and highly significant rise (at 1%) in the level of inorganic phosphate from calving to 8 hours postpartum. This was followed by a secondary fall and then a return to normal values at about 3-7 days postpartum.


PLASMA INORGANIC PHOSPHATE
FIGURE 6
COMPARISON OF THE DIFFERENT GROUPS DURING THE PARTURIENT PERIOD
mgm. INORGANIC PHOSPHATE per 100 ml. PLASMA
7
6
5
4
3
2
FIRST CALF (7)
SECOND CALF (8)
THIRD CALF (9)
FOURTH CALF (8)
7
6
5
4
3
2
1
12
0
12
24
36
48
3
4
5
6
7
10-20
← DAYS FROM CALVING →
← HOURS FROM CALVING →
← DAYS AFTER CALVING →

Comparison of the Changes in Different Groups. Figure 6 shows the comparison of the differences in behaviour of plasma inorganic phosphate in the different groups in graphic form.

A comparison of the mean values at 2 days prepartum with the mean values at calving, in the different groups, is shown in Table 16.

TABLE 16

Mgm. Inorganic Phosphate per 100 ml. plasma.

	First Calf. (7)	Second Calf. (8)	Third Calf. (9)	Fourth Calf. (8)
2 days prepartum	5.76	6.19	5.60	5.64
Calving.	4.56	4.07	3.41	3.08
Mean Change	-1.20	-2.12	-2.19	-2.56
Significance.	1%	1%	1%	1%

It will be seen, that all groups showed a highly significant fall in the values of inorganic phosphate when the values at calving are compared with the values at 2 days prepartum.

Analysis of the differences between groups (t test) showed that there was a significant difference between first and third and between first and fourth calf animals. The differences between other groups were not significant.

Table 17 shows the comparison of the mean values at 2 days prepartum with the mean values at 8 hours postpartum.

TABLE 17.

Mgm. Inorganic Phosphate per 100 ml. plasma.

	First Calf. (7)	Second Calf. (8)	Third Calf. (7)	Fourth Calf (8)
2 days prepartum	5.76	6.19	5.60	5.64
8 hours postpartum	6.65	5.89	4.89	4.34
Mean Change.	+0.89	-0.30	-0.71	-1.30
Significance.	N.S.	N.S.	N.S.	5%

At 8 hours postpartum there were no significant changes in the levels of inorganic phosphorus in first, second and third calf animals when the levels were compared with the levels at 2 days prepartum. Fourth calf animals showed a significant fall.

Analysis of the differences between groups showed that there was a highly significant difference between first calf animals and fourth calf animals; and a significant difference between first calf animals and third calf animals. The differences between other groups were not significant.

Table 18 shows the comparison between 2 days prepartum and 2 days postpartum.

TABLE 18.

Mgm. Inorganic Phosphate per 100 ml. plasma.

	First Calf (7)	Second Calf. (8)	Third Calf. (9)	Fourth Calf. (5)
2 days prepartum	5.76	6.19	5.60	5.64
2 days postpartum	4.90	5.34	4.75	4.57
Mean Change	-0.86	-0.85	-0.85	-1.07
Significance.	N.S.	1%	N.S.	N.S.

At 2 days postpartum there was a significant fall in the level of inorganic phosphate in second calf animals, but no significant change in the other groups.

Analysis of the differences between groups showed that there were no significant differences in this comparison.

Table 19 shows the comparison between 2 days prepartum and 3 - 7 days postpartum for 27 cows with samples available in the latter period.

TABLE 19.

Mgm. Inorganic Phosphate per 100 ml. plasma.

	First Calf. (6)	Second Calf. (8)	Third Calf. (8)	Fourth Calf (6)
2 days prepartum	5.51	6.19	5.53	5.55
3-7 days postpartum	4.91	5.69	5.56	5.51
Mean Change	-0.60	-0.50	+0.03	-0.04
Significance	N.S.	5%	N.S.	N.S.

At 3-7 days postpartum there was a significant fall in the level of inorganic phosphate in second calf animals, but no significant change in the other groups.

Analysis of the differences between groups showed that there were not significant differences in this comparison.

Table 20 shows the comparison between 2 days prepartum and 10-20 days postpartum for 29 cows with samples available in the latter periods.

TABLE 20

Mgm. Inorganic Phosphate per 100 ml. plasma.

	First Calf (7)	Second Calf (8)	Third Calf (9)	Fourth Calf (5)
2 days prepartum	5.76	6.19	5.60	4.94
10-20 days postpartum	5.95	5.72	6.26	5.74
Mean Change	+0.19	-0.47	+0.66	+0.80
Significance.	N.S.	N.S.	N.S.	N.S.

At 10-20 days postpartum none of the groups showed a level significantly lower than at 2 days prepartum.

Analysis of the differences between groups showed that there were highly significant differences between second and third and between second and fourth calf animals.

Maximum Changes. In order to demonstrate the magnitude of the changes in plasma inorganic phosphate the same method was used as was used for serum calcium and magnesium, i.e., the 2 day prepartum level was compared with the lowest level reached by plasma inorganic phosphate in the period 12 hours prepartum to 3 days postpartum.

The results of this analysis are shown in Table 15 (Appendix 1, p.xv).

The means for the various groups are shown in Table 21.

TABLE 21.

Plasma Inorganic Phosphate in Normal Parturient Cows.

Mean Values at 2 days Prepartum compared with Means of the lowest value reached during the period 12 hours prepartum to 3 days postpartum.

Plasma Inorganic Phosphate mgm.per cent.

	First Calf (7)	Second Calf (8)	Third Calf (9)	Fourth Calf (8)
2 days prepartum	5.76	6.19	5.60	5.64
Lowest value	3.79	3.81	3.00	2.93
Difference	1.95	2.38	2.60	2.71
%age Fall.	33.85	38.45	46.42	48.05

The data in Table 21 show that plasma inorganic phosphate showed the same progressive increase in the magnitude of the change with each succeeding parturition, as was shown by serum calcium and magnesium. Analysis of the data, however, showed that the differences between groups was not statistically significant.

The results for plasma inorganic phosphate, therefore, show that, when the 32 cows were considered as one group, there was a highly significant fall in the level of plasma inorganic phosphate at calving. The fall at calving was followed immediately by a marked rise in the level of inorganic phosohorus, with the result that, 8 hours after calving, the level was not significantly lower than at 2 days prepartum. There was a secondary fall in the phosphate level from 8 hours postpartum to 2 days postpartum, so that in the latter period, the level was significantly lower that at 2 days prepartum. At 3-7 days postpartum the level was still lower than at 2 days prepartum, but not significantly so. By 10-20 days postpartum the normal prepartum levels had been regained.

The tendency for a fall in plasma inorganic phosphate at calving was more consistent than the fall in serum calcium or the rise in serum magnesium.

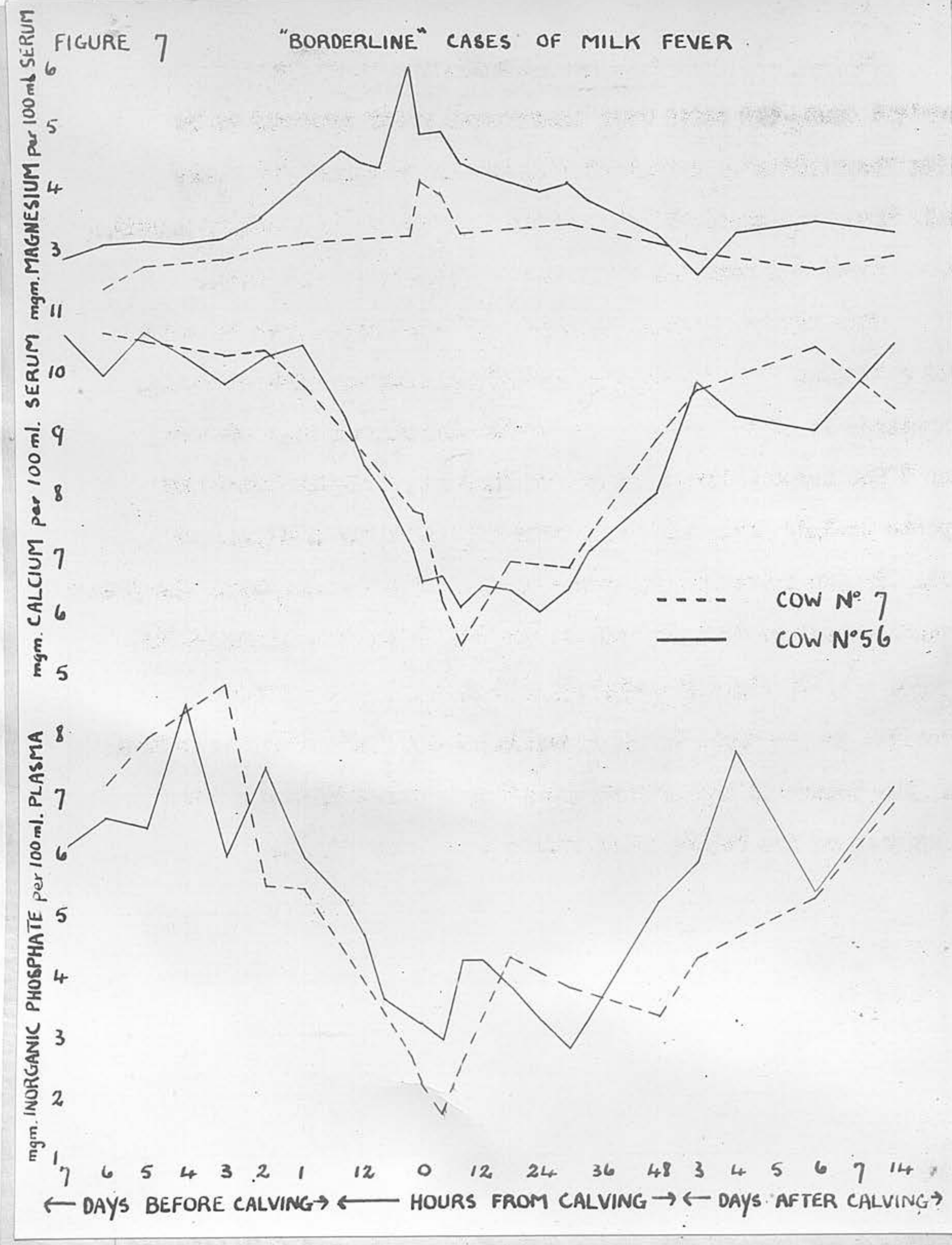
Comparison of the different groups showed that all showed a highly significant decrease in the level of inorganic phosphate at calving. The fall was at its maximum in all groups at calving, and was significantly greater in third and fourth calf animals than in first calf animals. Second calf animals occupied an intermediate position.

The behaviour of inorganic phosphate in the various groups differed in the immediate postpartum period. (Figure 6). While all groups showed a rise in the values of plasma inorganic phosphate from calving to approximately 8 hours postpartum, the levels in the third and fourth calf groups remained below the prepartum levels until approximately 3-7 days postpartum. Second calf animals showed a rise from calving to 8 hours postpartum to a level slightly lower than their prepartum level and remained fairly constant thereafter. The first calf animals showed a very marked rise in the level of inorganic phosphate from calving to 8 hours postpartum, and during the period 4 hours postpartum to 20 hours postpartum the mean values were actually above those of the prepartum period. From approximately 20 hours postpartum the level in first calf animals again fell so that at 3-7 days postpartum the level of inorganic phosphate in first calf animals tended to be below that of the other groups. By 10-20 days postpartum first calf animals had regained their prepartum levels.

It is evident, therefore, that the behaviour of inorganic phosphate in first calf animals differed significantly from that of third and fourth calf animals during the postpartum period. The second calf animals occupied an intermediate position.

The lowest levels reached by inorganic phosphate in the investigation of normal cows were 2.70 mgm. in first calf animals, 2.90 mgm. in second calf animals, 2.00 mgm. in third calf animals, and 1.55 mgm. in fourth calf animals.

FIGURE 7 "BORDERLINE" CASES OF MILK FEVER



"Borderline" Cows.

During the course of the investigation on normal parturient cows, two cases were encountered which appeared to be showing "borderline" symptoms of milk fever. The data for these animals have been presented separately in Table 16 (Appendix I,p.xvi). Figure 7 shows the data for these two animals in graphic form.

Cow number 7 was a sixth calf cow and cow number 56 was a second calf cow. Both cows appeared dull and uneasy after calving, and appeared as if they were going to develop milk fever. In cow number 7 the serum calcium dropped to 5.42 mgm., plasma inorganic phosphate to 1.70 mgm., and serum magnesium rose to 4.10 mgm. per 100 ml. In cow number 56 the serum calcium fell to 6.00 mgm., the plasma inorganic phosphate to 2.75 mgm., while the serum magnesium rose to 6.00 mgm. per 100 ml. - the highest level of serum magnesium encountered in the whole of the investigation of normal and milk fever cows. The return of the cows to normal was co-incident with the commencement of the return to normal of the blood levels.

DISCUSSION OF RESULTS.

In general, the results of this investigation confirm those of the previous workers who have noted a fall in the levels of serum calcium and of plasma inorganic phosphate, and a rise in the level of serum magnesium in normal cows at parturition.

The failure of some workers to note these changes associated with parturition is probably due to insufficient frequency of blood sampling. It will be evident from inspection of the date presented, that daily sampling, as has been practised by many workers, will often fail to demonstrate the changes occurring, and will give no indication of the rapid alteration which may take place in the levels of calcium, magnesium and inorganic phosphate at, or around calving. The increased frequency of blood sampling probably accounts also for the fact that the changes which have been found in the levels of calcium, magnesium and inorganic phosphate are greater than those found by most previous workers.

Of the three blood constituents examined, the greatest relative changes have been noted in the plasma inorganic phosphate, and the results support the statment of Godden and Allcroft (1932) that the fall in inorganic phosphate is a reliable indication of impending parturition.

The most interesting aspect of the results of the investigation is the finding that the changes in all three constituents became significantly more marked with each succeeding parturision. Since milk fever practically never occurs in first calf heifers and is rarely seen in second calf animals, (Henderson 1938, Allcroft 1947a) this finding would seem to support the hypothesis of Allcroft and

Godden (1934) that milk fever is a pathological exaggeration of the normal physiological changes occuring at parturition. This hypothesis is also supported by the fact that the changes occurred during the period when milk fever is most common i.e., 12 hours prepartum to 2-3 days postpartum. It is of interest to note that, in this study, it has been found that there was a good deal of individual variation within this period in the time, relative to calving, at which the serum calcium reached its lowest level, as is to be expected if milk fever is to be regarded as a pathological exaggeration of the normal changes at parturition. Further, the fact that two cases of "borderline" milk fever were encountered also adds weight to the hypothesis. These cases can be regarded as transitional cases between on the one hand, normal cows where the fall in blood calcium is arrested before clinical signs of milk fever becomes evident, and, on the other, cases where the fall in calcium progresses to such an extent that clinical milk fever results.

The fact that there was a certain amount of overlapping between the levels of serum calcium in normal cows at parturition and the levels in clinical cases of milk fever suggests that there may be individual variation between cows in the level of serum calcium which can be tolerated without the exhibition of clinical signs. Alternatively, it may be that a lowered level of blood calcium is not the only requisite for the production of clinical milk fever and that other metabolic changes must occur concurrently. The evidence resulting from this study suggests that, if this second premise be accepted, the other necessary metabolic changes are not merely a concurrent fall in the inorganic phosphate level or a rise in the serum magnesium; e.g. in Cow 56 (Table 16, Appendix I p.xvi) the serum calcium fell to 6.00 mgm. per cent., while the inorganic phosphate fell to 1.70 mgm. and the serum magnesium rose to 6.00 mgm. without the cow developing clinical milk fever. It seems, therefore, that the levels of calcium, magnesium and inorganic phosphate in the blood may not be the only factors in determining whether clinical milk fever occurs. The scope of the present study does not warrant speculation as to what these factors may be.

The acceptance of the theory that milk fever is an exaggeration of the normal changes occurring in cows at parturition does not, of course, get one very far. It does, however, suggest that investigation of the causes of the changes in normal cows might help to elucidate the ultimate cause of milk fever. Limited studies on the causation of these changes have formed part of this investigation and the results are reported in Part 2 and 3 of this Section.

Certain points arising from the study of serum magnesium and plasma inorganic phosphate in normal parturient cows can be elaborated here.

<u>Sérum Magnesium</u>. The results of the present study amply confirm the findings of those previous workers who have noted a rise in the level of serum magnesium at normal parturition. Very little is known about the mechanisms which control the level of serum magnesium. The pharmacologically antagonistic actions of calcium and magnesium ions are, however, well understood. Thus Meltzer and Auer (1908) showed that the parenteral injection of magnesium salts produces narcosis and anaesthesia, and that this can be countered by the administration of calcium salts. This has been confirmed by Moore and Wingo (1942), working with dogs. Seekles, Sjollema & van der Kaay (1932a) have demonstrated the same effects in cattle. This led Kloubek (1932),

Pribyl (1933) and Schulhof (1933) to postulate that magnesium narcosis is the cause of milk fever. While it is possible that the calcium : magnesium ratio is of importance in the symptomatology of milk fever, as has been suggested by Barker (1939), it is unlikely that the level of blood magnesium has a direct aetiological significance. It is more likely that, so far as the level of serum magnesium at normal parturition is concerned, the primary determining factor is the lowered level of serum calcium. In support of this it may be said that while it is difficult to suggest reasons for a primary change in serum magnesium, it is possible to postulate fairly plausible reasons for the fall in serum calcium at parturition, e.g. the drain in blood calcium from the onset of lactation. That there is a reciprocal relationship between serum calcium and magnesium is demonstrated by the increased level which accompanies the decrease in serum calcium in oxalate poisoning, and by the hypocalcaemia which accompanies the hypermagnesaemia induced by the parenteral administration of magnesium salts (Duncan 1942). The fact that, as shown in the present study, the changes in both elements became progressively more marked with each succeeding parturition, lends support to the view that there is an inter-relationship between the two, and suggests that the reciprocal relationship may be of a quantitative nature.

<u>Plasma Inorganic Phosphate</u>. Comparison of Figures 1 and 5 suggests that there is no such close inter-relationship between the blood calcium and the inorganic phosphate, although both fractions showed a distinct and significant drop at calving. In the case of serum calcium, however, the drop continued post-partum, to reach a minimum level at about 24 hours after parturition. Plasma inorganic phosphate, on the other hand, showed a marked and spectacular rise

commencing at parturition and reaching a maximum between 8 and 20 hours postpartum. The impression given is that the act of parturition had little effect on the level of serum calcium - the fall continued for another 24 hours; in the case of inorganic phosphate, however, the act of parturition was followed by an immediate reversal of the trend - the fall was arrested and a rapid rise in the blood level substituted. It appears, therefore, that the level of plasma inorganic phosphate is influenced directly by the act of parturition.

Study of Figure 6 shows that the postpartum rise in plasma inorganic phosphate was most marked in first and second calf animals. In the case of first calf animals the rise was such that the levels attained during the period 4 hours postpartum to 20 hours postpartum were above the normal prepartum levels. In second calf animals the inorganic phosphate regained its prepartum level about 4 hours postpartum and remained fairly constant thereafter. In the case of third and fourth calf animals there was a smaller but distinct rise in the immediate postpartum period, followed by a more gradual return to normal levels. These findings suggest that first calf animals are capable of reacting more strongly than older animals to a fall in the level of blood phosphate. They appear to be able to mobilise phosphate more quickly than second, third or fourth calf animals. This may be the reason for the fact that the fall in the inorganic phosphate at parturition is least marked in first calf animals, and becomes more marked with each succeeding parturition. This ability of first calf animals to compensate more readily for alteration in the level of a blood constituent may be one of the reasons for the fact that milk fever never occurs in first calf animals.

Following the initial rise in the level of inorganic phosphate in the immediate postpartum period in first calf animals there was a secondary fall, so that from about 2 days postpartum to about 6 days postpartum, the level in these animals tended to be lower than in the other groups. The possible significance of this secondary fall in first calf animals will be discussed later.

<u>The Cause of the Drop in Blood Calcium</u>. The most generally accepted explanation of the drop in blood calcium at normal parturition, and in milk fever, is the drain produced by the onset of milk secretion. The removal of calcium from the blood by the secretion of colostrum was one of the postulates on which Dryerre and Greig (1925) based their parathyroid dysfunction theory. Little and Wright (1926) suggested that the drain of calcium in the colostrum was sufficient to account for the fall in the level of blood calcium.

A prima facie case for this theory can be argued on the following lines:-

1. The fall in blood calcium is co-incident with the onset of lactation.

2. As was pointed out by Little and Wright (1926) half a gallon of colostrum contains as much calcium as the blood at any one time. It is evident, therefore, that the onset of lactation results in a considerable demand on the available blood calcium. If the mechanisms for the mobilisation of calcium from the body reserves were incapable of meeting this demand, a lowering of blood calcium would result.

3. The fall in blood calcium at parturition is greatest in

cows which have had at least three previous parturitions i.e. in cows which are at the peak of their milk production.

4. The dairy cow is an animal in which milk production has reached a very high level. Hypocalcaemia is rare in beef breeds where milk production is not so well developed.

In order further to elucidate the effect of the onset of lactation on the levels of blood calcium, magnesium and inorganic phosphate it was decided to study the effect of prepartum milking and of mastectomy on the levels of these blood fractions. The results of these studies are reported in Parts 2 and 3 of this Section.

PART 2.

A Study of the Effect of Prepartum Milking on the blood Calcium, Magnesium and Inorganic Phosphate in Parturient Cows.

Introduction.

If the fall in blood calcium and phosphorus in normal cows at parturition is related to the sudden demand for these elements produced by the onset of lactation, prepartum milking, by stimulating a more gradual onset of milk secretion, should tend to prevent the abrupt fall in these elements. If drainage into the milk is a potent factor in influencing the level of blood calcium and phosphorus it is to be expected that the gradually increasing drain produced by pre-partum milking would have one of two effects. Firstly, it could produce a gradual fall in calcium and phosphorus co-incident with the gradual rise in the secretion rate; or secondly, by allowing the mobilisation mechanisms time to compensate for the loss via the milk, it could abolish the fall altogether.

The changes in blood calcium and phosphorus in prepartum milked cows have been studied by two groups of American workers. Johnson et al (1948) studied the effects of prepartum milking on the blood calcium and phosphate. Two groups of cows were used; one group was milked for ten days prior to the expected calving date, and the other milked only after calving. Blood samples were taken at weekly intervals, commencing four weeks prior to calving, immediately after calving, and ending four weeks after calving. These workers noted no apparent differences between cows milked prepartum and those milked only postpartum. In both groups blood calcium and phosphate dropped appreciably at calving and returned to the prepartum level within a week after calving.

As a result of a study of 46 cows Smith and Blosser (1947) concluded that prepartum milking had no significant effect on the incidence of milk fever. They stated that it would seem that factors in addition to the initiation of milk secretion are involved in the causation of parturient paresis. They presented data on serum calcium levels for 3 days prepartum, the day of calving, and for 3 days postpartum.

In both of these investigations blood samples were taken at rather wide intervals. As has been noted earlier it is necessary to take very frequent blood samples in order to detect the changes which may be occurring in the blood picture at parturition. Accordingly, it is possible that differences between prepartum milked and normal cows may have been missed in the investigations quoted.

In Great Britain there have been no reports of investigations of the effects of prepartum milking on the blood calcium, magnesium or phosphorus. Boutflour (1949) has asserted that the practice of prepartum milking lowers the incidence of milk fever. His opinion is apparently based on the observations of Hill, Widdowson and Maggs (1950) that in 86 parturitions in pre-milked cows there were only 3 cases of milk fever. It is impossible, however, to determine the significance of these figures as no information is given as to the susceptibility of the population e.g. how many of the parturitions were first or second calvings?

The present investigation was undertaken to determine whether frequent blood sampling would show any differences between prepartum milked and normal cows in the behaviour of serum calcium, magnesium and plasma inorganic phosphate.

Materials and Methods .

The effect of prepartum milking was studied in seven cows. As is explained later it was possible to utilise the data from only two of these animals.

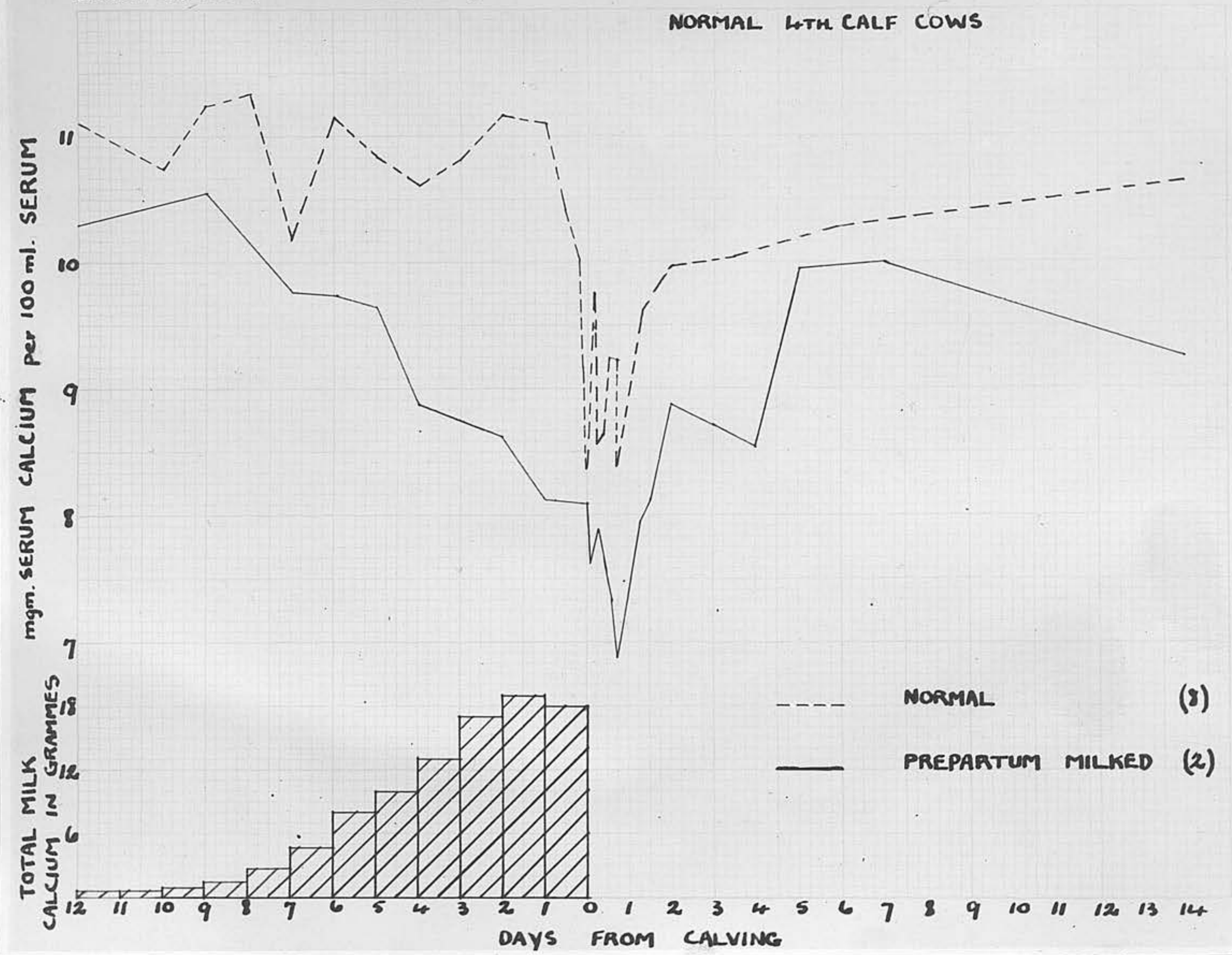
The object in the investigation was to get the cows giving an appreciable quantity of milk for some time before calving so that any effect on the blood picture produced by the drain in calcium, magnesium or phosphate could become manifest. Accordingly, milking was started three weeks before the expected calving date. During the prepartum period the cows were fed a liberal concentrate ration as in the "steaming-up" process. Blood samples were taken daily as far as possible during the prepartum period. Postpartum, the same frequency of blood sampling was adopted as was used in the study of normal cows. Representative samples of milk were collected daily and analysed for their total calcium, magnesium and phosphate contents.

Total milk calcium and magnesium were estimated by the methods of A.O.A.C. (1945) for food analysis, modified for milk. Total milk phosphate was estimated by the micro-method of Graham and Kay (1934).

RESULTS.

Of the seven cows in which prepartum milking was practised only two gave appreciable quantities of milk during the prepartum

SERUM CALCIUM FIGURE 8 COMPARISON OF PREPARTUM MILKED AND NORMAL 4TH CALF COWS



period. Five of the cows gave negligible quantities of milk prepartum despite liberal feeding and frequent stripping of the udder. In three of these five cows the blood picture was identical with that obtained in the study of normal cows at calving. Accordingly the results from these animals have not been presented. The other two cows which gave negligible quantities of milk prepartum developed clinical milk fever. The data from these animals are presented in the section devoted to the study of clinical cases of milk fever.

Two cows gave appreciable quantities of milk during the prepartum period. Both these animals were at their fourth parturition. Cow number 1 gave a total of 82.72 kg. milk during the prepartum period, and Cow number 2 a total of 46.86 kg.

The data for the blood analyses for these animals is presented in Table 17 (Appendix I, p.xvii), and for the milk analyses in Table 18 (Appendix I, p. xviii). Figure 8 shows the mean data for serum calcium for the two prepartum milked cows compared with the data obtained from the 8 normal fourth calf animals which were used in the investigation described in Part 1 of this Section. The histogram shows the mean daily excretion of calcium in the milk during the prepartum period for the two cows. Figure 9 is a similar graph for plasma inorganic phosphate and Figure 10 for serum magnesium. The magnesium secretion in the milk has not been plotted as the quantities secreted were so small.

DISCUSSION OF RESULTS.

Great care must be exercised in drawing conclusions from these results as only two cows are involved. The results are, however, consistent for the two animals studied.

In the case of serum calcium and plasma inorganic phosphate, instead of the abrupt fall in the last 12-24 hours before calving seen in normal postpartum milked cows, the prepartum milked cows showed a gradual fall during the last 5-6 days prepartum, co-incident with the rise in the level of milk secretion. The recovery phase in the postpartum period was delayed in the prepartum milked cows as compared with the normal cows; especially was this so in the case of plasma inorganic phosphate.

The parturient rise in serum magnesium in the prepartum milked cows tended to commence earlier and to be more restrained than in normal cows of the same age.

So far as they go, the results suggest that the artificial initiation of lactation before parturition does have an effect on the levels of blood calcium and inorganic phosphate. The results do not agree with those of American workers who have found no apparent differences between prepartum milked and normal cows.

The fact that the fall in blood calcium and inorganic phosphate is co-incident with, and proportional to, the rise in the level of secretion of these elements in the milk, suggests that the blood is being depleted by the demands of lactation. Within its limits, therefore, the evidence supports the theory that drainage into the milk is the cause of the fall in these elements at normal partubition.

No definite opinion can be expressed on the influence of prepartum milking on the incidence of milk fever. In this connection, however, it is of interest to note that the two prepartum milked cows showed levels of calcium and inorganic phosphate at parturition which were lower than the average levels for normal cows of the same age and that the recovery of normal levels in the postpartum period was more prolonged. Such evidence would tend to suggest that prepartum milking increased the likelihood of milk fever developing. On the other hand it might be argued that prepartum milking, by substituting a gradual drop in blood calcium and inorganic phosphate for the sudden change seen in normal cows, may allow the tissues time to accommodate, and enable the animal to tolerate lower levels of these fractions than is normally the case. Alternatively, the gradual change may allow the mobilisation mechanisms more time to compensate, and thus prevent the calcium and inorganic phosphate from falling to levels at which clinical milk fever occurs. The answer to these questions depends on further investigations; it does seem that the detailed study of prepartum milked cows may be valuable in elucidating the aetiology of milk fever.

In conclusion, it must be emphasised that the present experiment can only be regarded as a pilot one. Further work with larger numbers of animals is indicated before definite conclusions can be drawn.

PART 3.

A Study of the Effect of Mastectomy on the Serum Calcium and Magnesium and Plasma Inorganic Phosphate in Parturient Cows.

Introduction.

The complete removal of the udder from a pregnant animal provides a method of allowing parturition to take place without the concurrent complication of the onset of milk secretion. In the study of the blood chemistry at parturition, therefore, mastectomy provides a possible means of differentiating the changes associated with the act of parturition per se, from those associated with the onset of milk secretion.

Two reports have appeared in the literature on the effect of mastectomy on the blood calcium and inorganic phosphate at parturition. Wilson and Hart (1932) studied the effect of mastectomy on one parturient goat. They found that the serum calcium stayed level at parturition. Plasma inorganic phosphate dropped from a prepartum level of 7.66 mgm. to 5.59 mgm. per cent at parturition. This was followed by a marked rise to 11.52 mgm. per cent. one day after parturition.

When the present investigation was started no reports had appeared in the literature on the effects of mastectomy on the blood chemistry of parturient cows. Niedermeier, Smith and Whitehair (1949) have since reported a controlled experiment on the effect of mastectomy on the blood calcium, magnesium, inorganic phosphate and plasma fat in eight parturitions in five cows. These workers reported that mastectomised cows showed only a very slight drop in serum calcium and plasma fat at parturition. The rise in serum magnesium was abolished in mastectomised cows. Plasma inorganic phosphate was reported as falling at parturition in both mastectomised and normal cows with a slightly smaller decrease in the mastectomised animals.

Materials and Methods.

Three cows were used in this study. Mastectomy was performed as described by Frank (1944), under intravenous chloral hydrate anaesthesia, when the cows were approximately six months pregnant.

Blood samples were collected from the jugular vein. The same frequency af blood sampling was adopted as was used in the studies of normal cows at parturition. The normal parturient cows can be regarded as controls as the methods of feeding and management were essentially the same.

Cow number 1 was a third calf animal, cow number 2 a second calf animal. In the case of Cow number 3 the exact number of the parturition was unknown but it was at least the fourth.

RESULTS.

Calving was normal and unaided in all three cows. The complete data are presented in Table 20 (Appendix I. p.xx). Cow number 2 calved somewhat unexpectedly with the result that the data for this animal in the prepartum are somewhat meagre.

Figure 11 shows the data ingraphic form. Figure 12 shows the comparison of the mean data for the three mastectomised cows with the mean data for three normal parturient cows, viz. Cows number 53, 55 and 26. These cows were chosen because they were

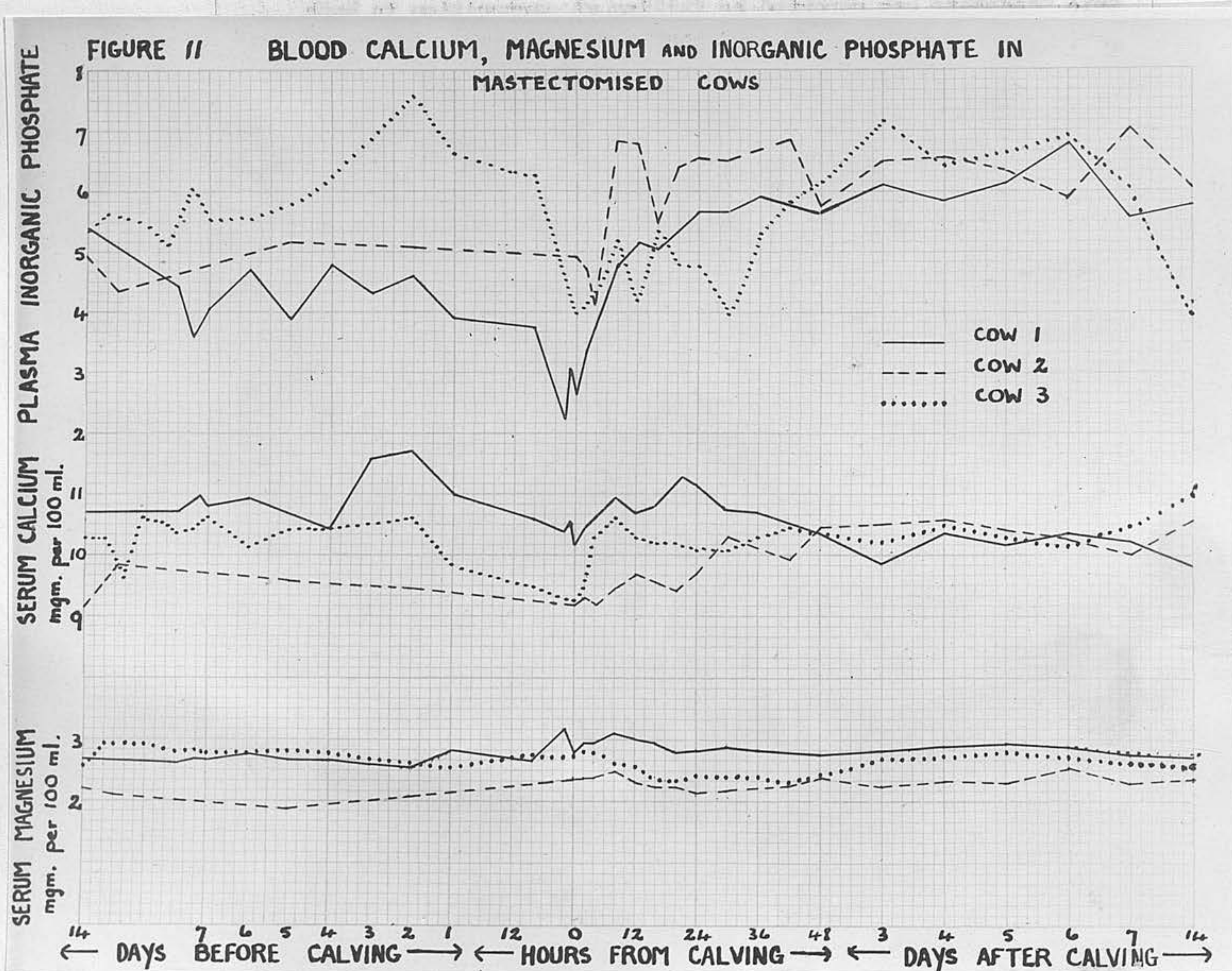


FIGURE 11 BLOOD CALCIUM, MAGNESIUM AND INORGANIC PHOSPHATE IN MASTECTOMISED COWS
PLASMA INORGANIC PHOSPHATE
SERUM CALCIUM mgm. per 100 ml.
SERUM MAGNESIUM mgm. per 100 ml.
COW 1
COW 2
COW 3
14 7 6 5 4 3 2 1 12 0 12 24 36 48 3 4 5 6 7 14
← DAYS BEFORE CALVING → ← HOURS FROM CALVING → ← DAYS AFTER CALVING →

comparable in number of calving with the mastectomised cows, and because they had a similar number of samples available. In order to avoid undue bias, only period containing samples from at least two of the three cows in each group, have been plotted on Figure 12.

Serum Calcium.

Study of Figure 11 shows that all three cows did show a drop in the level of serum calcium at parturition. The fall was, however, very slight and was followed immediately after parturition by a rise in the level of serum calcium. The postpartum rise was most marked in Cow 3 which showed a rise from 9.30 mgm. at calving to 10.30 mgm. at 8 hours postpartum.

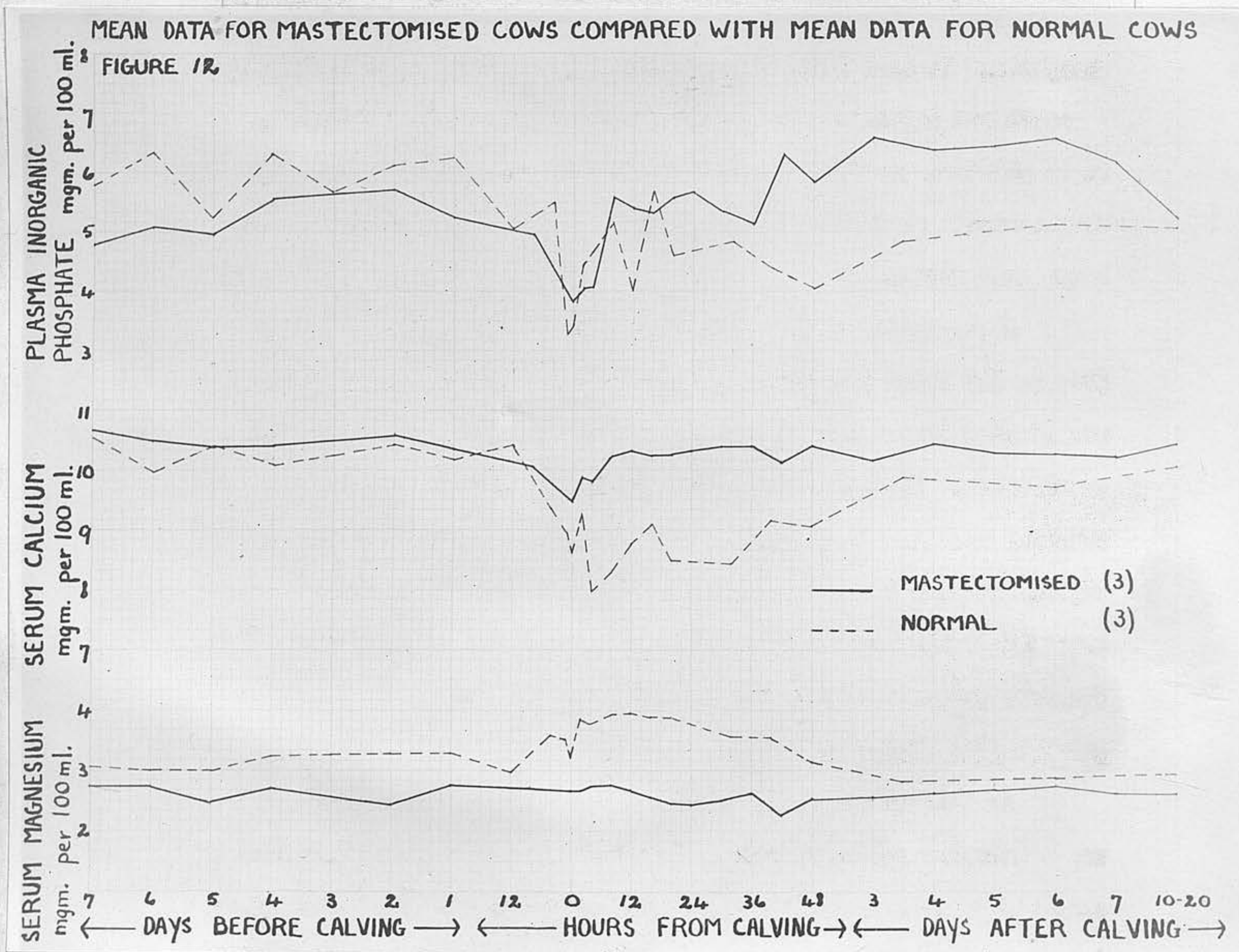
Comparison of the mean data for mastectomised cows with the mean data for normal cows (Figure 12) shows that the fall in serum calcium at parturition was smaller in mastectomised cows. The main difference between the two groups was evident in the postpartum period. In normal cows the lowered level of serum calcium persisted until 3-4 days postpartum, while in mastectomised cows the prepartum level had been regained by 8 hours postpartum.

Serum Magnesium.

In this constitutent the changes associated with normal parturition were almost completely abolished. In Cows 1 and 2 there was a very slight rise in the level of serum magnesium at parturition.

Plasma Inorganic Phosphate.

Study of Figure 11 shows that, in all three mastectomised cows, there was a fall in the level of this constituent around parturition. In Cow 1 the lowest point was reached at 2 hours prepartum, in Cow 2 and 4 hours postpartum, and in Cow 3 at calving.


MEAN DATA FOR MASTECTOMISED COWS COMPARED WITH MEAN DATA FOR NORMAL COWS
FIGURE 12
PLASMA INORGANIC PHOSPHATE mgm. per 100 ml.
SERUM CALCIUM mgm. per 100 ml.
SERUM MAGNESIUM mgm. per 100 ml.
MASTECTOMISED (3)
NORMAL (3)
7 6 5 4 3 2 1 12 0 12 24 36 48 3 4 5 6 7 10-20
← DAYS BEFORE CALVING → ← HOURS FROM CALVING → ← DAYS AFTER CALVING →

Cow 3 showed a marked transient rise in inorganic phosphate in the 1 to 2 day prepartum period. The immediate postpartum period was marked in all three cows by a rise in the level of plasma inorganic phosphate. In Cows 1 and 2 the level rose immediately after calving to reach and maintain a level higher than in the prepartum period. Cow 3 showed a smaller rise in the immediate postpartum period and then a more marked rise after a secondary fall which occurred at 30 hours postpartum.

A comparison of the mean data for the two groups of cows (Figure 12) shows that the behaviour of plasma inorganic phosphate was similar in the two groups in the period immediately around parturition. The fall in the mastectomised cows tended to be slightly smaller and the rise following calving to be slightly more rapid. It will be noted, however, that, while mastectomised cows showed a higher level in the later postpartum period, normal cows showed a level lower than in the prepartum period.

DISCUSSION OF RESULTS.

In general the results of the investigation agree with those of Niedermeier et al. (1949). The fall in blood calcium has been slightly greater in this study. Niedermeier et al. reported no rise in serum magnesium at parturition in mastectomised cows while in this study a slight but definite rise took place in all three cows. In both studies a fall in plasma inorganic phosphate at parturition slightly less in mastectomised cows than in normal cows, has been observed. The postpartum rise in inorganic phosphate was more rapid in the cows studied in the present investigation. A similar, but slightly less well marked, tendency is evident in the data of Niedermeier et al., although they do not comment on this aspect. Wilson and Hart (1932) also reported a rapid rise in the postpartum level of inorganic phosphate in their study of one mastectomised goat. The tendency for plasma inorganic phosphate to attain a higher level in the postpartum period as compared with the prepartum period, is not evident in the data presented by Niedermeier et al., except in the case of two cows.

The results of these studies on mastectomised cows suggest that the onset of milk secretion is the major factor in producing the drop in serum calcium in normal cows at parturition.

Two possible explanations of the diminution of the fall in serum calcium in mastectomised cows present themselves. Firstly, it could be argued that the diminution in the fall in serum calcium is due to the abolition of hormonal or nervous mechanisms normally operating from the udder at parturition. There is no evidence, however, that the udder has any endocrine function. With regard to nerve mechanisms, it is well known that nerve reflexes, originating in the udder and acting via the posterior pituitary, are concerned in the phenomenon of the "let down" of milk. There is no evidence that these mechanisms are associated directly with the control of mineral metabolism.

Secondly, it is possible that the diminution of the fall in serum calcium in mastectomised cows is due to the abolition of the drain in blood calcium resulting from the onset of milk secretion. This seems the more rational explanation. As will be shown later, a good deal of experimental evidence has accumulated suggesting that milk secretion does have an influence on the level of serum calcium.

While drain of calcium into the milk appears to be the major factor in causing the fall in serum calcium at calving, the fact that a slight fall still persists after complete removal of the udder, indicates that it is not the only factor involved.

The very slight changes in serum magnesium observed in mastectomised cows are in keeping with what would be expected if we accept the suggestion that serum calcium and magnesium are in inverse relationship, so that the findings lend indirect support to such a hypothesis.

The persistence of a fall in plasma inorganic phosphate at parturition despite mastectomy is interesting. This supports the suggestion made in Part 1 that the level of inorganic phosphate is directly influenced by the act of parturition. Neidermeier et al. suggest that the exercise involved in parturition may contribute to the drop in inorganic phosphate. They quote in support of this suggestion the findings of Palmer, Cunningham and Eckles (1930), who noted an appreciable increase in inorganic phosphate 15 minutes after exercise, followed by a distinct decrease which persisted for at least 2 hours after exercise. These changes described by Palmer et al. are very transitory and do not follow the same pattern as is observed in mastectomised or normal cows. Further, Gemmill and Ribeiro (1933) have reported no change in plasma inorganic phosphate in man and dogs as the result of severe exercise. If exercise were the only factor involved it is likely that similar changes would be observed at parturition in other species. The changes observed in inorganic phosphate at parturition in women (Oberts and Plass 1932) have been very slight and transitory. Mull and Bill (1932) have observed a slight rise in inorganic phosphate during the later stages of pregnancy in women, reaching a peak at delivery, followed by a short decline. This is the reverse of the findings of van Landingham, Henderson and Bowling (1942) who noted a progressive lowering of the plasma inorganic phosphate in heifers with advancing gestation. It is also the reverse of the well established finding of a fall in the level of inorganic phosphate at calving in cattle.

The fact that similar changes have not been recorded in other species makes it unlikely that exercise is a major cause of the changes in inorganic phosphate at calving in normal and mastectomised cows. That the fall is not due merely to a transference of inorganic to organic phosphate in the blood is shown by the findings of Moodie (1952) who has recorded a fall in the total blood phosphate in these mastectomised cows.

GENERAL DISCUSSION ON SECTION 1.

A good deal of experimental evidence has now accumulated suggesting that the drain of calcium into the colostrum is a major factor in causing the lowering of blood calcium in normal cows at parturition.

The most direct evidence in support of this hypothesis is given by the experiments of Niedermeier et al. (1949) on mastectomised cows. Their findings in respect of serum calcium have been largely confirmed in this study. Complementary evidence tending to add weight to the theory has also been forthcoming in recent years. Thus Niedermeier and Smith (1948) reported higher than normal levels of serum calcium at 3-4 days postpartum following an initial drop at calving, in cows which were not milked after parturition. They reported that the highest levels occurred at the time of greatest intramammary pressure. Mercer et al. (1949) reported that the interruption of milking for a 10 day period during lactation resulted in a significant elevation of serum calcium.

The finding in this study that the fall in serum calcium at parturition is most marked in cows which have reached the peak of their milk production, also gives indirect support to the hypothesis. Further support is given by the fact that in the two prepartum milked cows which gave appreciable quantities of milk before calving, the serum calcium showed a gradual fall co-incident with the rise in the level of calcium secretion in the milk, and in contrast to the abrupt fall in serum calcium seen in normal cows. As has been pointed out before, however, great care must be taken in arguing too forcibly from this limited data, especially as it is in contradiction to American results which, while they are less detailed in respect of frequency of blood sampling, are based on much larger numbers of animals.

When all the evidence is taken into account, however, it does seem probable that the drain of calcium into the colostrum is the main cause of the lowering of blood calcium in normal cows at parturition.

If we accept the hypothesis, which seems reasonable, that milk fever is an exaggeration of the normal physiological changes associated with parturition, it is clear that the drain of calcium from the blood into the milk is one of the major factors in the aetiology of milk fever. That it is not the only factor is obvious from the fact that, if it were, every heavy milking cow would be subject to the disease as was pointed out by Greig (1930a). Hibbs et al. (1946) have shown that the colostrum of cows developing milk fever is no higher in calcium content than the colostrum of normal cows. These workers have also shown (Hibbs, Pounden and Krauss 1951) that milk fever cases secrete no more colostrum than normal cows. It is logical to assume, therefore, as is suggested by Hibbs et al. (1951) that the fundamental difference between cows which develop milk fever and those which do not, lies in a difference in the functioning of the calcium regulating mechanisms.

The evidence resulting from the present study suggests that the mechanisms controlling the mobilisation of calcium become progressively impaired with each succeeding parturition. A point is reached, usually after the third or subsequent parturition, when, in a proportion of animals, the mechanisms fail to counteract the fall in blood calcium resulting from the demands of milk secretion, with the result that clinical milk fever ensues.

Ward, Blosser and Adams (1952) have recently reported the results of a study which may help to elucidate the causes of the progressive increase, with advancing age, in the magnitude of the changes in serum calcium in parturient cows. These workers conducted complete calcium balance studies on a limited number of cows for a period of three weeks before, and three weeks after parturition. Four groups of cows were used viz. primiparous Jersey cows, mature normally calving Jersey cows, mature normally calving Guernsey and

Holstein cows, and mature Jersey cows which developed milk fever. The cows subsequently developing milk fever were found to be in a definitely and consistently negative calcium balance throughout the whole of the experimental period; the two groups of mature normally calving cows were in abborderline calcium balance during the prepartum period and changed to a negative calcium balance just before calving; the primiparous cows remained in a definitely and consistently positive calcium balance during the prepartum period and switched to a negative balance about 1 to 2 days postpartum.

These results suggest that the failure of mature animals to mobilise calcium as quickly as young animals, and thus to counteract the falling blood calcium level, may be associated with depletion of the body reserves resulting from a prolonged negative calcium balance.

In this connection the recent study of Thomas, Litovitz, Rubin and Geschickter (1952) on the time distribution of intravenously injected radiocalcium (calcium 45) may be of some significance. In a comparison of young and adult rabbits injected intravenously with very small quantities of calcium 45 these workers found that the rate of bone uptake of the injected calcium was approximately 89 per cent of the injected dose for young animals, and 41 per cent for the adults, in four hours. They point out that the uptake of calcium by the bones must be in the nature of an exchange process as the rate of uptake is far too rapid to be explained as a growth process in the building up of osseous tissues. They therefore suggest that the difference in magnitude of the uptake by the bones in young and adult animals could be explained in terms of a difference in "effective bone area" available for exchange in the two age groups.

While it is dangerous to argue by analogy from rabbits to cattle, the apparent ability of young dairy cattle to mobilise calcium more readily than older cattle could be explained by postulating a similar difference in "effective bone area" available for interchange of calcium between the blood and the bony reserves. Experiments on cattle with radiocalcium, designed to test this suggestion would seem to offer a hopeful line of future research.

The sudden reversal of the trend of plasma inorganic phosphate from a fall at calving to a rise immediately after calving, has suggested that the changes in inorganic phosphate are more intimately associated with the act of parturition per se than are the changes in serum calcium. That this is so is proved by the fact that changes in the level of inorganic phosphate persist in parturient mastectomised cows. That milk secretion does have an effect on the level of plasma inorganic phosphate is, however, suggested by the fact that normal control cows showed a lower level of inorganic phosphate in the postpartum period as compared to the prepartum period, while in mastectomised cows the opposite was the case i.e. the level was higher in the postpartum than in the prepartum period. This higher level of inorganic phosphate following calving in the mastectomised cows may be explained by the cessation of the demands of the growing foetus, and the absence of the demands of lactation. The behaviour of the plasma inorganic phosphate in the two prepartum milked cows which gave appreciable quantities of milk before calving, also supports the hypothesis that milk secretion plays some part in influencing the inorganic phosphate level at parturition.

The findings of Ward et al. (1952) on the calcium balance of primiparous cows may be of some significance in explaining the secondary fall in inorganic phosphate which occurs in first calf animals following the marked initial rise after calving (Figure 6). This secondary fall in first calf animals occurs about a day after the period when, according to Ward et al., primiparous animals go into a negative calcium balance, and may possibly be related to a similar change in the phosphate balance.

The results obtained in the study of serum magnesium in normal, prepartum milked, and mastectomised cows confirm the well recognised inverse relationship normally existing between serum calcium and serum magnesium. In these animals the level of serum calcium appears to be the primary determining factor, and the magnitude of the rise in serum magnesium appears, in general, to be a reflection of the magnitude of the fall in serum calcium.

In conclusion it may be said that it is unlikely that the fundamental cause of milk fever will be ascertained until more is known concerning the control of the blood calcium in the bovine, and the factors which infouence it. A point which should be borne in mind is that the study of the calcium regulating mechanisms in other animals may not necessarily be of much assistance, as it is doubtful if a disease strictly comparable with milk fever occurs in any animal apart from the cow and the ewe. Milk fever has been commonly diagnosed in the sow, but Hogg (1952) has recently cast doubts on whether the condition does in fact occur in this species, and the author's clinical experience tends to confirm these suspicions. It may be that the higher level of milk secretion in ruminants, particularly the dairy cow, is sufficient to account for the occurrence of the disease. On the other hand, it is possible that in addition there may be pecularities in the calcium regulating mechanisms in ruminants, which have a bearing on the causation of milk fever.

SUMMARY AND CONCLUSIONS.

1. The variations in the levels of serum calcium, serum magnesium, and plasma inorganic phosphate have been studied in normal, prepartum milked, and mastectomised cows, from 14 days prepartum until 14 days postpartum.

2. Normal cows showed a significant decrease in the level of serum calcium at calving. The fall commenced about 12 to 24 hours before calving, and continued postpartum to reach a minimum level at about 24 hours after calving. This was followed by a gradual return to normal levels at about 3 to 7 days postpartum. Limited observations on prepartum milked cows showed that the prepartum fall in serum calcium commenced earlier, and was more gradual, than the fall in cows milked only after parturition. The fall in serum calcium tended to co-incide with the increase in the level of secretion of calcium in the milk. In mastectomised cows the fall in serum calcium at parturition was greatly diminished in comparison with normal cows of the same age. It is concluded that the drain of calcium from the blood to the colostrum is a major factor in producing the fall in serum calcium at parturition in normal cows.

3. Normal cows showed a significant increase in the level of serum magnesium at parturition, broadly inverse to the decrease in serum calcium. The rise in serum magnesium was at its maximum at

about 8 hours postpartum. In prepartum milked cows the rise in serum magnesium tended to commence earlier, and to be more sustained, than in cows milked only postpartum. Only very slight and transient changes were observed in serum magnesium in mastectomised cows at parturition. The changes in serum magnesium in normal cows at parturition are regarded as being secondary to a primary change in serum calcium.

4. Normal cows showed a significant decrease in the level of plasma inorganic phosphate at parturition. The fall commenced some 12 to 24 hours before calving. Parturition was followed by an immediate reversal of the trend so that at 8 hours postpartum the level was not significantly lower than the prepartum level. This peak at 8 hours postpartum was succeeded by a secondary fall to 2 days postpartum, and then by a gradual return to normal levels. In first calf animals the postpartum rise was very marked. This was followed by a secondary fall so that from 3 to 7 days postpartum the levels in first calf animals were lower than in the other groups.

In prepartum milked cows the behaviour of plasma inorganic phosphate was similar to that of serum calcium i.e., there was a gradual fall during the prepartum period co-incident with the rise in the level of phosphate secretion in the milk. In mastectomised cows plasma inorganic phosphate showed a decrease at calving which was almost as great as that seen in normal cows of the same age. The postpartum period was marked in mastectomised cows by a rapid rise in the level of plasma inorganic phosphate so that levels above the prepartum values were attained.

The fall in plasma inorganic phosphate seemsto be more intimately associated with the act of parturition than it is with the onset of milk secretion, although the evidence does suggest that the latter plays some part in the lowering of inorganic phosphate at parturition.

5. In normal cows the changes in all three constituents became progressively more marked with each succeeding parturition.

6. During the investigation of normal cows at parturition two cows were encountered which appeared to be showing "borderline" symptoms of milk fever, with spontaneous recovery.

7. The results of the investigation support the hypothesis that milk fever can be regarded as a pathological exaggeration of the normal physiological changes associated with parturition. It has been concluded that the drain in calcium from the blood to the colostrum is a major factor in producing the fall in serum calcium at parturition in normal cows. If the hypothesis that milk fever is an exaggeration of the normal changes is accepted, it follows that the drain in calcium in the colostrum is a major factor in the aetiology of milk fever. It is obvious that this cannot be the only factor, and it is suggested that clinical milk fever may be the result of a progressive interference with the mobilisation of calcium such as would result from a progressive decrease in the availability of calcium in the body reserves.

SECTION 2.

STUDIES OF THE EFFECTS OF SOME MILK FEVER TREATMENTS ON NORMAL COWS.

PART 1.

The Effect of Injection of a) Calcium borogluconate and b) Acid sodium phosphate in Normal Parturient and Non-parturient Cows.

INTRODUCTION.

The parenteral injection of calcium salts is the treatment most widely employed in milk fever therapy. Calcium borogluconate is the salt almost exclusively used in this country, most commonly in doses of four ounces given intravenously, although there has been a tendency in recent years to use larger doses; many veterinary surgeons now use doses of up to six ounces, frequently three ounces intravenously and three ounces subcutaneously.

During the investigation of the effect of treatment of clinical cases of milk fever by the intravenous injection of calcium borogluconate, described in Section 3, it was observed that the injection of calcium was almost invariably followed by a rise in the level of plasma inorganic phosphate, and frequently by a fall in the level of serum magnesium. It was decided to see if these effects occurred in normal parturient and non-parturient cows.

Craige (1947b) stated that the intravenous injection of calcium gluconate was followed in parturient cows by a rise in the level of the blood inorganic phosphate, and in non-parturient cows by a fall in the level of this constituent. He claimed that in non-parturient cows artifically "alkalinised" by the oral administration of sodium carbonate, calcium injection was followed by a rise in the level of blood inorganic phosphate. He called this phenomenon "Phosphorus Reversal" and regarded it as evidence in favour of his conception of milk fever as a parturient alkalosis.

Allcroft (1947b) has studied the effects of intravenous and subcutaneous injections of calcium and magnesium salts in normal cattle. He did not study the effects of these salts on the blood phosphate levels.

In recent years Barker (1948) has advocated the use of acid sodium phosphate along with calcium borogluconate in the treatment of milk fever, and has claimed a reduction in the incidence of relapses following treatment by the use of this therapy. No reports have been found in the literature on the effects of injection of acid sodium phosphate in ruminants. As there is evidence (Binger 1917, Tisdall 1922) that in dogs, the injection of this salt results in a fall in the level of serum calcium, it was decided to study the effect of injections of the salt in normal parturient and non-parturient cows.

MATERIALS AND METHODS.

A study was made of the effect of the intravenous injection of a) four ounces of calcium borogluconate (equivalent to 8.00 g. Ca); and b) two ounces of acid sodium phosphate (equivalent to 8.40 g. Na and 11.30 g. P.) on the levels of serum calcium and magnesium and plasma inorganic phosphate in six parturient and six non-parturient cows. The cows used were normal animals on the Field Station of the Veterinary School.

The non-parturient cows used in this experiment had calved for a minimum period of six weeks. The experiments on parturient cows were carried out within a maximum period of fifteen hours after calving. It was felt that a slight staggering of the time of injection relative to calving, would be an advantage, as this would tend to cancel out the effect of any consistent changes in the levels of the blood fractions which might normally be occurring at this time. The changes resulting from the injections would therefore be more easily recognised. In the case of the six parturient cows given calcium injections the average period between calving and the start of the experiment was seven hours, with a maximum of fifteen hours and a minimum of five minutes. For the six cows given phosphate injections the average period was five hours, with a maximum of twelve hours and a minimum of one hour.

The salts were dissolved in approximately 16 ozs. of distilled water and were injected into the jugular vein by gravity, using a "flutter valve" injection apparatus. Injection occupied about five to ten minutes. The pre-injection blood sample was taken from the jugular vein and the solution injected before removal of the needle. The majority of the post-injection samples were taken from the mammary vein. If post-injection samples were taken from the jugular vein, the vein opposite to the one used for injection was taken, to avoid any risk of contamination of the needle by the injected solution.

Post-injection samples were taken at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours and 10 hours following injection.

The analyses were done as described in "General Materials and Methods"

RESULTS.

A. The Effect of the Intravenous Injection of four ounces Calcium Borogluconate on Normal Parturient and Non-Parturient Cows.

The complete data for serum calcium, serum magnesium and plasma

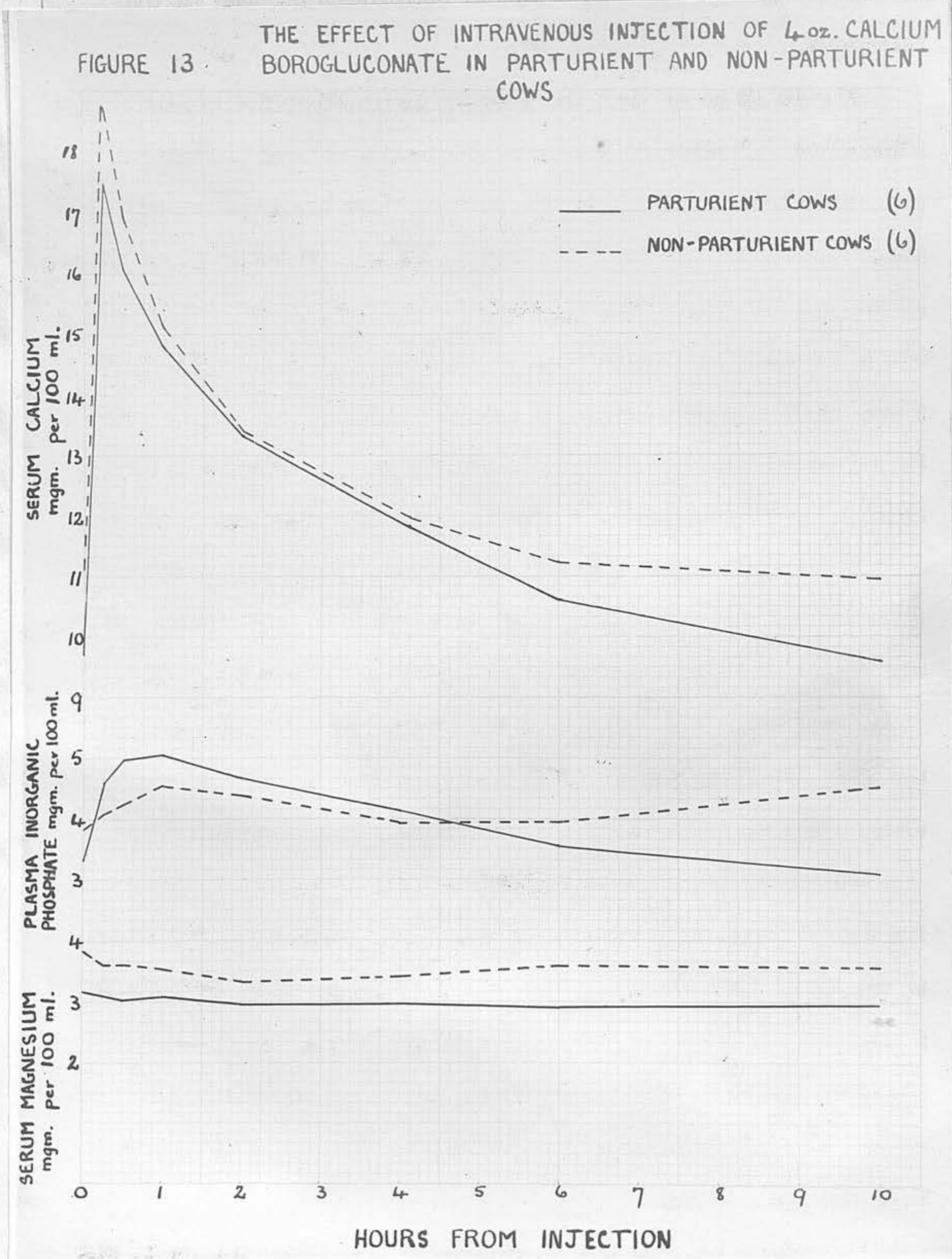


FIGURE 13.
THE EFFECT OF INTRAVENOUS INJECTION OF 4 oz. CALCIUM BOROGLUCONATE IN PARTURIENT AND NON-PARTURIENT COWS
PARTURIENT COWS (6)
NON-PARTURIENT COWS (6)
SERUM CALCIUM mgm. per 100 ml.
PLASMA INORGANIC PHOSPHATE mgm. per 100 ml.
SERUM MAGNESIUM mgm. per 100 ml.
18
17
16
15
14
13
12
11
10
9
5
4
3
4
3
2
0
1
2
3
4
5
6
7
8
9
10
HOURS FROM INJECTION

inorganic phosphate, resulting from this experiment are presented in Tables 21, 22 and 23 (Appendix 2, pp.xxi and xxii). The mean data for the two groups of cows are shown in Figure 13.

Figure 13 shows that the rise in serum calcium produced by the intravenous injection of 4 ozs. of calcium borogluconate was of approximately the same magnitude in parturient and non-parturient cows. The average level 15 minutes after injection was about 18 mgm. and the minimum 16.00 mgm. The subsequent fall was closely parallel in the two groups, with a slightly quicker fall in the parturient group. Normal levels were regained about six hours after injection; the effect of the injection, as judged by the serum calcium levels, had completely disappeared by ten hours post-injection.

There were, therefore, no apparent differences in between parturient and non-parturient cows in the behaviour of serum calcium, following the intravenous injection of calcium borogluconate.

There was a slight tendency for serum magnesium to fall following the injection of calcium borogluconate. Comparison of the means, however, showed that the fall was not statistically significant.

Following the injection of calcium borogluconate, there was a rise in the level of plasma inorganic phosphate in both parturient and non-parturient cows. The phosphate level reached its maximum on the average, one hour after injection, although there was a certain amount of variation in individual animals. After the peak at one hour the phosphate level fell to pre-injection levels at about the sixth hour.

The rise in the inorganic phosphate level was greater in the parturient than in the non-parturient group. Analysis of the data (t test) showed that, in parturient animals, there was a highly significant rise (at 1%) in the level at one hour post-injection. The levels at 15 minutes, 30 minutes and 2 hours post-injection were significantly higher (at 5%) than the pre-injection level.

The rise in inorganic phosphate in non-parturient cows was not so marked. Analysis showed that it was not significant.

Study of the data for individual animals (Table 23, Appendix 2, p. xxii) shows that in parturient cows the rise in the phosphate level was consistent and that it was very marked in some animals within a short time of the injection. Thus cow C 4 showed a rise from 1.29 mgm to 3.97 mgm. in 15 minutes, and to 4.56 mgm in 30 minutes. This was followed by a marked fall to 2.10 mgm. at one hour post-injection. Cow C 6 is of interest in that the pre-injection level of 6.14 mgm. is high for a parturient cow. This is probably due to the fact that, at the start of the experiment, the cow was calved seven hours, so that the phosphate level was probably at the peak of the normal postpartum rise. It may be noted that despite the high initial level of inorganic phosphate, this cow still showed a rise following calcium injection.

In the non-parturient group the tendency for a rise in the phosphate level was not so consistent. Two cows, C 8 and C 11 showed a transient fall in inorganic phosphate following calcium injection. One cow, C 12, showed an abnormally low inorganic phosphate level throughout the experiment.

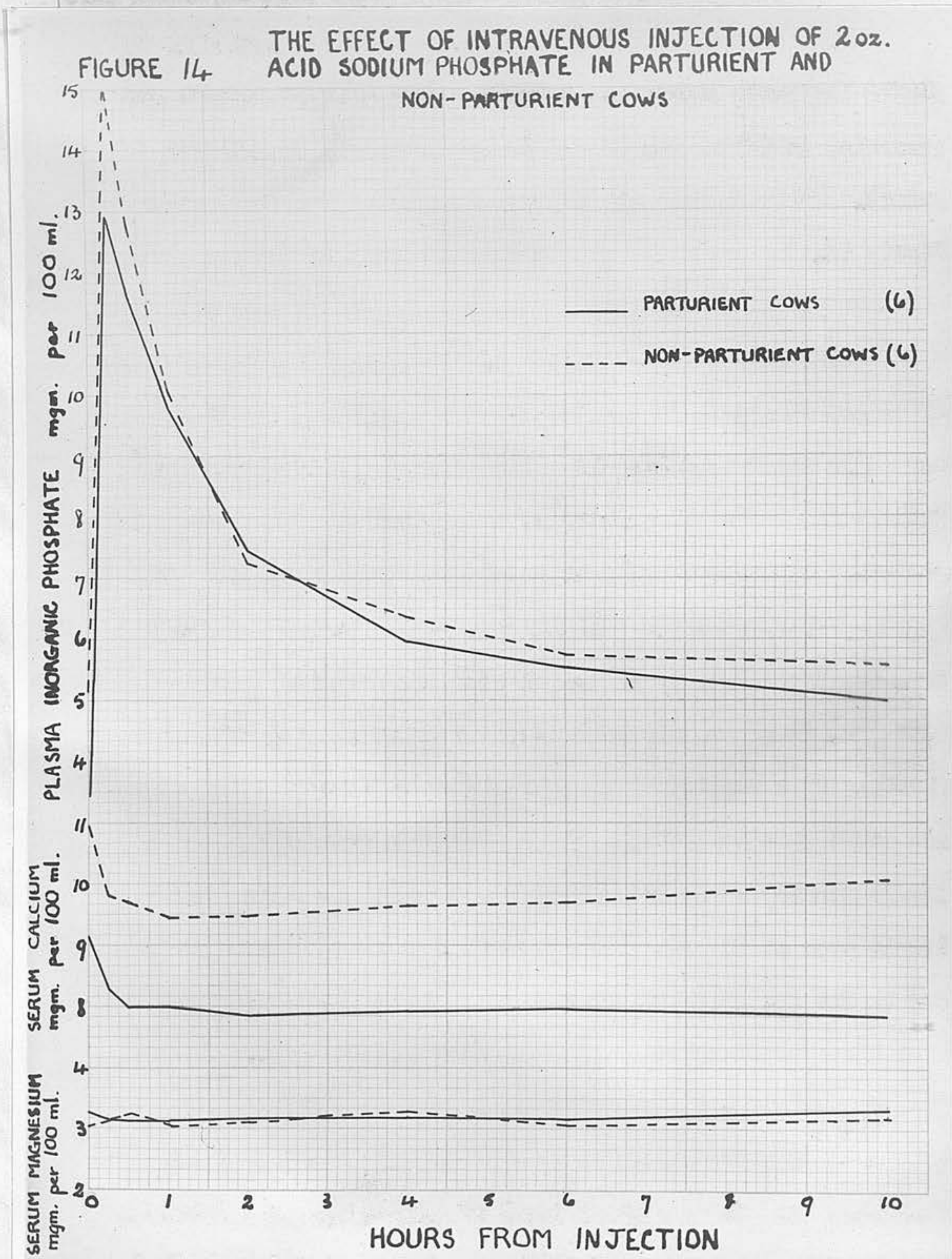


FIGURE 14
THE EFFECT OF INTRAVENOUS INJECTION OF 2oz. ACID SODIUM PHOSPHATE IN PARTURIENT AND NON-PARTURIENT COWS
PARTURIENT COWS (6)
NON-PARTURIENT COWS (6)
PLASMA INORGANIC PHOSPHATE mgm. per 100 ml.
SERUM CALCIUM mgm. per 100 ml.
SERUM MAGNESIUM mgm. per 100 ml.
HOURS FROM INJECTION
15
14
13
12
11
10
9
8
7
6
5
4
11
10
9
8
4
3
2
0
1
2
3
4
5
6
7
8
9
10

B. <u>The Effect of the Intravenous Injection of two ounces Acid Sodium Phosphate on Normal Parturient and Non-parturient Cows.</u>

The complete data for serum calcium, serum magnesium and plasma inorganic phosphate, resulting from this experiment are presented in Tables 24, 25 and 26 (Appendix 2, pp. xxii and xxiii). The mean data for the two groups of cows are shown in Figure 14.

Study of the data shows that the injection of 2 ozs. of acid sodium phosphate resulted in an immediate rise in the level of plasma inorganic phosphate. The rise was slightly more marked in the non-parturient cows, in keeping with the higher initial levels in these animals. The mean level at 15 minutes post-injection was 12.88 mgm. in parturient cows and 14.97 mgm. in non-parturient cows. The highest level attained was 17.50 mgm. in a non-parturient cow, and the smallest rise was to 8.77 mgm. in a parturient animal. The post-injection rise was followed by a rapid fall so that normal levels were regained about six hours post-injection. In parturient cows, however, the levels tended to remain slightly above their pre-injection levels even at 10 hours post-injection. This was undoubtedly due to the low initial levels in these cows, and to the fact that the level would normally be rising at this time.

Non-parturient cows had regained their pre-injection levels by 10 hours post-injection, with the exception of cow P 12 which showed an initial level of 4.15 mgm., and a level at 10 hours after injection of 7.27 mgm. This cannot be ascribed directly to the phosphate injection, however, as the levels at four and six hours post-injection were lower than the 10 hour post-injection level. Cow P 11 showed a very rapid fall in the phosphate level, with a level at 2 hours post-injection lower than the pre-injection level.

It appears, therefore, that the rise in the level of plasma inorganic phosphate produced by the injection of 2 ozs. acid sodium phosphate persists, on the average, for six hours. In some cows, however, elimination of the injected phosphate may be extremely rapid.

Serum Calcium.

The injection of acid sodium phosphate was followed, in both parturient and non-parturient cows, by a fall in the level of serum calcium. There was an immediate fall of approximately 1 mgm. per cent in serum calcium fifteen minutes after injection of the phosphate solution. The maximum mean fall was of the order of 1.50 mgm. and occurred at about one to two hours following injection. From one to ten hours post-injection the level of serum calcium remained fairly constant so that it was still below the pre-injection level at the end of the experiment.

Comparison of the means showed that there was a significant fall (at 5%) in serum calcium in parturient cows 15 minutes after injection, and a highly significant fall (at 1%) when the remainder of the periods were compared with the pre-injection levels. In non-parturient cows all the post-injection periods showed a highly significant fall (at 1%) in serum calcium when compared with the pre-injection levels.

Apart from the fact that, as is to be expected, the levels of serum calcium were lower in the parturient than in the non-parturient group, there were no significant differences between the groups in the behaviour of serum calcium.

Study of the data for individual animals shows that the tendency for a fall in serum calcium was consistent. The maximum depression produced by the phosphate injection in an individual cow was 2.35 mgm. in the non-parturient cow P 7. while the minimum was 1.10 mgm. in the parturient cow P 2 and the non-parturient cow P 8.

Serum Magnesium.

The injection of 2 ozs. acid sodium phosphate produced no consistent or significant change in serum magnesium in parturient or non-parturient cows.

DISCUSSION OF RESULTS.

Injection of Calcium Borogluconate. The results indicate that the rise in serum calcium produced by the intravenous injection of four ounces of calcium borogluconate is of very short duration, in both parturient and non-parturient cows. In both groups the serum calcium had returned to its pre-injection level some six to ten hours following injection. These results agree with those of Allcroft (1947) and, in this respect, with those of Craige (1947) who found that the serum calcium had returned to its pre-injection level by 24 hours after injection, no samples having been taken between 4 and 24 hours after injection. The transient effect of intravenous injection suggests that the practice of supplementing the intravenous injection of calcium with a subcutaneous injection, as is practiced by many veterinary surgeons in the treatment of milk fever, is a sound one.

The injection of calcium was followed by a very slight fall in the level of serum magnesium, as was found by Allcroft

(1947). This is another example of the reciprocal behaviour between the two ions.

The injection of calcium was followed in parturient cows by a distinct increase in the concentration of plasma inorganic phosphate. In non-parturient cows this was less well marked. These results are in disagreement with those of Craige (1947) who claims to have observed a rise in plasma inorganic phosphate in parturient cows, and the reverse in non-parturient cows, although study of the data he presents makes it doubtful if his conclusions are justified. For instance, his observations on parturient cows were made at times varying between 11 days pre-partum and 20 days postpartum. This seems to presume a rather wide interpretation of the parturient period. The alleged difference between parturient and non-parturient cows, which he designates "phosphorus reversal", is one of the main bases for his conception of milk fever as a manifestation of alkalosis, as he claims to have induced a rise in the plasma inorganic phosphate following calcium injection in non-parturient cows which had been given sodium carbonate by oral administration. The finding, in this study, that only two non-parturient cows showed very slight decreases in inorganic phosphate following calcium injection, and that the other four showed fairly distinct increases, contradicts one of Craig's main arguments in favour of the alkalosis theory.

It is well known that the concentration of circulating blood calcium is related to the concentration of circulating plasma protein and plasma phosphate, and various attempts have been made to derive equations to express these relationships (Sholl 1939). It seems possible that the rise in blood phosphate following calcium injection is the result of mobilisation of phosphate in an attempt to restore the calcium - protein - phosphate equilibrium. The greater increase in the plasma inorganic phosphate in parturient cows following calcium injection is most probably due to the fact that the initial levels are lower, and to the fact that the level tends to be increasing anyhow in normal parturient cows in the immediate postpartum period i.e., at the time when these experiments were being conducted.

Injection of Acid Sodium Phosphate. The rise in the level of plasma inorganic phosphate produced by the intravenous injection of two ounces acid sodium phosphate was completely dissipated by six hours after injection. The result is therefore similar to that found in the study of the effect of injection of four ounces calcium borogluconate on the serum calcium level.

The most interesting result of this part of the investigation was that the injection of acid sodium phosphate was followed by a reduction in the concentration of serum calcium. The decrease in serum calcium was of the order of 1.50 mgm. per cent. This is a smaller fall than that produced by Binger (1917) in his experiments on dogs. He found that doses equivalent to 150 mgm. P per Kg. reduced blood calcium from about 10 mgm. to about 6 mgm. per cent. The differences can be explained in terms of the difference in dosage. In the present experiments the dosage (assuming the weight of the cows to be about 400 Kg.), was approximately at the rate of 18 mgm P per Kg. Another factor which may help to explain the difference is that, as was pointed out by Greenwald (1931), Binger's results can be accounted for largely on the basis of haemo-dilution; in some of his experiments he injected quantities of solution almost equal to the blood volume of the dog. Haemo-dilution will not explain the fall in ser um calcium in this study as the quantity of injected solution (approximately 16 ozs.) is insignificant in relation to the blood volume of the cow.

Greenwald (1931) also points out that the decrease in serum calcium which follows acid sodium phosphate injection, is not necessarily due to any specific action of the P ion. He had demonstrated (Greenwald 1918) that injection of sodium sulphate produced quite as marked decreases in the concentration of blood calcium as did injection of sodium phosphate. Tisdall (1922) had also concluded that the sodium ion was the more important one, and that the Na/Ca ratio was more important than the Ca/P ratio in the production of tetany following injection of sodium phosphate salts. Schmidt and Greenberg (1935) in discussing Greenwald's objections to Binger's work, suggest that, despite his criticism, there is evidence of a reciprocal relationship between calcium and inorganic phosphate. They instance the inverse relationship between the two in parathyroidectomised dogs, and in human nephritis. The fact that there may be a reciprocal relationship between the two ions under certain conditions does not invalidate Greenwald's suggestion that the Na ion may be equally as important as the P ion in producing the fall in serum calcium which follows injection of acid sodium phosphate.

So far as the present investigation is concerned, therefore, care must be taken not to attribute the fall in serum calcium directly and solely to the increased concentration of the blood phosphate. It seems equally possible, in view of Greenwald's work, that it is related to the increased concentration of the blood sodium, or to a combination of the increased concentration of both ions.

The reduction in blood calcium would seem to be an argument against the use of acid sodium phosphate as a supplement to calcium therapy in the treatment of milk fever. On the other hand, the decrease is slight with the dosage employed. Further, this argument ignores any possible beneficial effect which may follow the raising of the blood phosphate level.

PART 2.

Studies on the Effect of Udder Inflation on The Serum Calcium and Magnesium and Plasma Inorganic Phosphate in Normal Cows.

INTRODUCTION.

The udder inflation treatment of milk fever developed, as has been described in the general Introduction to this thesis, in a somewhat fortuitous manner from the udder infusion treatment evolved by Schmidt at the close of the 19th century. The dramatic results obtained with udder inflation in the treatment of milk fever, while they stimulated a great deal of speculation as to its mode of action, have not led to much experiment investigation of its effects.

Dryerre and Greig (1925) in the paper in which they originally suggested that milk fever might be related to parathyroid dysfunction, suggested that udder inflation "by initiating powerful afferent stimuli reflexly causes an excessive secretion of adrenaline, and possibly prevents further calcium loss. The adrenaline now exerts its power of hastening oxidation, the toxins are burned up and the blood pressure raised." Following on the demonstration by Little and Wright (1925) and Dryerre and Greig (1928) that milk fever was accompanied by a hypocalcaemia, it was shown by Dryerre and Greig (1928) that in clinical cases of milk fever udder inflation was followed by an increase in the blood calcium. They also showed that udder inflation caused a rise in the blood calcium in normal lactating ewes. Fish (1929) showed that in milk fever cases udder inflation produced a rise in the inorganic phosphate fraction of the blood. This has been confirmed by Smith and Niedermeier (1948) and Niedermeier and Smith (1950). It is now well established that inflation of the udder will raise the blood calcium and phosphate in animals affected with milk fever.

Many veterinary surgeons have found that cases of milk fever which do not respond to calcium injection therapy, will often respond to udder inflation. The exact reason for this has not been apparent.

Robertson et al. (1948) and Robertson (1949) found that a limited number of cases of milk fever occurring on one farm responded to udder inflation after showing an unsatisfactory response to calcium therapy. Analysis of blood samples from these cases suggested that the beneficial effects of udder inflation might be due to its ability to cause a consistent rise in the blood phosphate level as well as in the blood calcium level. Results obtained in the treatment of clinical cases of milk fever in the present investigation tended to support this finding.

It was felt that a detailed study of the effects of udder inflation on the levels of calcium, magnesium, and inorganic phosphate in normal cows might be of help in explaining why udder inflation may be successful when calcium injection therapy fails. It was also felt that such a study might be of help in elucidating the exact mode of action of udder inflation.

It has generally been assumed that the main action of udder inflation in raising the blood calcium and phosphorus is in stopping the secretion of these elements and in forcing them back into the circulation from the udder. This theory was lent support by the findings of Hayden (1927) and of Fish (1928) that udder inflation is followed by the appearance in the blood of lactose, a constituent of milk not normally present in the blood. The theory has , however, been criticised. For example Boddie (1949) has suggested that cows which have been recumbent for some hours with clinical milk fever have presumably stopped secreting milk. He postulated that udder inflation might produce its beneficial effects by the pressure on nerve endings in the udder producing remote effects through the release of hormones. A similar theory has been suggested by Green (1939). Smythe (1930) has postulated a mammary hormone, the release of which following udder inflation causes a rise in blood calcium. These nerve reflex and hormonal theories, while possible, remain pure theories as yet entirely unsupported by experiment evidence.

In this investigation it was decided to test the hypothesis that udder inflation acts by forcing calcium and phosphate back into the circulation. It was argued that if this theory were true, inflation of the udder while that organ was distended with milk should result in a greater increase in blood calcium and phosphate than inflation of an udder which had been emptied of milk as completely as possible. Moreover, inflation of the udder of a dry non-lactating cow should result in no increase, or a very slight increase, in the level of blood calcium and phosphate if the rise in these elements is due to either stopping of milk secretion, or pushing back of calcium and phosphate into the circulation.

As udder inflation has also been used in the theatment of grass tetany, a condition accompanied by a hypomagnesaemia, it was felt that a study of the effect of udder inflation on the blood magnesium levels in normal cows might be of interest.

Materials and Methods.

The effect of udder inflation on the serum calcium and magnesium and plasma inorganic phosphate was studied on 18 normal cows of the Ayrshire breed. All of the cows were at their third or subsequent parturition.

Six cows were parturient. In these the inflation of the udder was carried out as soon as possible after calving. The maximum period which elapsed between calving and the inflation of the udder was fifteen hours. These cows were not milked before inflation.

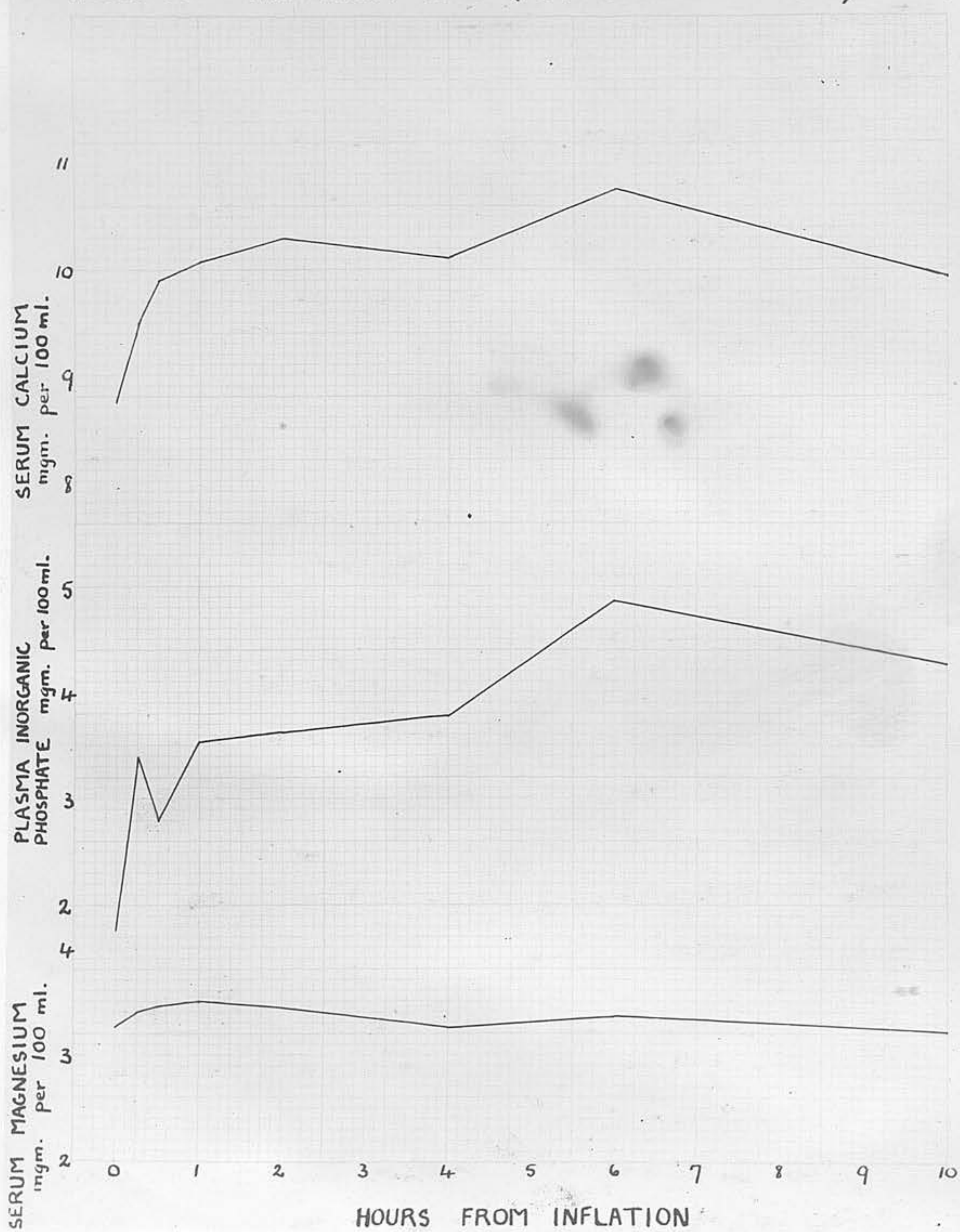
Twleve cows were non-parturient and had calved for a minimum period of six weeks. Of these 8 were lactating and in 4 of these the udder was inflated at the usual time of milking without milking out the udder before inflation. In the other 4 non-parturient lactating cows the udder was milked out as completely as possible immediately before inflation. The remaining 4 cows were non-parturient, non-lactating animals.

In all cases the udder was inflated with air. The apparatus used consisted of a hand pump connected to a teat cannula by means of rubber tubing, with an air filter interposed between the pump and the teat cannula.

In each case the udder was inflated until it had attained the amount of distension normall y employed in the treatment of clinical cases of milk fever by this method. After. inflation of eachquarter of the udder the teat was tied with bandage to prevent the escape of air. The bandages were removed about one hour after the end of inflation. There was usually no escape of air at this time.

Blood samples were collected immediately before

FIGURE 15 THE EFFECT OF UDDER INFLATION IN NORMAL PARTURIENT COWS (MEAN DATA FOR 6 COWS)



inflation, and at 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours and 10 hours after the end of inflation. All blood samples in this experiment were taken from the mammary veins.

RESULTS.

A. Udder Inflation in Parturient Cows.

The complete data for serum calcium, serum magnesium and plasma inorganic phosphate, resulting from this experiment are presented in Table 27 (Appendix 2 p. xxiv.) The mean data for the six cows are shown Figure 15.

The data show that udder inflation resulted in parturient cows in an immediate increase in the level of serum calcium. The rise was evident within 15 minutes of inflation and attained its maximum between 2 and 6 hours after inflation.

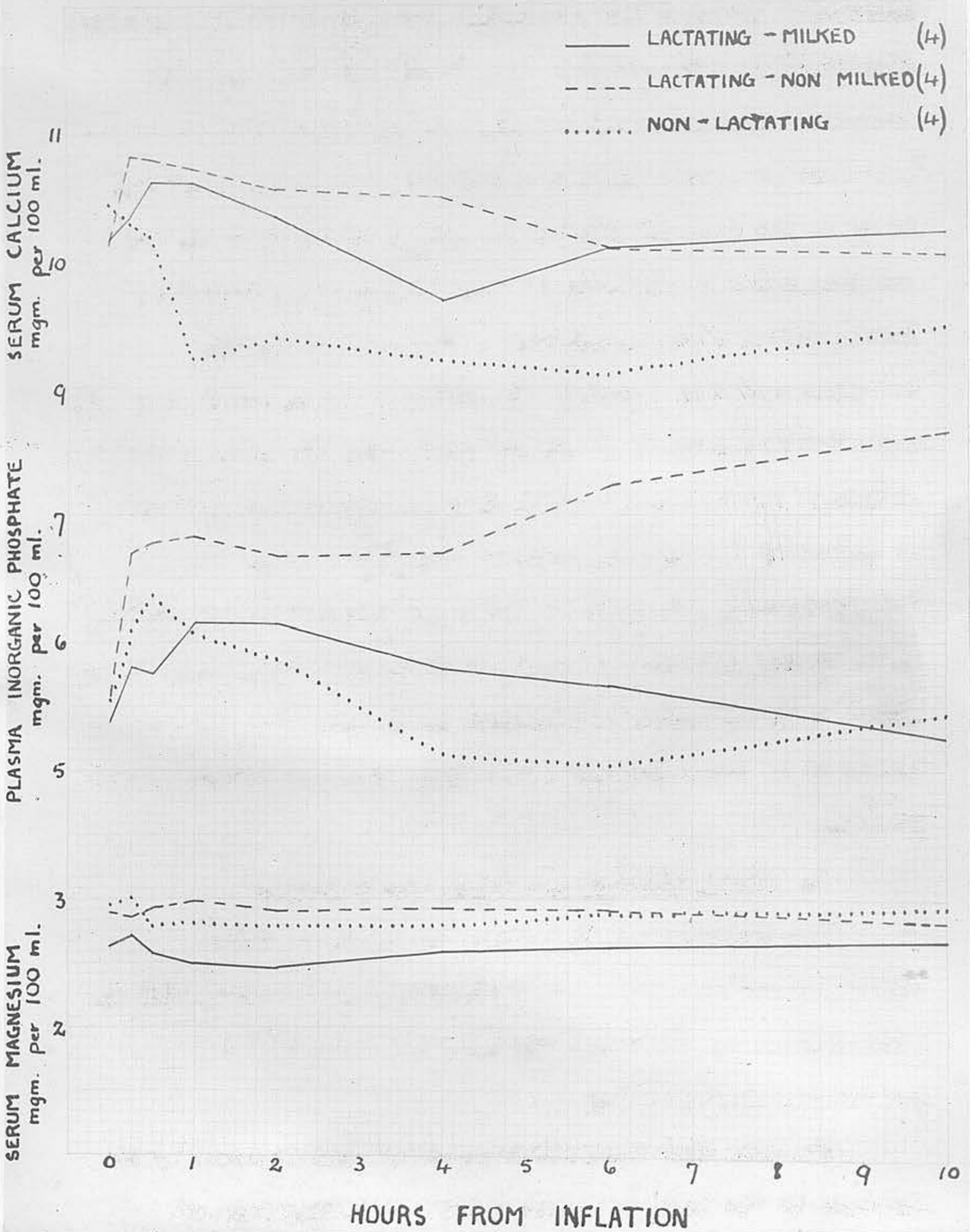
Comparison of the mean pre-inflation level with the mean values after inflation showed that the mean values at 15 minutes, 30 minutes, 1 hour and 2 hours after inflation were significantly higher (at 5%) than the mean pre-inflation level. The differences at 4 hours, 6 hours and 10 hours were highly significant (at 1%).

The maximum increase was shown by cow U 5 which showed a rise from a pre-inflation level of 7.80 mgm. to 10.45 mgm. at 2 hours after inflation.

Although there was a tendency for serum magnesium to rise following inflation, statistical analysis showed that the rise was not significant.

There was a tendency for plasma inorganic phosphate to increase after udder inflation. Analysis of the differences between the mean pre-inflation level and the mean post-

FIGURE 16 THE EFFECT OF UDDER INFLATION ON NON-PARTURIENT COWS



inflation levels showed that at 15 minutes there was a significant increase (at 5%), at 1 hour and 6 hours there was a highly significant increase (at 1%); the levels at 30 minutes, 2 hours, 4 hours and 10 hours after inflation were not significantly higher than the pre-inflation levels. This analysis suggests that there was considerable variation in the behaviour of plasma inorganic phosphate following inflation. Study of the data for individual animals supports this. For example, Cow U 1, which had a remarkably low pre-inflation level, showed a marked increase immediately following inflation and then a sudden fall at 30 minutes, and subsequently a fluctuating level of inorganic phosphate. In cow U 3 the phosphate behaved in a similar manner, except that it showed an extremely low level at 4 hours after inflation. In cow U 4 the level at 10 hours was below the pre-inflation level, while in all the other cows the 10 hour post-inflation level was higher than the pre-inflation levels. In cows U 5 and U 6 the levels at 10 hours were markedly higher than the pre-inflation levels.

B. Udder Inflation in Non-parturient Cows.

The complete data for the three groups of non-parturient cows are shown in Tables 28, 29 and 30 (Appendix 2, pp. xxv, xxvi and xxvii). The mean data are presented graphically in Figure 16.

The data show that udder inflation was followed by an increase in the level of serum calcium in both groups of lactating cows. The increase was, however, slight and transient. The maximum elevation was attained in the majority of cases within 15 to 30 minutes after inflation, and pre-inflation levels were regained about 4 to 6 hours after inflation.

Analysis of the data for the two groups of lactating cows showed that the increase in serum calcium was not statistically significant.

Although the cows which were not milked before inflation did show a greater increase in serum calcium than the cows which were milked out before inflation, the differences are small and are not statistically significant for the numbers of cows involved.

In non-lactating cows udder inflation was followed by a consistent decline in the level of serum calcium. The fall in serum calcium commenced immediately after inflation and tended to persist until the end of the experiment. This fall in serum calcium was significant (at 5%) when the mean values for the periods 2 hours and 4 hours post-inflation were compared with the mean pre-inflation level.

Udder inflation had no consistent or significant effect on serum magnesium in any of the groups of normal non-parturient cows.

Study of the data shows that udder inflation was followed in all groups by an increase in the level of plasma inorganic phosphate. In lactating cows milked before inflation and in non-lactating cows the rise was slight. In the former the maximum rise was attained between 1 and 2 hours after inflation, and in the latter the maximum rise occurred between 15 and 30 minutes after inflation.

Analysis of the data for these two groups (i.e., lactating milked and non-lactating) showed that the changes were not statistically significant, when the post-inflation levels were compared with the pre-inflation levels.

In the lactating cows not milked before inflation the rise in inorganic phosphate was more marked and tended to persist until the end of the experimental period.

Analysis of the data for this group showed that there was a highly significant increase in the level of inorganic phosphate 15 minutes after inflation. The increases shown at 2 hours and 6 hours after inflation were significant (at 5%).

Lactating cows not milked before inflation, therefore, showed a significant increase in plasma inorganic phosphate following inflation, while lactating cows milked before inflation and non-lactating cows showed an increase which was not statistically significant.

DISCUSSION OF RESULTS.

The only previous investigation of the effect of udder inflation on the level of serum calcium in normal animals was by Dryerre and Greig (1928) who studied the effect of inflation in 4 normal lactating ewes. They found that inflation caused a rise of about 10% in blood calcium. The results of the present study support this finding.

The most interesting aspect of the results is that while udder inflation was followed by an increase in the concentration of serum calcium in parturient cows and in lactating cows, in non-lactating cows it was followed by a decrease in serum calcium.

The fact that there was a rise in lactating cows and not in non-lactating cows suggests that the rise in serum calcium following udder inflation is due either to the stopping of milk secretion or to the resorption of calcium from the mammary gland, or to a combination of these effects. There seems to be no doubt that udder inflation will stop milk secretion. Peterson and Rigor (1932) and Garrison and Turner

(1936) have shown that milk secretion is practicallyy completely inhibited when air pressures equivalent to 25 to 40 mm. of Hg are maintained in the udder. The pressure usually attained in udder inflation is 60 to 70 mm. Hg (Niedermeier and Smith 1950).

Two facts seem to suggest that actual resorption of calcium takes place. Firstly, the rise in blood calcium occurs within a very short time of inflation e.g. there is an appreciable increase within 15 minutes of inflation. This cannot be attributed entirely to the stopping of milk secretion. Secondly, the rise in serum calcium is more marked in parturient cows than in non-parturient lactating cows. This might be explained by the fact that colostrum is much higher in calcium content than is normal milk and that a greater rise was to be expected if this resorption hypothesis is accepted. The fact that the rise in serum calcium was greater in lactating cows not milked before inflation than in lactating cows milked before inflation also suggests that resorption from the mammary gland is taking place.

The results obtained for plasma inorganic phosphate are similar to those for serum calcium and also tend to suggest that resorption from the mammary gland is taking place. The fact that in non-parturient lactating cows not milked before inflation, the phosphate level was still increasing at the end of the experiments, suggests that the stopping of milk secretion is also influencing the blood picture.

In the light of the above there would seem to be little need to postulate any special hormonal or nerve reflex mechanisms in explanation of the blood changes observed after udder inflation.

SUMMARY AND CONCLUSIONS.

1. The intravenous injection of four ounces of calcium borogluconate in both normal parturient and non-parturient cows resulted in an immediate rise in the level of serum calcium. This was followed by a rapid fall so that normal levels were regained some six hours after injection. There was a tendency for serum magnesium to fall slightly following calcium injection. In both parturient and non-parturient cows there was a rise in the level of plasma inorganic phosphate following calcium injection. The peak of the rise occurred about an hour after injection; this was followed by a return to pre-injection levels at about the sixth hour. The increase in the phosphate was more marked in parturient than in non-parturient cows.

It is concluded that, in view of the transient effect of intravenous injection on the serum calcium level, the practice of supplementing intravenous calcium therapy by a subcutaneous injection, in the treatment of clinical milk fever, is a sound one. The rise in plasma inorganic phosphate following calcium injection is attributed to an attempt to restore the normal calcium-protein-phosphate equilibrium. The greater increase in plasma inorganic phosphate in parturient cows is attributed to the lower initial levels, and to the fact that the phosphate level tends to be increasing anyhow in normal parturient cows at the time at which the experiments were conducted.

2. The injection of two ounces of acid sodium phosphate resulted in an immediate rise in the level of plasma inorganic phosphate, with a return to pre-injection levels about six hours after injection. There was a reduction of approximately 15 per cent in the concentration of serum calcium following acid sodium phosphate injection. No consistent change in the level

of serum magnesium was observed.

It is suggested that the reduction in the concentration of serum calcium may be the result of the increase in phosphate ions, the increase in sodium ions, or a combination of these effects. The reduction in serum calcium would seem to be an argument against the use of acid sodium phosphate in the therapy of milk fever. This may, however, be counteracted by a beneficial effect from the raising of the blood phosphate level.

3. Udder inflation resulted in an increase in the concentration of serum calcium in parturient cows, and in non-parturient, lactating cows. In non-parturient, non-lactating cows a slight but consistent fall in serum calcium followed udder inflation. A rise in the level of plasma inorganic phosphate followed udder inflation in all groups of cows with the smallest rise in the non-lactating group. The increases in the concentration of serum calcium and plasma inorganic phosphate were more marked in parturient than in non-parturient cows, and more marked in non-parturient, lactating cows not milked before inflation than in non-parturient lactating cows milked before inflation. No consistent changes in serum magnesium were observed following udder inflation in any of the groups of normal cows.

It is concluded that udder inflation acts essentially by the stopping of milk secretion and by causing the resorption of calcium and phosphate from the milk. The greater increases in serum calcium and plasma inorganic phosphate in parturient than in non-parturient cows can be explained by the greater concentration of calcium and phosphate in colostrum than in normal milk.

There seems to be no need to postulate hormonal or or nerve reflex mechanisms to explain the action of udder inflation.

SECTION 3.

STUDIES OF CLINICAL CASES OF MILK FEVER WITH PARTICULAR REFERENCE TO RESPONSE TO TREATMENT.

INTRODUCTION.

The observation of Little and Wright (1925) that milk fever is accompanied by a hypocalcaemia, was subsequently confirmed by numerous workers. As a result, calcium substitution therapy came to be employed. This treatment was often so spectacular in its action, and so generally satisfactory in its results, that there was a tendency to assume that the hypocalcaemia was the only important biochemical manifestation of the disease. Moreover, the success of the treatment tended to discourage any detailed biochemical investigation of its effects.

Quite soon after the work of Little and Wright it was shown by Sjollema (1928) and by Fish (1929) that the hypocalcaemia of milk fever was accompanied by a hypophosphataemia. This also has been amply confirmed. Until recent years, however, it has been assumed that the hypophosphataemia was relatively unimportant, and there has been little discussion on the significance of the low blood phosphate levels in milk fever. Fish (1930) had suggested that the hypophosphataemia and hyperglycaemia in milk fever might have a bearing on the muscular paralysis observed, as a result of disturbance of the hexosephosphate relationships. Accordingly, he suggested that phosphate as well as calcium should be used in milk fever therapy, and advocated the use of a solution of calcium glycerophosphate as a therapeutic agent. This therapy was never used on any wide scale and little attention was paid to the role of the blood phosphates in milk fever until Barker (1948) described the use of acid sodium phosphate as a supplement to calcium borogluconate therapy. He claimed to have observed fewer relapses and fewer cases of persistent paresis following combined calcium and phosphate treatment, than following calcium therapy alone, but he has published no data in support of these assertions.

There is very little published data on the effect of treatment with calcium on the blood phosphate level. Observations by Robertson (1949), and by Robertson et al. (1948) suggested that where a satisfactory response to calcium therapy was obtained, there was an increase in the level of blood inorganic phosphate after treatment. Conversely, cases which did not respond satisfactorily to treatment, tended to show a persistently low blood phosphate level. Treatment by udder inflation resulted in a consistent rise in the level of plasma inorganic phosphate as well as in the serum calcium. Robertson suggested that the success of udder inflation in cases which had failed to respond to calcium therapy might be due to its ability to cause a consistent rise in the blood phosphate level.

The part played by serum magnesium in milk fever has aroused considerable interest. Sjollema and Seekles (1930), and Sjollema and Seekles (1932) showed that in milk fever the serum magnesium is above the level found in normal cows. Kloubek (1932), Pribyl (1933) and Schulof (1933) regarded milk fever as a form of magnesium narcosis, due to the lowering of the Ca/Mg ratio. Hibbs (1950) has claimed that the narcotic effect of magnesium is elicited both by the raising of the effect of inflation on the blood phosphate level. He found that inflation produced a slight initial fall in the phosphate level and then a gradual rise to normal which was more rapid than the return to normal of the blood calcium. Niedermeier and Smith (1950) studied the effect of udder inflation in seven cases of milk fever. In their cases recovery took place when the serum calcium had reached about 6 mgm. per cent; plasma inorganic phosphate was still below the normal range at the time of recovery. They suggested that the relative levels of calcium, magnesium and phosphorus were more important than the actual blood level of any one constituent.

The present study was undertaken in an attempt to clarify the significance of the part played by the serum magnesium and plasma inorganic phosphate in the symptomatology of milk fever, and in the nature of the response to treatment. Accordingly, it was decided to take pre-treatment blood samples from clinical cases of milk fever, and, where possible, to take frequent blood samples following treatment so that the behaviour of serum calcium, serum magnesium and plasma inorganic phosphate during the recovery phase could be ascertained.

MATERIALS AND METHODS.

Data have been collected from 96 cows affected with milk fever. Twenty-eight cows were affected more than once at one parturition so that a total of 128 pre-treatment blood samples were obtained. One hundred of these cases were attended by the author personally and occurred in the clinical practice of the veterinary school. The remaining 28 cases occurred in the clinical practice of the veterinary school but were not attended by the author, or were cases from which blood samples were sent to the laboratory by practising veterinary surgeons. The cows were predominantly of the Ayrshire breed but a few Jersey cross, Shorthorn cross, and Friesian cows are included.

Blood Sampling. Blood samples were taken immediately before treatment usually from the jugular vein as this vein was normally used for injection. Post-treatment blood samples were taken as frequently as poss ible. In nearly all cases, seen by the author, a blood sample was taken about 15 to 30 minutes after treatment. As the cases were all routine ones encountered in clinical practice it was not always possible to take as frequent blood samples after treatment as was desired. The frequency of post-treatment sampling depended largely on the co-operation of the owner and on the accessibility of the farm to the laboratory. Accordingly, there is great variation in the numbers of post-treatment samples taken, and in the time relative to treatment at which they were taken. This has rendered difficult the orderly presentation of the data in tabular form.

Methods of Treatment. For the purpose of the study only two forms of treatment were used, viz. the intravenous injection of four ounces of calcium borogluconate, and the inflation of the udder with air.

Statistical Analysis. In the comparison of means two methods have been used. 1) Student's t test in the comparison of small groups (Mainland 1938), and 2) In groups containing large numbers of samples (over thirty) a comparison has been made using the Standard Error of the Difference between Means (Mainland 1938). Where the difference between groups was more than three times its Standard Error the difference has been regarded as highly significant (at 1%); where the difference was greater than twice its Standard Error the difference has been regarded as significant (at 5%).

RESULTS.

a) Levels of Serum Calcium, Serum Magnesium and Plasma Inorganic Phosphate before Treatment. The complete pre-treatment data are presented in Table 31 (Appendix 3 pp. xxviii, xxix and xxx).

Table 22 gives the mean values for 1) All pre-treatment levels (128 cases), 2) Levels before first treatment (96 cases), and 3) Levels before second or subsequent treatments (32 cases).

TABLE 22.

Milk Fever Cases.

Mean Blood Levels Before Treatment.

Mgm. per cent.

	Calcium		Magnesium		Inorganic P.	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
a) All Treatments (128)	5.03	0.120	3.21	0.068	1.62	0.085
b) 1st Treatment (96)	4.82	0.120	3.19	0.085	1.55	0.099
c) Subsequent Treatments (32)	5.71	0.299	3.25	0.109	1.81	0.164
Difference c) - b)	0.89	0.272	0.06	0.170	0.26	0.192
Significance	1%		N.S.		N.S.	

N.S. = Not Significant.

It will be seen that analysis of the differences between groups showed that there was a highly significant difference between the level of serum calcium before first treatment and the level of serum calcium before second or subsequent treatment. There were no significant differences between the groups in the values of serum magnesium or plasma inorganic phosphate.

It is obvious that the levels of serum calcium and plasma inorganic phosphate are significantly lower in milk fever cases than they are in normal cows at parturition. The levels of serum magnesium in the cases of milk fever encountered in this study did not appear to differ greatly from those in normal parturient cows. A comparison was therefore made between the mean pre-treatment levels of serum magnesium in milk fever cases, and the mean level in the 32 normal parturient cows studied in Section 1. The mean serum magnesium level in milk fever cases was 3.21 mgm. per cent. The mean serum magnesium level in the 32 normal cows at eight hours post-partum i.e. at the time of the maximum mean increase, was 3.17 mgm. There was no significant difference between these means. Since milk fever occurs predominantly in third or subsequent calf animals a fairer comparison is between the milk fever levels and the levels at eight hours postpartum in third and fourth calf animals. The mean for third and four calf cows at eight hours postpartum was 3.34 mgm. per cent i.e., higher than the mean level in milk fever cases. The difference was, however, not significant. The level of serum magnesium in milk fever, therefore, did not differ significantly from the level in normal parturient cows.

b). <u>Pre-treatment Blood Levels in Relation to Severity of Clinical Signs.</u> Each attack of milk fever has been classified as mild, moderate or severe according to the nature of the clinical signs. Complete clinical histories were available for 116 treatment attacks of milk fever in 85 cows. The classification into mild, moderate or severe was made by study of the clinical history without reference to the blood picture: The general guiding principle was that cases which were still on their feet at the time of treatment were classified as "Mild"; cases in sternal recumbency at the time of treatment were classified as "moderate", and cases in lateral recumbency were classified as "severe": The only exceptions to this rule were cases which were in sternal recumbency but which were described in the clinical history as being "alert" or "showing mild sumptoms." These cases were classified in the "mild" category. The classification of cases is shown in Table 31 (Appendix 3, pp. xxviii, xxix and xxx).

A comparison was made of the pre-treatment levels of serum calcium, serum magnesium and plasma inorganic phosphate in the three different groups. The mean levels in the three groups are shown in Table 23.

TABLE 23.

Milk Fever Cases

Pre-treatment Blood Levels in Relation to Clinical Nature.

Mgm. per cent.

	Calcium		Magnesium		Inorganic P.	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Mild (45)	5.95	0.201	3.20	0.096	2.03	0.148
Moderate (55)	4.62	0.138	3.26	0.111	1.45	0.098
Severe (16)	3.82	0.184	3.30	0.222	1.10	0.170

Significance of Differences Between Groups.

	Mean S.E. Sig.	Mean S.E. Sig.	Mean S.E. Sig.
Mild and Severe	2.13 0.272 1%	0.10 0.242 N.S.	0.93 0.225 1%
Mild and Moderate	1.33 0.244 1%	0.06 0.147 N.S.	0.58 0.178 1%
Moderate and Severe	0.80 0.230 1%	0.04 0.248 N.S.	0.35 0.194 N.S.

N.S. = Not Significant.

Nos. of cases in each group are shown in parenthesis.

It will be seen that analysis of the differences between groups showed that the differences in the levels of serum calcium were highly significant between all three groups. There were no significant differences between groups in the levels of serum magnesium. In the case of plasma inorganic phosphate, there was a highly significant difference between cases showing mild symptoms and cases showing severe symptoms, and between cases showing mild symptoms and cases showing moderate symptoms.

It would appear, therefore, that the clinical picture was influenced mainly by the levels of serum calcium and plasma inorganic phosphate. The level of serum magnesium did not appear to have an important influence on the severity of the clinical signs.

It may be noted that, while there was a general tendency for the severity of the clinical signs to be significantly related to the extent of the depression in serum calcium and plasma inorganic phosphate, there were a few exceptions to the rule. For example, Cow 64 (Table 32, Appendix 3, pp. xxviii, xxix and xxx) showed a serum calcium level of 2.90 mgm. and a plasma inorganic phosphate level of 1.10 mgm. although she was on her feet at the time of treatment. Cow 47 showed moderately severe symptoms of clinical milk fever with a serum calcium level of 8.13 mgm. and an inorganic phosphate level of 2.35 mgm. Cow 45 showed severe clinical signs of milk fever with a serum calcium level of 5.25 mgm. and an inorganic phosphate level of 0.80 mgm. It is evident, therefore, that the serum calcium and plasma inorganic phosphate levels were not the sole factors in determining the severity of the clinical picture.

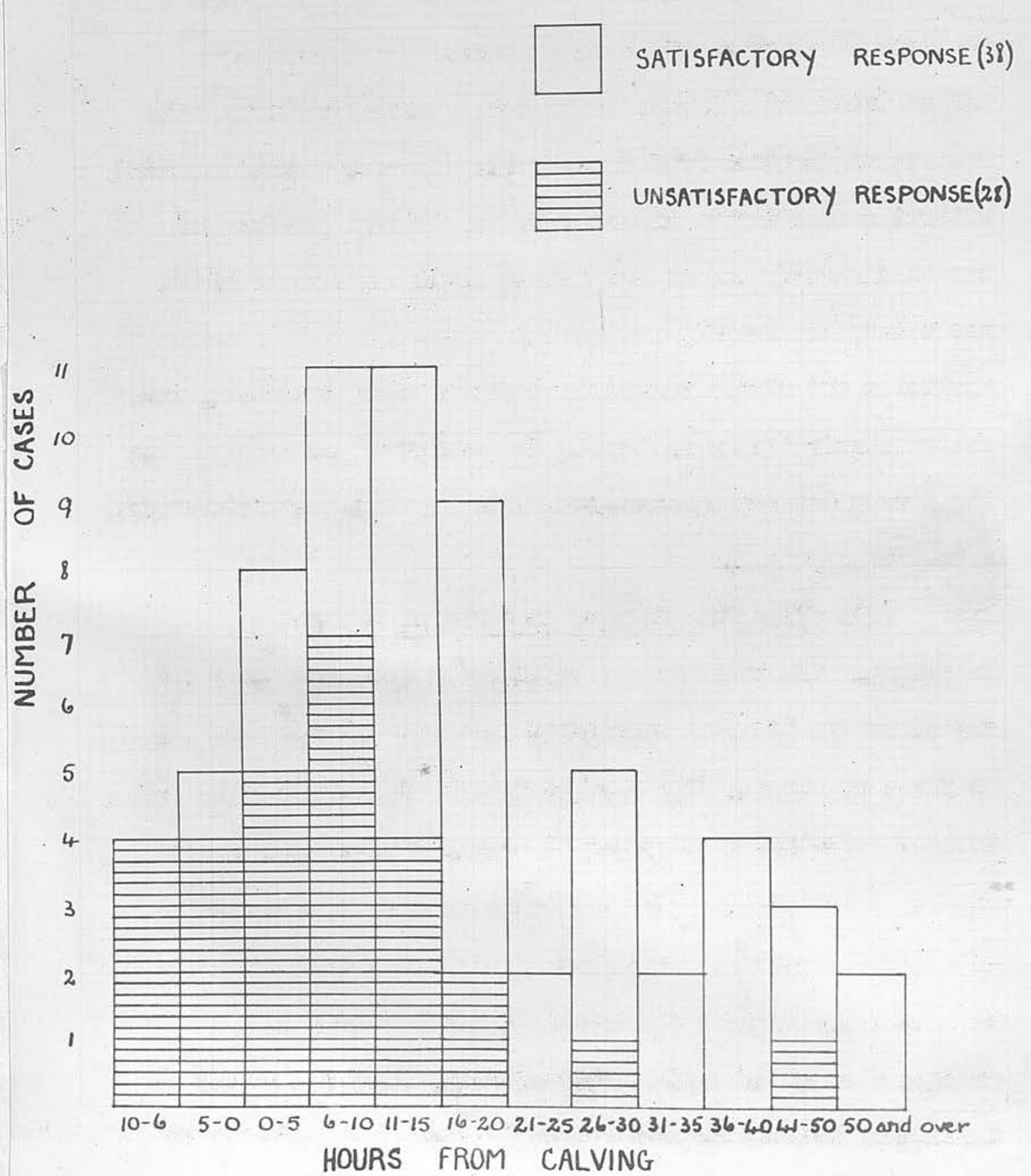
c). <u>Number of Calving at which Milk Fever Occurred.</u>

Reasonable accurate information on this was available in 93 of the milk fever cases. Of these, two cases occurred at the second calving, twenty-one at the third, thirty-five at the fourth, nineteen at the fifth, nine at the sixth, six at the seventh, and one at the eight calving.

d). Time from Calving at which Milk Fever Occurred. This information is presented in Table 31 (Appendix 3 pp. xxviii, xxix and xxx). The time between calving and first treatment was known in 93 cows. This interval will not differ greatly from the time between calving and the onset of the disease as there was usually little or no delay in reporting and attending these cases. Of the 93 cases 85, or 91.4% occurred between ten hours before calving and 96 hours after calving. The average interval between calving and treatment for these 85 cases was 16.5 hours. The distribution of the other eight cases is shown in Table 31 (Appendix 3 pp. xxviii, xxix and xxx).

e). Response to Treatment. (1) Classification of Cases. Cases have been classified as showing a satisfactory or an unsatisfactory response to treatment. In order, as far as possible, to avoid subjective impressions on the nature of the response the only criterion which was used was whether or not the case required further treatment. In the majority of cases classified as showing an unsatisfactory response there was a definite relapse after first treatment. In a few cases further treatment was given although the cows did not show marked clinical signs, but showed merely dullness and anorexia, without signs of any other disease. The initial blood levels before second or subsequent treatments (Table 31, Appendix 3, pp. xxviii, xxix and xxx) show that the diagnosis was

FIGURE 17 RELATION BETWEEN TIME FROM CALVING AND RESPONSE TO FIRST TREATMENT IN MILK FEVER CASES



substantially correct. Only cases attended by the author personally have been classified on this basis.

It was possible to classify on this basis, 68 cows with clinical milk fever, Forty cows, or 59%, showed a satisfactory response to treatment i.e., they required only one treatment. Twenty-eight cows, or 41%, showed an unsatisfactory response to first treatment. Of these 28 cows, 23 responded to a second treatment, three responded to a third treatment, and one responded after four treatments. One cow, number 90, died without responding to two treatments, although biochemical analysis showed that at the time of death the blood picture was within the normal range so far as serum calcium, serum magnesium and plasma inorganic phosphate were concerned. Post mortem examination showed multiple petechial haemorrhages on the serous membranes, suggesting that death was associated with a "shock" syndrome.

(2). Time from Calving in Relation to Response to Treatment. The time between calving and treatment was known for 66 of the 68 cases classified according to their response to first treatment. The average interval between calving and treatment for the 66 cows was 16 hours. Thirty-eight cows showing a satisfactory response were treated at an average time of 23 hours after calving; the 28 cows which responded unsatisfactorily to first treatment, were treated at an average time of 6.5 hours after calving. Analysis of the difference between the two groups (t test) showed that it was highly significant. The distribution of the times, relative to calving, in the two groups is shown in Figure 17. It will be seen that eight of the nine cases occurring before calving showed an unsatisfactory response to first treatment.

(3). Initial Blood Levels in Relation to Response to Treatment. The mean pre-treatment levels for the three blood constituents in the two groups are shown in Table 24.

TABLE 24.

Milk Fever Cases - Response to First Treatment.

Mean Pre-treatment Blood Levels in Satisfactory and Unsatisfactory Cases.

Mgm. per cent.

	Calcium		Magnesium		Inorganic P.	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Satisfactory Response (40)	4.54	0.186	3.50	0.087	1.41	0.153
Unsatisfactory Response (28)	4.71	0.189	3.30	0.104	1.32	0.137
Difference	0.17	0.265	0.20	0.136	0.09	0.205
Significance	N.S.		N.S.		N.S.	

N.S. = Not Significant.

Analysis showed that there were no significant differences between the groups in this comparison. The pre-treatment levels of the blood fractions gave no indication, therefore, of the nature of the response to be anticipated.

(4) Blood Levels after Treatment with Calcium Borogluconate in Relation to Response to Treatment. Tables 32 to 35 (Appendix 3, pp. xxxi to xxxiv) give the post-treatment data for 34 treatments with calcium borogluconate which resulted in a satisfactory response, and for 23 treatments which resulted in an unsatisfactory response to calcium therapy. Only the data between treatment and 12 hours after treatment have been included in these Tables. In the case of cows showing an unsatisfactory response only the data within this

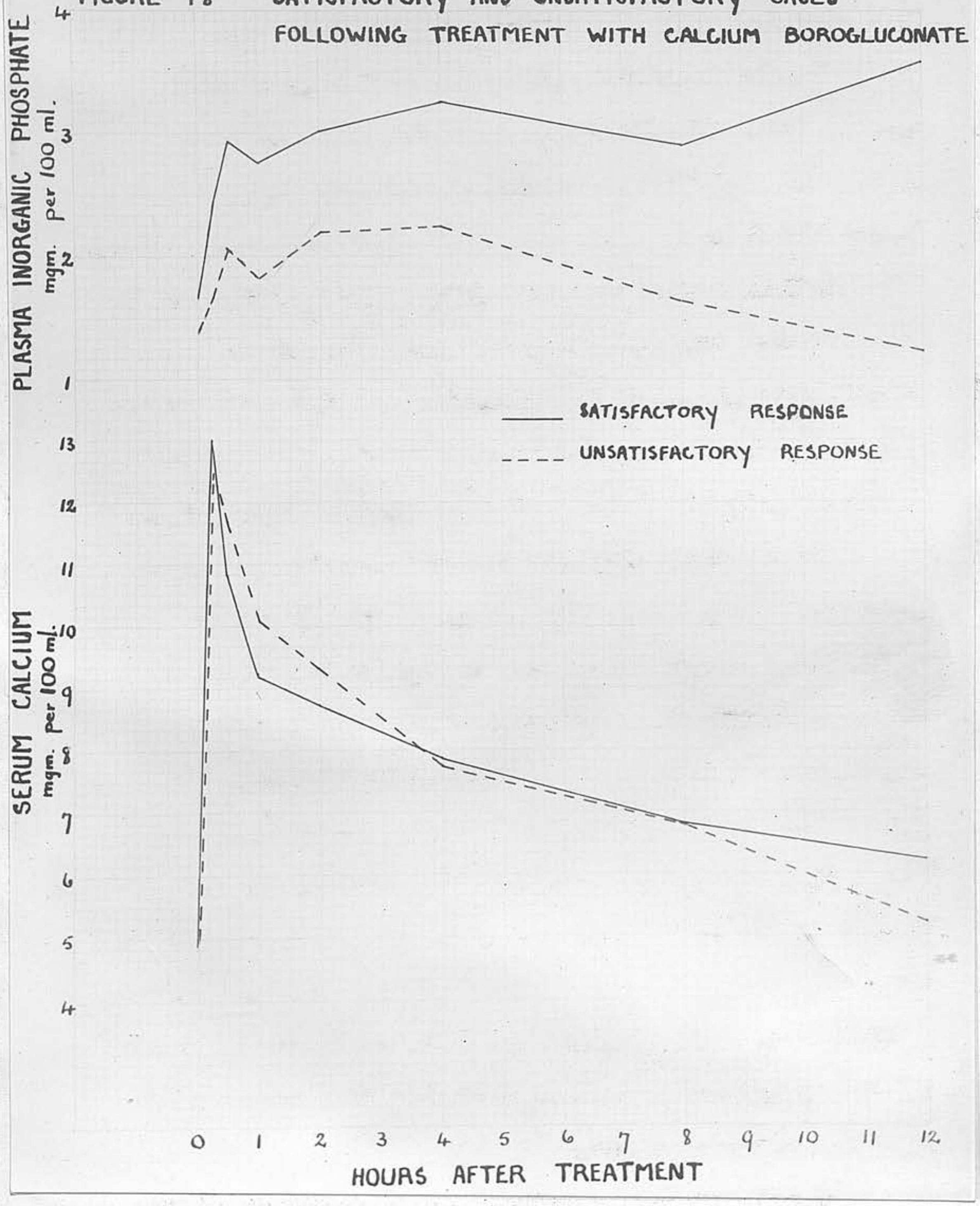


FIGURE 18 BLOOD CALCIUM AND INORGANIC PHOSPHATE IN SATISFACTORY AND UNSATISFACTORY CASES FOLLOWING TREATMENT WITH CALCIUM BOROGLUCONATE

(5). Response to Treatment after Udder Inflation. A total of eleven treatments by udder inflation, in ten cows, was studied. The complete data for these cases are presented in Table 34 (Appendix 3 p. xxxiii). The mean data are shown in Figure 19.

Figure 19 shows that udder inflation was followed by a rise in the level of serum calcium. The increase was at first rapid, to reach a peak at four hours after inflation. This was followed by a slight decline, and then a gradual rise from one day after inflation until 3-7 days after inflation. Study of the data for individual cases (Table 34 Appendix 3 p. xxxiii) shows that the increase in serum calcium was consistent. The amount of the increase depended mainly on the initial level i.e., the increase was most marked in cases with the lowest pre-inflation levels. Thus Cow 89(2) showed a rise from 3.80 mgm. before inflation to 8.20 mgm. at one hour after inflation; Cow 44(4) showed a rise from a pre-inflation level of 2.93 mgm. to 5.96 mgm. one hour after inflation. In the three cows with samples in the 3-7 days post-inflation period there was no evidence of a hypercalcaemia at this time; in two of the cows the levels were below the normal range.

Plasma inorganic phosphate after inflation behaved in a similar manner to serum calcium i.e., there was a rapid rise to four hours post-inflation, a more or less level trend from four hours until 24 hours, and a gradual rise to 3-7 days after inflation. Study of the data for individual cases shows that the rise in plasma inorganic phosphate in the initial stages tended to be very rapid. For example, Cow 44(2) showed an increase from an initial level of 0.95 mgm. to a level of 2.20 mgm. fifteen minutes after inflation; Cow 89(2) showed a rise from 0.19 mgm. before inflation to 1.15 mgm. fifteen minutes after inflation, and to 2.20 mgm. thirty minutes after inflation. The general tendency was for plasma inorganic phosphate to double its pre-inflation value by one hour after inflation. One Cow, 81 (2), showed a slight and transient fall in the phosphate level fifteen minutes after inflation, and then a sharp rise to one hour after inflation. Cow 89 (2) after a marked initial rise, showed a sharp fall to 0.04 mgm. at one hour after inflation and then a rise to 3.26 mgm. at two hours. The maximum change in an individual case following inflation was in Cow 89 (2), which showed a rise from a pre-inflation level of 0.19 mgm. to 6.58 mgm. six hours after inflation.

Serum magnesium showed a slight increase after udder inflation and remained above the pre-inflation levels for ten hours and then showed a gradual decline. Study of the data for individual cases shows that the rise in serum magnesium, alghough small, was consistent.

In order to test the significance of the changes in serum calcium, serum magnesium and plasma inorganic phosphate following udder inflation in milk fever cases the values in the post-inflation periods were compared by t test with the corresponding values in the pre-inflation periods. This analysis showed that, in the cases of serum calcium and plasma inorganic phosphate, there was a highly significant difference (at 1%) between all of the mean values in the post-inflation periods and the corresponding mean values in the pre-inflation period. In the case of serum magnesium the mean values at fifteen minutes, thirty minutes, one hour, two hours and ten hours showed a highly significant difference (at 1%) when compared with the corresponding pre-inflation values. The value at four hours were significantly higher than the corresponding pre-inflation value. The values at 12-24 hours and at 3-7 days were not significantly different from the corresponding pre-inflation values.

The times of recovery, as judged by the time the cows rose to their feet, are shown in Table 36 (Appendix 3, p. xxxv). The average time for the ten cases which did recover was 3.72 hours, with a range of fifteen minutes to ten hours.

The levels of the blood constituents at the time of recovery were - serum calcium - 7.59 mgm. (5.90 to 10.18 mgm.); Serum magnesium - 3.29 mgm. (2.36 to 3.85 mgm.); and plasma inorganic phosphate - 2.69 mgm. (0.04 to 4.50 mgm.). It will be seen, therefore, that there was considerable variation in the levels of the three constituents at the time of recovery.

Of the ten cows treated by udder inflation, one cow, No. 90. died after receiving further treatment (See p. 116). One cow, No. 44, relapsed following initial treatment by calcium injection, was treated by udder inflation, relapsed again, was treated by a second calcium injection, relapsed again, and was finally treated by udder inflation and recovered. Of the remaining eight cows none relapsed following udder inflation, but five of these cases had previously been treated by calcium injection.

(6) Detailed Studies of Response to Treatment. In fourteen cases of milk fever the post-treatment blood levels were studied in considerable detail. Most of the data from these cases have been abstracted and used in the preparation of the above results. However, the complete data are valuable in showing the trends in the behaviour of the blood fractions following treatment. The concise and orderly presentation of the complete data from these cases in tabular form is rendered

impossible by the fact that while some cows required only one treatment for recovery, others required two, three and in one case, four treatments before eventual recovery. This made it impossible to take the post-treatment samples at exactly the same intervals. Accordingly, graphs have been prepared from the data of some of these animals, showing the different types of cases encountered and are used to illustrate points in the discussion of results which follows.

DISCUSSION OF RESULTS. AND GENERAL DISCUSSION.

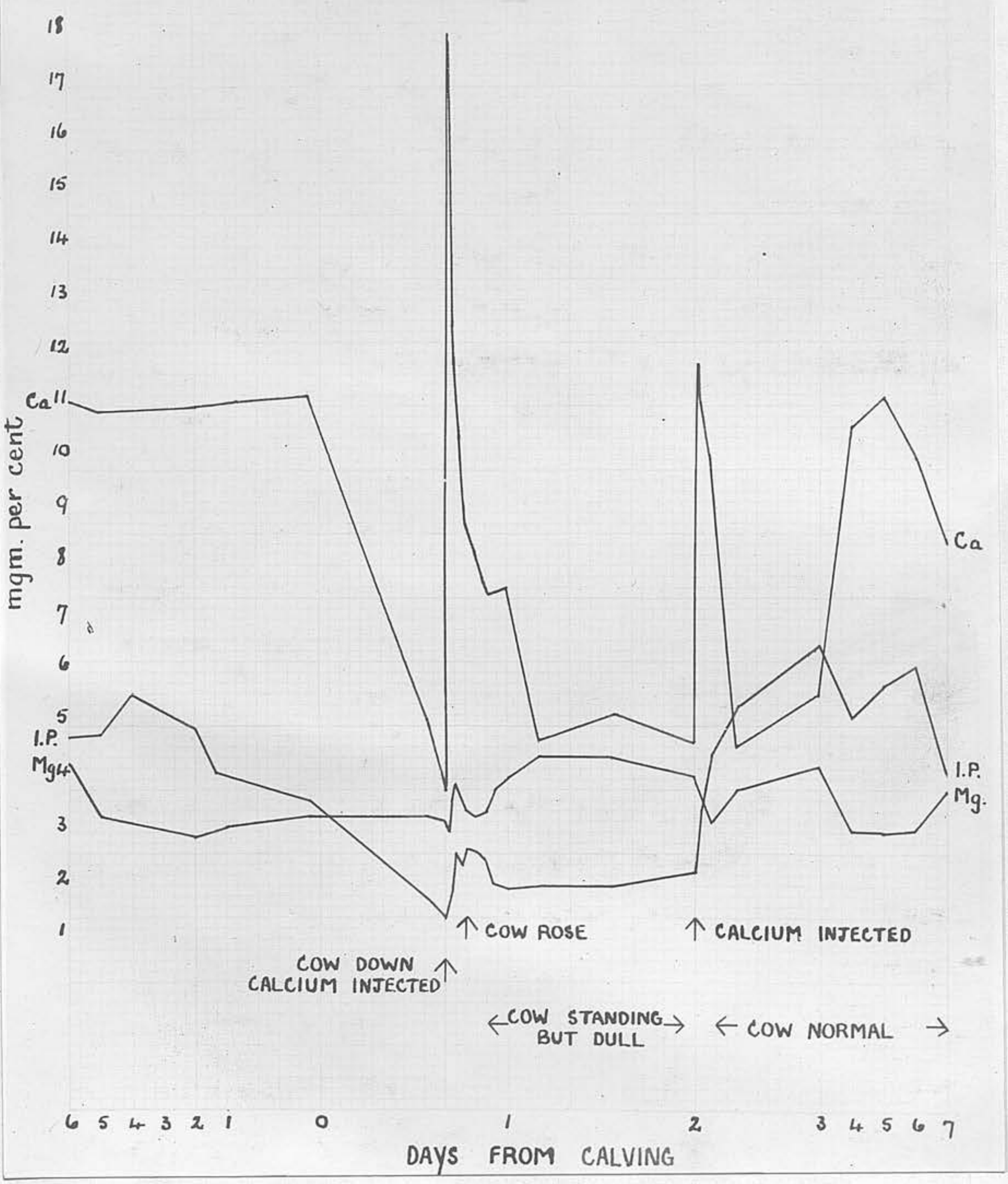
The pre-treatment levels of serum calcium, serum magnesium and plasma inorganic phosphate in milk fever cases in this investigation agree fairly closely with those of previous workers. The results confirm the already well-established fact that milk fever is usually associated with a hypocalcaemia, hypophosphataemia, and hypermagnesaemia. The hypocalcaemia and the hypophosphataemia are more marked in milk fever than in normal parturient cows. In the cases investigated here, however, the level of ser um magnesium was lower than it was in normal parturient cows of an age susceptible to milk fever. It follows, therefore, that the statement that milk fever is associated with hypermagnesaemia can be regarded as true only if by hypermagnesaemia is meant a level of serum magnesium higher than in normal non-parturient cows.

The results of the studies on normal cows in the earlier sections suggested that the increase in the serum magnesium was a reflection of the decrease in the serum calcium, and that the extent of the rise in serum magnesium was proportional to the extent of the fall in serum calcium. This quantitative relationship does not appear to hold in clinical milk fever. It is logical to assume that there is a limit to the ability of serum magnesium to rise in response to a falling blood calcium level and that this limit is reached in clinical milk fever. It is probable also that in clinical milk fever the mechanism which stimulates the rise in serum magnesium becomes impaired in keeping with the general slowing down of metabolic processes which accompanies the disease.

The fact that the level of serum magnesium is no higher in milk fever than in normal parturient cows, and that there was no significant correlation between the level of serum magnesium and the severity of the clinical signs (Table 23) suggests that serum magnesium does not play a very important role in typical milk fever. However, as has been suggested by Hibbs (1950), it is possible that the relatively high serum magnesium level accounts for the lack of tetanic symptoms in typical milk fever and for the comatose condition which usually is seen in spite of the low blood calcium level.

As already mentioned, Barker (1939) has classified hypocalcaemia into three clinical groups according to whether it is accompanied by a raised, lowered or normal level of serum magnesium. He claims that cases of hypocalcaemia with a mean serum magnesium level of 3.30 mgm. per cent show "narcosis"; cases with a mean level of 2.30 mgm. show "coma"; and cases with a mean serum magnesium level of 0.80 mgm. per cent show "excitement and convulsions". It is possible to agree with the last of Barker's groups i.e. hypocalcaemia accompanied by hypomagnesaemia - essentially the blood picture in lactation tetany, where the clinical signs are usually recognisably different from those in true milk fever. It is less easy to classify cases into the other clinical groups suggested by Barker. The method is largely dependent on subjective impressions of the nature of the clinical signs and depends a great deal on the clinical acumen and experience of the observer. So far as the present study is concerned, all that can be said is that it was found impossible to classify cases clinically into a group showing "narcosis" and a group showing "coma". An argument against Barker's classification is that it ascribes a very definite difference in clinical manifestation to a very slight change in the level of serum magnesium. The results

FIGURE 20 MILK FEVER CASE No. 31

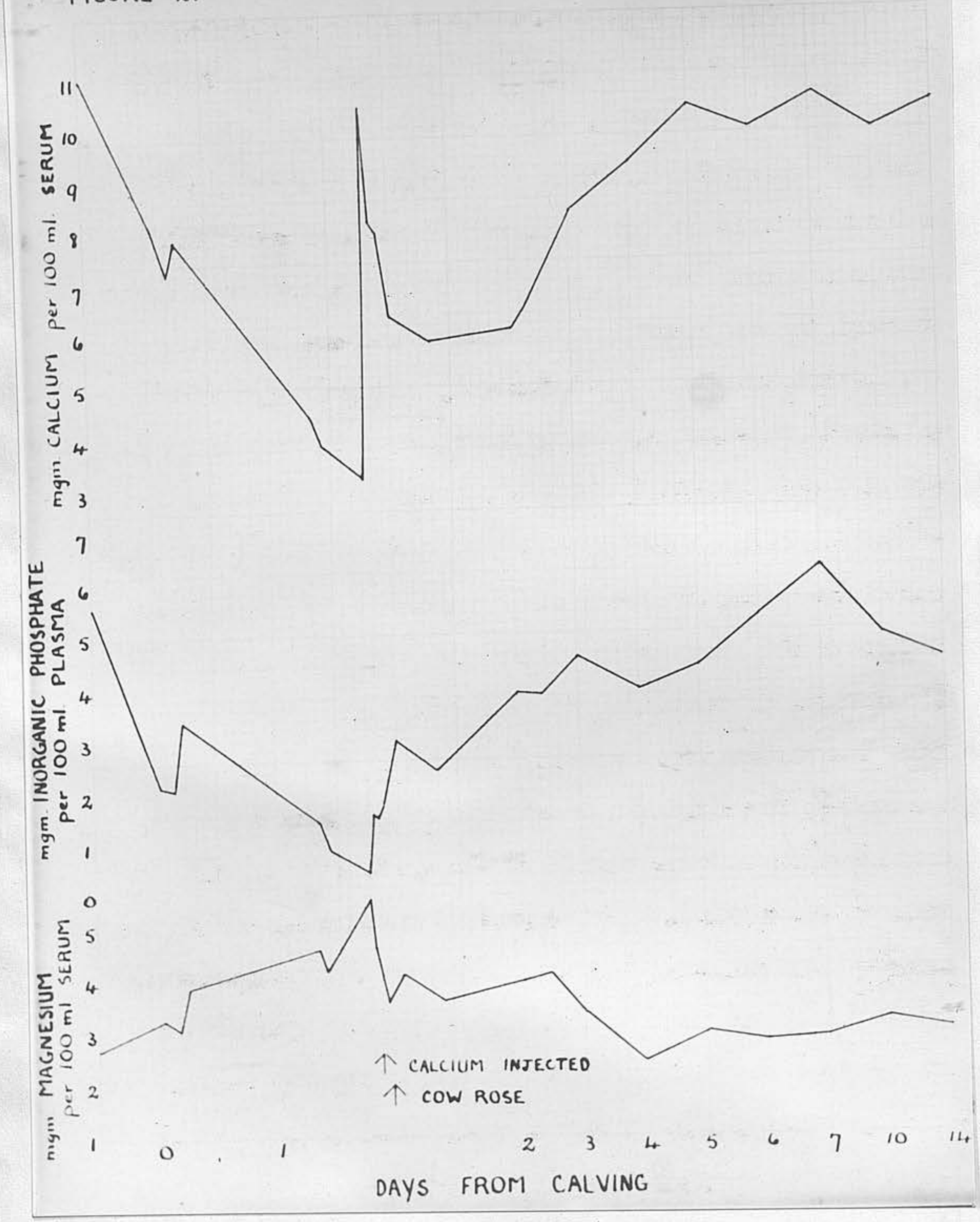


obtained in this study show that there may be considerable variation in the level of serum magnesium in an individual cow within a relatively short space of time without any alteration in the clinical picture. (e.g. case 31, Figure 20). Moreover, the group approaching most nearly Barker's "comatose" type, (the group showing severe symptoms of milk fever, Table 23), showed a slightly higher mean level of serum magnesium than the group most nearly approaching Barker's "narcotic" type (the group showing moderately severe symptoms).

The finding that the severity of the clinical signs was significantly related to the extent of the depression in serum calcium agrees with the original observations of Little and Wright (1925), and with those of Dryerre and Greig (1928). It disagrees with the recent finding of Blood, Larsen and White (1949) who found no correlation between the severity of the clinical signs and the extent of the depression in serum calcium in a study of twenty five cases of milk fever.

While there was, in the present study, a significant general tendency for the severity of the clinical signs to be related to the extent of the depression in serum calcium and also in plasma inorganic phosphate, there were certain exceptions to the general rule. The most marked example was Cow 64 which was showing only milk signs of milk fever with a serum calcium level of 2.90 mgm. and a plasma inorganic phosphate level of 1.10 mgm. per cent. That the clinical picture is not solely dependent on the levels of serum calcium and plasma inorganic phosphate is evident particularly in the study of the levels of serum calcium after treatment with intravenous calcium borogluconate. Thus Figure 21 shows that in the group

FIGURE 21 MILK FEVER CASE No. 29

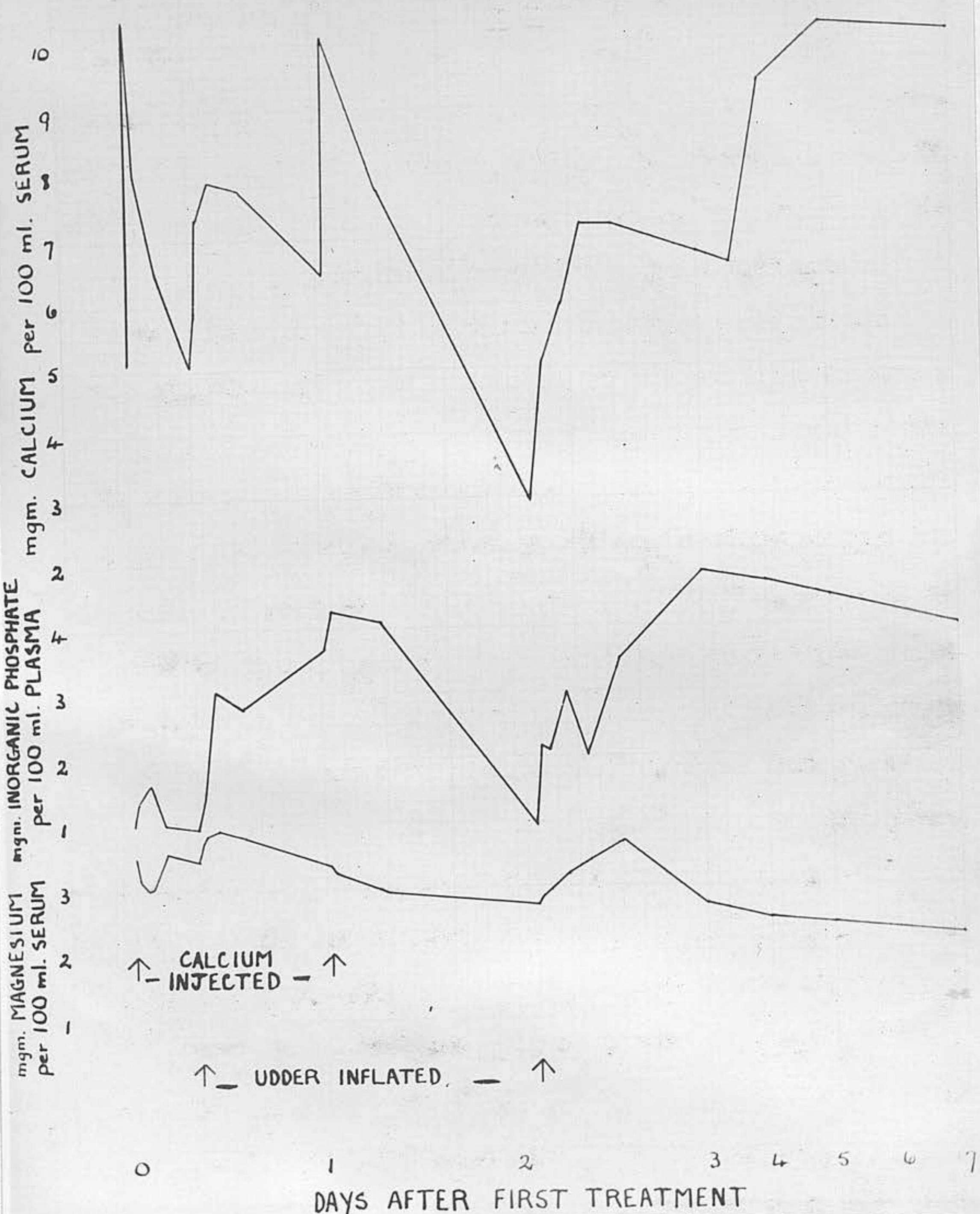


of cases which responded satisfactorily to calcium treatment the mean level of serum calcium had returned almost to its pre-injection level by 12 hours after treatment, although none of the cows were exhibiting clinical signs of milk fever at this time. The data for individual cows also show this point clearly. Thus in Cow 31 (Figure 20) the serum calcium had returned to a level of 4.57 mgm. per cent within 12 hours of injection. The serum calcium remained at this level for a further 24 hours although the cow showed no more definite signs of milk fever during this time than slight dullness. The rise in serum calcium in this cow produced by the second calcium injection, was also very transient, and within a further 12 hours the serum calcium had fallen to 4.41 mgm. although the cow was normal and eating at this time. A similar picture was evident in many of the other cows subjected to detailed study. (Figures 21, 23 and 24).

The finding that a distinct hypocalcaemia frequently recurs even in cases which do not relapse following treatment, does not agree with the views of Hibbs et al. (1946) and Hibbs et al. (1951). These workers state that the decline in serum calcium following treatment levels off somewhere near the normal level, and that in cases which relapse the fall in serum calcium continues until milk fever levels are again reached.

In searching for an explanation of the low serum calcium levels without clinical signs it should be remembered that only the total serum calcium levels have been studied here. Seekles, Sjollema and van der Kaay (1932c) claim to have cured an atypical case of milk fever by the injection of parathyroid extract and, although the total serum calcium was lower after recovery, the ionised calcium had increased to double its pre-

FIGURE 22 MILK FEVER CASE No. 44



treatment value. It is possible, therefore, that the ability of certain cows to tolerate lower levels of total serum calcium than others without the exhibition of clinical signs, may be due to differences in the partition of serum calcium.

Study of the behaviour of serum calcium following treatment by calcium borogluconate injection (Figures 20, 21, 23, 24 and 25) suggests that calcium injection, by virtue of raising the blood calcium level, abolishes the clinical signs, and allows the calcium regulating mechanisms time to re-establish normal levels. In other words calcium injection acts as a temporary expedient until the normal regulatory mechanisms come into play rather than by any direct specific curative action. The studies reported in Section 1 suggested that the main cause of the fall in blood calcium is the drain produced by the secretion of colostrum. It follows, therefore, that calcium injection may serve only to counteract the effects of the drain of mammary secretion until the calcium regulating mechanisms have time to restore normal levels. Hibbs and his co-workers (1951) have arrived at somewhat similar conclusions.

The study of normal parturient cows, reported in Section 1, showed that the fall in serum calcium commenced 12-24 hours before parturition, and that lower than normal levels persisted until 3-4 days postpartum. The results from the four cases in which the blood picture was studied in the prepartum period and which subsequently developed milk fever (Figures 20, 21, 25 and 26), show that the fall commenced in the last 12-24 hours prepartum i.e., the time at which the changes commenced in normal cows. It has been found that milk fever cases occurring before calving, or within a few hours after calving, are more likely to relapse following treatment than are cases occurring later in

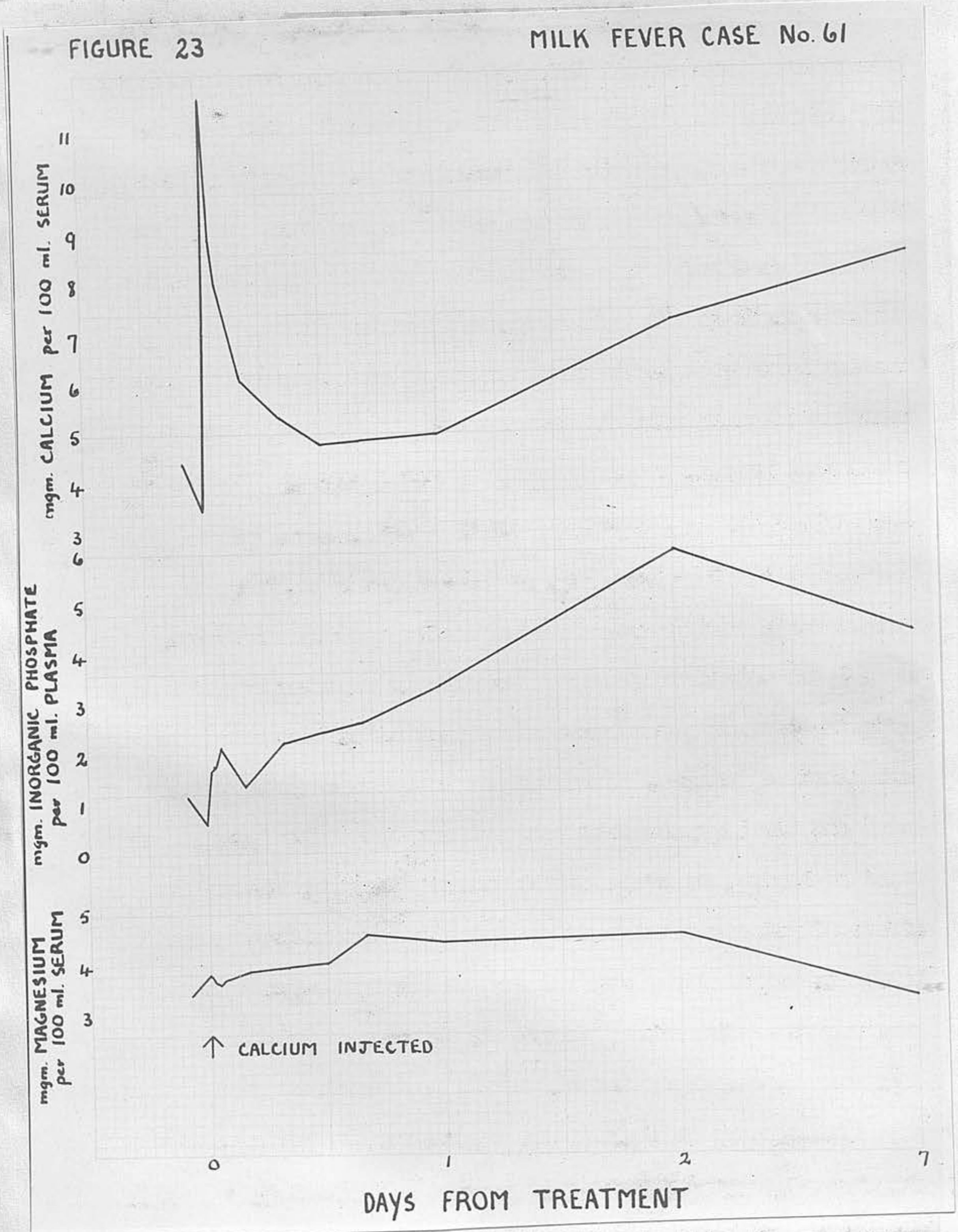


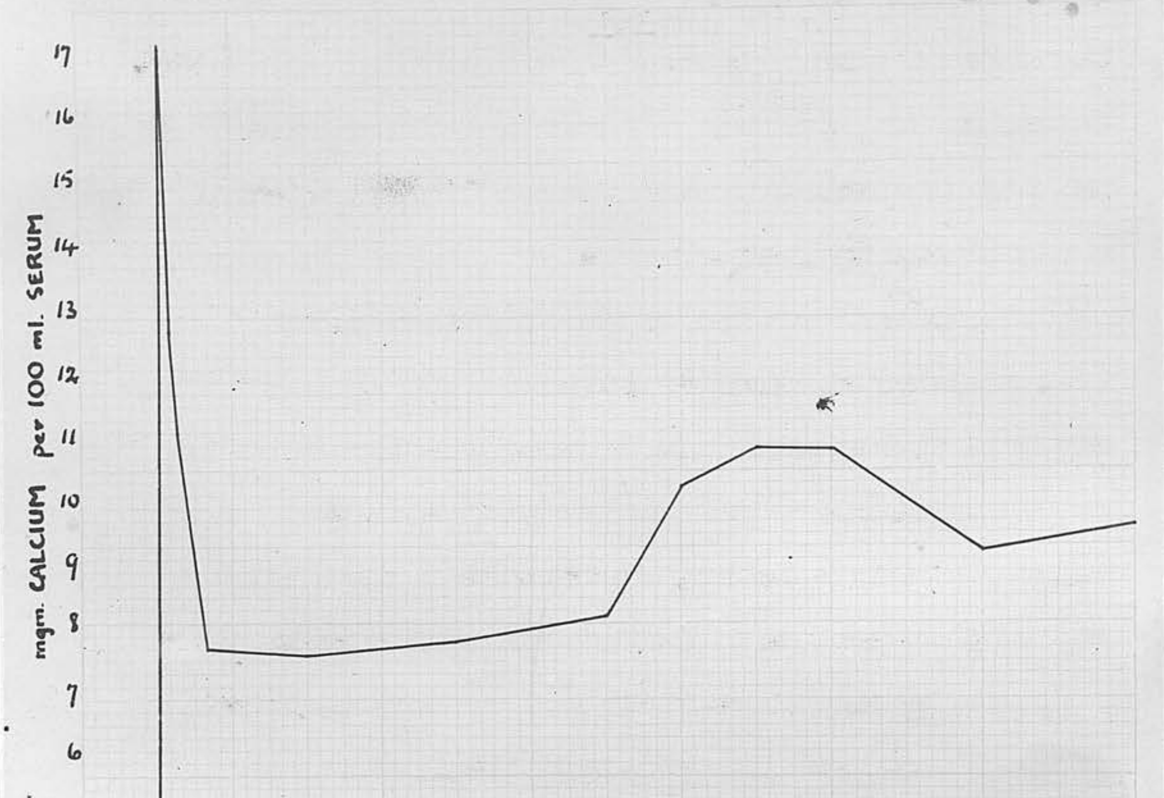
FIGURE 23
MILK FEVER CASE No. 61
mgm. CALCIUM per 100 ml. SERUM
11
10
9
8
7
6
5
4
3
mgm. INORGANIC PHOSPHATE per 100 ml. PLASMA
6
5
4
3
2
1
0
mgm. MAGNESIUM per 100 ml. SERUM
5
4
3
↑ CALCIUM INJECTED
0
1
2
7
DAYS FROM TREATMENT

relation to calving.

These facts lend further support to the hypothesis that milk fever is an exaggeration of the normal changes associated with parturition. They further suggest that it usually requires some 3-4 days for the normal calcium regulating mechanisms to compensate for the demands for calcium produced by the sudden increase in secretion of colostrum at parturition. Thus, in a case given calcium treatment either before or shortly after parturition it is likely that the artificial raising of the blood calcium will not be maintained sufficiently long for the normal regulating mechanisms to come into play and restore the normal levels. Conversely, in a case occurring, say 24 hours after calving, the raised blood calcium level is maintained sufficiently long for the calcium regulating mechanisms to restore normal levels. It follows, therefore, that, in cases occurring near parturition, anything which will serve to maintain the serum calcium level will lessen the likelihood of relapse. The rapid decline in the serum calcium level and the high percentage of relapses following intravenous calcium therapy, reported in this Section, and the transient effect of intravenous injection of calcium on the serum calcium levels in normal cows, reported in Section 2, suggest that the practice of supplementing intravenous therapy by subcutaneous injection of calcium is sound.

The results obtained in the treatment of clinical cases of milk fever by udder inflation would seem to support the commonly expressed belief that mammary inflation is a more reliable treatment than calcium injection therapy. Thus two out of 11 cases (18%) showed an unsatisfactory response to treatment by udder inflation , compared with 23 out of 57 (40%) treated by

FIGURE 24 MILK FEVER CASE No. 36



calcium therapy. The number of cases treated by udder inflation is, however, too small to warrant the drawing of firm conclusions. Further, most of the cases treated by udder inflation were treated some time after calving and were therefore more likely to respond satisfactorily to treatment. The results are, however, in keeping with general experience and it is poss ible to explain why udder inflation should result in less frequent relapses.

The results obtained in the study of the effect of udder inflation on normal cows reported in Section 2, Part 2, suggested that mammary inflation acts by causing resorption of calcium and phosphate from the mammary gland, and by the stopping of milk secretion. The results of the studies in Section 1 suggested that the drain into the colostrum was the main cause of the fall in blood calcium. Udder inflation, therefore, besides raising the blood calcium and phosphate levels, will prevent further milk secretion, and thus stop the basic cause of the fall in blood calcium. Calcium injection, on the other hand, by abolishing the clinical signs of milk fever, and in consequence allowing metabolic processes, including the secretion of milk, to return to normal, will be more likely than udder inflation to be followed by relapse. It would seem that the practice of non milking a cow after calcium treatment would have somewhat the same effect as udder inflation. Abstaining from milking, however, does not prevent the milk from being secreted, at least in the early stages when the danger of relapse is most acute. Niedermeier and Smith (1948) have shown that abstention from milking after calving does not prevent the fall in serum calcium at calving, and indeed one of their cases developed clinical milk fever. It was not until

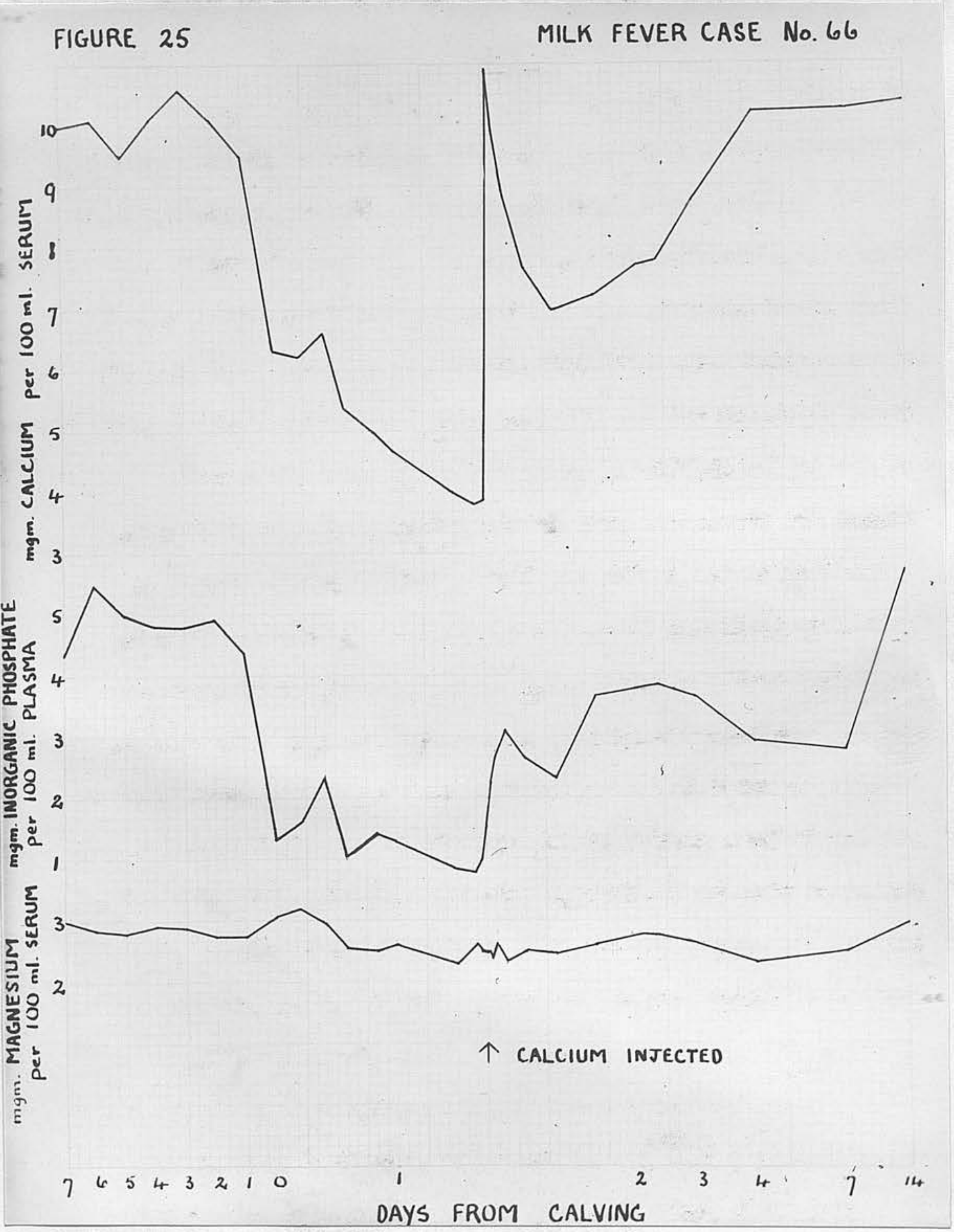


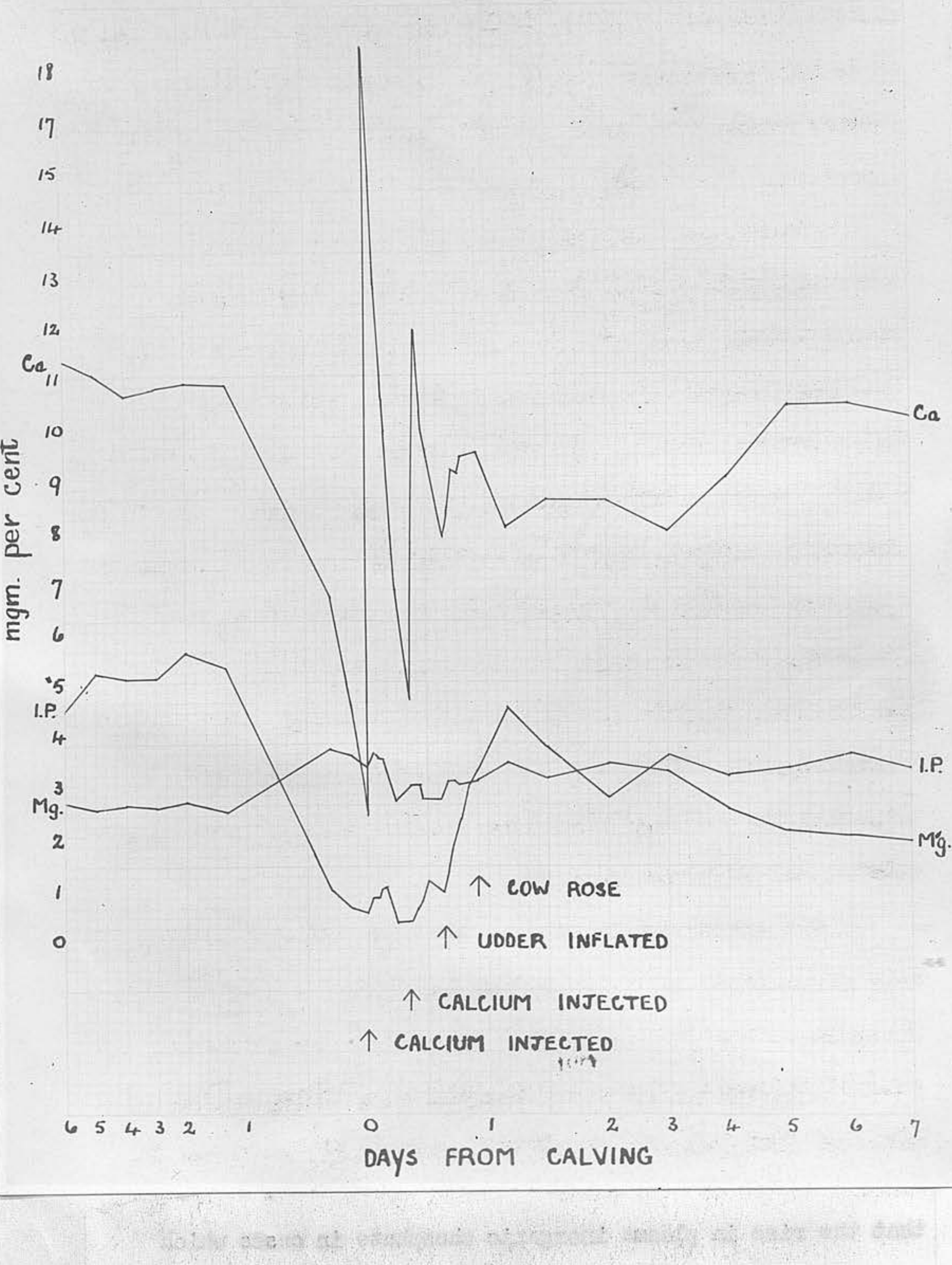
FIGURE 25
MILK FEVER CASE No. 66
mgm. CALCIUM per 100 ml. SERUM
10
9
8
7
6
5
4
3
mgm. INORGANIC PHOSPHATE per 100 ml. PLASMA
5
4
3
2
1
mgm. MAGNESIUM per 100 ml. SERUM
3
2
↑ CALCIUM INJECTED
7 6 5 4 3 2 1 0
1
2
3
4
7
14
DAYS FROM CALVING

three days later that higher than normal levels of serum calcium were observed.

The results obtained in the treatment of milk fever by udder inflation suggest that there may be another factor in explaining the apparent superiority of udder inflation as compared with calcium therapy. Udder inflation caused a more marked and consistent increase in the plasma inorganic phosphate than did calcium injection. This finding supports the findings of Robertson et al., (1948), and Robertson (1949) who suggested that the beneficial effect of udder inflation in cases which did not recover following calcium treatment might be due to the ability of udder inflation to cause a more marked and consistent rise in the plasma inorganic phosphate.

That a rise in the level of plasma inorganic phosphate following treatment is necessary for satisfactory recovery is suggested by the results obtained in the study of satisfactory and unsatisfactory responses to calcium therapy. (Figure 18). It will be noted that although the behaviour of serum calcium was similar in both groups, in the satisfactory group ther was a sustained rise in the level of plasma inorganic phosphate after calcium treatment, whereas in the group which responded unsatisfactorily to calcium therapy, the inorganic phosphate showed a slight initial rise and then a gradual decline. These results would seem to support the suggestion of Fish (1930) and of Barker (1948) that phosphate as well as calcium should be used in milk fever therapy. On the other hand, it may be that the rise in plasma inorganic phosphate in cases which responded satisfactorily to calcium therapy is merely an indication that the normal regulating mechanisms are coming into play. This suggestion is supported by the fact that in

FIGURE 26 MILK FEVER CASE No. 32



both groups there was an initial rise to four hours after injection. In satisfactory cases, occurring as has been shown, later in relation to calving than unsatisfactory cases, the phosphate level is possibly at a stage when it is going to rise as in normal parturient cows, while in unsatisfactory cases the factors causing the normal fall in blood phosphate are still operative.

It is possible, therefore, that the marked rise in the level of plasma inorganic phosphate in cases which respond satisfactorily to calcium therapy is more an indication that the normal regulating mechanisms are again functioning than that the actual phosphate level is of any intrinsic significance in the production of the clinical picture. If this premise is correct, it follows that the artificial raising of the blood phosphate level by the injection of phosphate salts, will not have any marked beneficial effect. The determination of the clinical significance of the blood phosphate level must, therefore, await the results of controlled experiments comparing the efficacy of calcium therapy alone with combined calcium and phosphate therapy.

The results of the studies of normal parturient cows, and of mastectomised parturient cows, suggested that the plasma inorganic phosphate level is influenced more by the act of parturition than by the onset of milk secretion, whereas, with serum calcium, the opposite appears to be the case. It seems, therefore, that there are fundamental differences in the factors which produce the changes in these two fractions in normal parturient and milk fever cows. Until more is known concerning the factors influencing the plasma inorganic phosphate it is not possible to arrive at a definite opinion on the significance of its role in milk fever.

The studies of mastectomised and prepartum milked cows suggested that the drain of calcium into the colostrum was the major cause of the fall in blood calcium in normal cows at parturition. It follows, therefore, if it is accepted that milk fever is an exagger tion of the normal changes that the drain of calcium into the colostrum is the major cause of the fall in blood calcium in cows with clinical milk fever.

It would seem, therefore, that clinical milk fever is the result of the animal being less able than the normal cow to cope with the sudden demands produced by the secretion of the calcium-rich colostrum. The fall in blood calcium progresses to such an extent that clinical milk fever ensues. In some animals, in the absence of treatment, the hypocalcaemia persists until death results, possibly from cellular damage consequent on the prolonged hypocalcaemia. In others, the hypocalcaemia results in a slowing of metabolic processes, including the secretion of colostrum, so that the basic cause of the fall in the blood calcium is removed. A gradual rise in the blood calcium is initiated by the normal regulating mechanisms. Calcium therapy appears merely to abolish the immediate symptoms and allow time for the mobilisation of calcium from the osseous tissues. The abolition of the symptoms may lead to further colostrum secretion and thus predispose to relapse. Udder inflation, on the other hand, by raising the blood calcium and at the same time, stopping further colostrum secretion, is less likely to be followed by relapse. The chances of relapse occurring after about the third day postpartum are less because the calcium regulating mechanisms have had more time to meet the demands, and because the secretion of the calcium-rich colostrum has been replaced by the secretion of normal milk.

The results, therefore, imply that, in milk fever, the calcium regulating mechanisms are essentially normal, and that the basic cause of the disease is an inability of the normal mechanisms to mobilize calcium sufficiently quickly to meet demands resulting from the onset of colostrum secretion. The primary dysfunction, therefore, is more likely to be an impairment in the mobilisation of calcium such as might result from a decrease in the availability of calcium in the body reserves for interchange with the blood. Thus, the normal regulating mechanisms require more time to meet demands on the blood calcium resulting from the onset of milk secretion.

In support of this hypothesis it may be said that it is difficult to explain certain aspects of the results, particularly the effects of calcium injection and udder inflation therapy, by accepting the hypothesis that milk fever is associated with an organic dysfunction of the calcium regulating mechanisms such as parathyroid activity, as was postulated by Dryerre and Greig. It is very difficult to comprehend the nature of an organic dysfunction which is of such a temporary nature, and which is so readily rectified by a treatment essentially replacement in effect. This criticism applies even more forcibly to the theories of Seekles (1948), who postulates a multiglandular dysfunction in milk fever.

It would seem that milk fever, and in particular the increased incidence in older animals, could be explained more simply and more rationally by postulating a decrease, with advancing age, in the availability of calcium in the osseous tissues for interchange with the blood. As is suggested in Section 1, a study of this aspect of the problem, using radiocalcium, would seem to offer a hopeful line of future research.

SUMMARY OF SECTION 3.

1) Pre-treatment levels of serum calcium, serum magnesium and plasma inorganic phosphate are presented for 128 cases in 96 cows. The levels of serum calcium and plasma inorganic phosphate were significantly lower than in normal parturient cows. The levels of serum magnesium, however, did not differ significantly from those in normal parturient cows.

2) The levels of serum calcium and plasma inorganic phosphate showed a significant correlation with the severity of the clinical signs; the level of serum magnesium did not.

3) Data are presented on the number of calving at which milk fever occurred, and the time from calving to the onset of the disease.

4) The response of cases to treatment by calcium injection and udder inflation therapy has been studied. Of 68 cases treated by calcium injection 28, or 41%, showed an unsatisfactory response to first treatment. Cases showing a satisfactory response to treatment were treated at an average time of 23 hours after calving, while cases showing an unsatisfactory response were treated at an average time of 6.5 hours. This difference was highly significant.

5) The pre-treatment blood levels of calcium, magnesium and inorganic phosphate gave no indication of the likely response to treatment. There was a significant difference, however, between satisfactory and unsatisfactory cases, in the behaviour of plasma inorganic phosphate following calcium therapy. The behaviour of serum calcium was similar in the two groups.

6) Eleven treatments by udder inflation were studied. Nine cases responded to one treatment. One cow died after receiving further treatment. The other case relapsed and responded finally to udder inflation therapy after relapsing to calcium treatment.

Udder inflation resulted in a significant increase in the levels of all three blood fractions.

7) In 14 cases the post-treatment blood levels were studied in considerable detail. These studies show that calcium therapy produced only a very transient rise in the level of serum calcium. Udder inflation resulted in a more sustained rise in the levels of the blood fractions. It is suggested that the essential effect of calcium injection and udder inflation therapy is to abolish the clinical signs and allow time for the normal regulating mechanisms to re-establish normal levels.

8) The significance of the results of the studies of the milk fever cases is discussed in relation to the results of the study as a whole.

GENERAL SUMMARY AND CONCLUSIONS.

1) The following aspects of the milk fever problem have been studied:-

a) the variations in the levels of serum calcium, serum magnesium and plasma inorganic phosphate in thirty two normal, two prepartum milked, and three mastectomised cows, from fourteen to seven days prepartum until fourteen days postpartum.

b) the effect of intravenous injection of calcium borogluconate and of acid sodium phosphate in six normal parturient and six normal non-parturient cows.

c) the effect of udder inflation on six normal parturient and twelve normal non-parturient cows.

and d) the initial levels of serum calcium, serum magnesium and plasma inorganic phosphate in one hundred and twenty eight cases in ninety six cows with clinical milk fever, and the variations in these blood fractions following treatment by calcium injection and udder inflation.

2) a) Normal cows showed a significant decrease in the level of serum calcium at parturition. The fall commenced some twelve to twenty four hours prepartum and lower than normal levels persisted until three to seven days postpartum.

b) Serum magnesium showed a significant increase at calving, broadly inverse to the decrease in serum calcium.

c) There was a significant decrease in the level of plasma inorganic phosphate at calving. Parturition was followed by an immediate reversal of the trend so that at 8 hours postpartum the levels were not significantly lower than the prepartum levels. This was succeeded by a secondary fall to two days postpartum and then a gradual return to normal levels at three to seven days postpartum.

and d) The changes in all three constituents became more marked with each succeeding parturition until at least the fourth.

3) Limited studies on prepartum milked cows showed that, in place of the abrupt fall in serum calcium and plasma inorganic phosphate seen in normal cows, a gradual fall resulted. The fall commenced in four to five days prepartum and was co-incident with the increase in the secretion of calcium and phosphate in the milk.

4) In mastectomised cows the changes in the levels of serum calcium and serum magnesium at parturition were practically completely abolished. Plasma inorganic phosphate showed a fall at calving similar to that seen in intact cows, but the postpartum rise was more rapid.

5) From the results of the above studies it is concluded:-

a) that the changes in serum magnesium are a reflection of the changes in serum calcium.

b) that the level of plasma inorganic phosphate is influenced more by the act of parturition than by the onset of milk secretion, although the latter seems to have some influence on its behaviour.

c) that the major cause of the fall in serum calcium at parturition is the drain produced by the onset of colostrum secretion.

and d) that milk fever can be regarded as an exaggeration of the normal changes at parturition.

It follows, therefore, that the onset of milk secretion is a major cause of the fall in blood calcium in milk fever.

6) The intravenous injection of calcium borogluconate resulted in a very transient rise in the level of serum calcium in normal parturient and non-parturient cows. A rise in the level of plasma inorganic phosphate, greater in parturient than in non-parturient cows, followed calcium injection. It is suggested that this is due to an attempt to restore the normal blood calcium : protein : phosphate equilibrium. The greater rise in parturient cows may be due to the lower initial levels and to the fact that the phosphate level may have been increasing anyhow at the time at which the experiments were conducted.

7) The intravenous injection of acid sodium phosphate in normal parturient and non-parturient cows was followed by a very transient rise in the level of plasma inorganic phosphate, and by a fall in the level of serum calcium. It is suggested that the fall in serum calcium may be due to the increase in the phosphate ions, or in the sodium ions, or to a combination of these effects.

8) Udder inflation was followed in normal parturient and in non-parturient lactating cows by a rise in the levels of serum calcium and plasma inorganic phosphate. The increases

were greater in parturient cows than in non-parturient cows, and greater in non-parturient lactating cows not milked before inflation than in non-parturient lactating cows milked before inflation. In non-parturient, non-lactating cows udder inflation was followed by a decrease in the level of serum calcium and by a very slight and transient rise in plasma inorganic phosphate. There were no significant changes in the levels of serum magnesium in any of the groups.

It is concluded that udder inflation acts essentially by stopping milk secretion and by causing the return of calcium and phosphate from the mammary gland to the blood.

9) Studies of milk fever cases showed that the levels of serum calcium and plasma inorganic phosphate were significantly lower than in normal parturient cows, while the levels of serum magnesium were not significantly different. There was a significant correlation between the severity of the clinical signs and the extent of the depression in serum calcium and inorganic phosphate, but no correlation with the levels of serum magnesium. It is concluded that, while the level of serum magnesium may be of importance in explaining the lack of tetanic symptoms in the disease, it is not of any great significance in typical milk fever.

10) In milk fever cases which responded satisfactorily to treatment there was a marked rise in the level of plasma inorganic phosphate, while in unsatisfactory cases the phosphate level showed a slight initial rise and then a gradual fall. This suggests that a rise in the level of this constituent is necessary for satisfactory recovery. It is pointed out,

however, that the rise in plasma inorganic phosphate may be only an indication that the normal regulating mechanisms are overcoming the low blood phosphate levels, and that the use of phosphate injections will not necessarily have any marked beneficial effect.

In considering the results for plasma inorganic phosphate of the study as a whole, it is concluded that more work is necessary before the exact significance of its role in milk fever can be appreciated.

11) It was found that cases of milk fever occurring either before, or shortly after calving, were more likely to relapse following calcium therapy than were cases occurring later in relation to calving. The behaviour of serum calcium following calcium therapy suggested that this acts essentially by abolishing the clinical signs and allowing time for the normal regulating mechanisms to meet the demands on the blood calcium resulting from the onset of milk secretion. Consequently, cases occurring before, or soon after calving, are more likely to relapse following calcium therapy than are cases occurring later. Cases treated by udder inflation are less likely to relapse since, besides causing resorption of calcium from the mammary gland, and thus abolishing the clinical signs, udder inflation stops further milk secretion and thus removes the basic cause of the fall in blood calcium.

12) The results of the investigation as a whole suggest that there is no primary dysfunction of the calcium regulating mechanisms in milk fever. It is suggested that the nature of the disease is more readily explained by postulating a decrease

with advancing age in the availability of calcium in the osseous tissues for exchange with the blood. In consequence, the animal is unable to cope with the demands for calcium produced by the secretion of colostrum, so that a fall in blood calcium to milk fever levels ensues. Treatment by calcium injection or udder inflation abolishes the clinical signs and allows the normal regulating mechanisms time to restore equilibrium.

REFERENCES.

Allcroft, W.M. (1947a). Vet. J. 103, 2.

Allcroft, W.M. (1947b). Vet. J. 103, 157.

Allcroft, W.M., & Godden, W. (1934). Biochem. J. 28, 1004.

Allcroft, W.M., & Green, H.H. (1934). Biochem. J. 28, 2220.

A.O.A.C. (1945). "Methods of Analysis of the Association of Official Agricultural Chemists". 6th.Edit. A.O.A.C. Washington.

Auger, M.L. (1926a). Rev. gen. Med. vet. 25, 353. (Trans. by Fish, P.A. Cornell Vet. 17, 64. 1927).

Auger, M.L. (1926b). Quoted by Hibbs (1950).

Auger, M.L. (1926c). Lyons Monograph, p. 81. (Quoted by Hibbs 1950).

Barker, J.R. (1939). Vet. Rec. 51, 575.

Barker, J.R. (1948). Vet. Rec. 60, 351. (and Personal Communication to Robertson et al. 1948).

Binger, J.C. (1917). J. Pharmacol. 10, 105.

Blood, D.C., & White, I.G. (1949). Aust. vet. J. 25, 114.

Blood D.C., Larsen, L.H., & White, I.G. (1949). Aust. vet. J. 25, 95.

Boddie, G.F. (1949). Vet. Rec. 61, 85.

Boutflour, R. (1949). Rep. on the National Milk Prod. Conf., Cirencester, p. 14.

Clark, E.P., & Collip, J.B. (1925). J. Biol. Chem. 63, 461.

Craige, A.H., Jr., (1947). Amer. J. vet. Res. 8, 260.

Craige, A.H., Jr., & Stoll, I.V. (1947). Amer. J. vet. Res. 8, 168.

Craige, A.H., Jr., Johnson, A.B., Blackburn, E.G. & Coffin, J.M. (1949). Amer. J. vet. Res. 10, 217.

Denis, W. (1922). J. biol. Chem. 52, 411.

Dryerre, H., & Greig, J.R. (1925). Vet. Rec. 5, 225.

Dryerre, H., & Greig. J.R. (1928). Vet. Rec. 8, 721.

Duckworth, J. & Godden, W. (1940). J. Dairy Res. 11, 9.

Duncan, G.G. (1942). "Diseases of Metabolism", W.B. Saunders Co., Philadelphia, p. 174.

Edwards, J.T. (1926). Vet. Rec. 6, 336.

Fish, P.A. (1927). Cornell Vet. 17, 99.

Fish, P.A. (1928). J. Amer. vet. med. Ass. 73, 10.

Fish, P.A. (1929). Cornell Vet. 19, 147.

Fish, P.A. (1930). 11th Int. Vet. Congr. Lond. 2, 330.

Fiske, C.H., & SubbaRow, Y. (1925). J. biol. Chem. 66, 375.

Frank, E.R. (1944). "Veterinary Surgery Notes". Burgess Publishing Co., Minneapolis. p. 180.

Gamm, O. (1950). Acta endocr., Copenhagen. 5, 413.

Garrison, E.R. & Turner, C.W. (1936). Mo. Agr. exp. res. Sta. Bull. 234. (Cited by Niedermeier & Smith. 1950).

Gemmill, C.L., & Ribeiro, B.A. (1933). Amer. J. Physiol. 103, 367.

Godden, W., & Allcroft, W.M. (1932). Biochem. J. 26, 1640.

Godden, W., & Duckworth, J. (1935). Biochem. J. 29, 445.

Graham, W.R., & Kay, H.D. (1934). J. Dairy Res. 5, 54.

Green, H.H. (1939). Vet. Rec. 51, 1179.

Greenwald, I. (1918). J. Pharmacol. 11, 281.

Greenwald, I. (1931). J. biol. Chem. 93, 551.

Greig, J.R. (1929). Vet. Rec. 9, 509.

Greig, J.R. (1930a). 11th. Int. Vet. Congr. Lond. 3, 306.

Greig, J.R. (1930b). Vet. Rec. 10, 301.

Harding, V.J. (1929). J. Amer. vet. med. Ass. 75, 702.

Hawk, P.B. (1947). "Practical Physiological Chemistry". 12th Edit. J.&.A. Churchill, Lond.

Hayden, C.E. (1927). Cornell Vet. 17, 121.

Hayden, C.E. (1929). Cornell Vet. 19, 285.

Hayden, C.E. (1938). Cornell Vet. 28, 152.

Hayden, C.E., & Sholl, L.B. (1924). Rep. N.Y. State Vet. Coll. p. 91. 1923-24.

Hayden, C.E., & Sholl, L.B. (1925). Rep. N.Y. State Vet.Coll. p. 200. 1924-25.

Henderson, J.A. (1938). Cornell Vet. 28, 173.

Hibbs, J.W. (1950). J. Dairy Sci. 33, 758.

Hibbs, J.W., Krauss, W.E., Monroe, C.F., & Sutton, T.S. (1946a). J. Dairy Sci. 29, 617.

Hibbs, J.W., Krauss, W.E., Pounden, W.D., Monroe, C.F., & Sutton, T.S. (1946b). J. Dairy Sci. 29, 767.

Hibbs, J.W., Pounden, W.D., & Krauss, W.E. (1947). J. Dairy Sci. 30, 564.

Hibbs, J.W., Pounden, W.D., & Krauss, W.E. (1951). J. Dairy Sci. 34, 855.

Hill, R., Widdowson, R.W., & Maggs, B.E. (1950). Agriculture. Lond.57, 351.

Hogg, A.H. (1952). Vet. Rec. 64, 39.

Hutyra, F., & Marek, J. (1926). "Special Pathology and Therapeutics of the Diseases of Domestic Animals". 3rd Edit. Bailliere, Tyndall & Cox, Lond. 3, 514.

Johnson, R.E., Eaton, H.D., Spielman, A.A., Matterson, L.D. & Slate, R.J. (1948). Abs. in J. Dairy Sci. 31, 684.

Kloubek, A. (1932). Zverol. Rozpravy. 6, 13. (Vet. Bull. 2, 567, 1932).

Little, W.L., & Keith. (1926). Cited by Little, W.L., & Wright, N.C., Vet. J. 82, 185, 1926.

Little, and Mattick, E.C.V. (1933a). Vet. Rec. 13, 238.

Little, W.L., & Mattick, E.C.V. (1933b). Vet. Rec. 13, 1091.

Little, W.L., & Wright, N.C. (1925). Brit. J. exp. Path.(1925).

Little, W.L. & Wright, N.C. (1926). Vet. J. 82, 185.

McBarron, E.J. (1952). Austr. vet. J. 28, 36.

McLeod, J.R. (1926). J. Lab. clin. Med. 11, 400.

Maguire, L.C. (1926a). Vet. Rec. 6, 484.

Maguire, L.C. (1926b). Vet. Rec. 6, 52.

Mainland, D. (1938). "The Treatment of Laboratory and Clinical Data", Oliver & Boyd, Edin. p. 151 et seq.

Meigs, E.B., & Blatherwick, W.R. (1917). J. biol. Chem. 29, 11.

Meltzer, S.J., & Auer, J. (1908). Amer. J. Physiol. 21, 400.

Mercer, D.W., Eaton, H.D., Johnson, R.E., Spielman, A.A., Plastridge, W.W., Matterson, L.D., & Nesvesky, L. (1949). J. Dairy Sci. 32, 977.

Moodie, E.W. (1952). Thesis submitted for Ph.D. degree, Univ. of Edin., July 1952.

Moore, R.M., & Wingo, W.J. (1942). Amer. J. Physiol. 135, 492.

Moussu, G. & Moussu, R. (1926). Cited by Hibbs (1950).

Mull, J.W. & Bull, A.H. (1932). Amer. J. Obstet. Gynec. 23, 807.

Niedermeier, R.P., & Smith, V.R. (1948). Abs. in J. Dairy Sci. 31, 684.

Niedermeier, R.P., & Smith, V.R. (1950). J. Dairy Sci. 33, 38.

Niedermeier, R.P., Smith, V.R., & Whitehair, C.K. (1949). J. Dairy Sci. 32, 927,

Oberts, W.F., & Plass, E.D. (1932). J. clin. Invest. 11, 123.

Palmer, L.S., Cunningham, H.S., & Eckles, C.H. (1930). J. Dairy Sci. 13, 174.

Petersen, W.E., Hewitt, E.A., Boyd, W.L., & Brown, W.R. (1931). J. Amer. vet. med. Ass. 79, 217.

Petersen, W.E., & Rigor, F.V. (1932). Proc. Soc. exp. Biol., N.Y. 30, 254.

Pribyl, E. (1933). Sverol. Rozpravy. 7, 61. (Nutr. Abs. Rev. 4, 88, 1934-35.)

Robertson, A. (1949). Vet. Rec. 61, 333.

Robertson, A., Burgess, J.W., Marr, A., & Milne, B.J.C. (1948). Vet. Rec. 60, 505.

Schmidt, C.L.A., & Greenberg, D.M. (1935). Physiol. Rev. 15, 287.

Schmidt, J.J. (1902). Maanedsskr. Dyrlaeg. 9, 228, 1897. (Trans. Vet. Rec. 15, 210, 1902-03.)

Schulof, A. (1933). Klin. spisy Sk. zverolek., Brno. 10, 23. (Nutr. Abs. Rev. 4, 699, 1934-35).

Seekles, L. (1937). Tijdschr. Diergeneesk. 64, 866. (Nutr. Abs. Rev. 7, 796, 1937-38).

Seekles, L. (1940a). Tijdschr. Diergeneesk. 67, 231. (Nutr. Abs. Rev. 10, 250, 1(40-41).

Seekles, L. (1940b). Tijdschr. Diergeneesk. 67, 379. (Nutr. Abs. Rev. 10, 445, 1940-41).

Seekles, L. (1948). Brit. Vet. J. 104, 203.

Seekles, L., Sjollema, B., & van der Kaay, F.C. (1932a). Biochem. Z. 244, 1.

Seekles, L., Sjollema, B., & van der Kaay, F.C. (1932b). Biochem. Z. 244, 167.

Seekles, L., Sjollema, B., & van der Kaay, F.C. (1932c). Biochem. Z. 244, 424,

Selye, H. (1946). J. clin. Endocrin. 6, 117.

Schlotthauer, C.F. (1928). Cornell Vet. 18, 217.

Sholl, A.T. (1939). "Mineral Metabolism". Reinhold Pub. Co. N.Y. p. 143.

Sjollema, B. (1928). Biochem. Z. 200, 300.

Sjollema, B. (1932). Nutr. Abs. Rev. 1, 621, 1931-32.

Sjollema, B., & Seekles, L. (1930). Biochem. Z. 229, 358.

Sjollema, B., & Seekles, L. (1932). Klin. Wschr. 11, 989. (Nutr. Abs. Rev. 2, 291. 1932-33).

Sjollema, B., Seekles, L., & van der Kaay, F.C. (1931). Tijdschr. Diergeneesk. 58, 14. (Cited by Sjollema. 1932).

Smith, V.R., & Blosser, T.H. (1947). J. Dairy Sci. 30, 861.

Smith, V.R., & Niedermeier, R.P. (1948). J. Dairy Sci. 31, 683.

Smythe, R.H., (1930). Vet. Rec. 10, 842.

Thomas, R.O., Litovitz, T.A., Rubin, M.I., & Geschickter, C.F. (1952). Amer. J. Physiol. 169, 568.

Tisdall, F.F. (1922). J. biol. Chem. 54, 35.

Van Landingham, A.H., Henderson, H.S., & Bowling, G.S. (1942). J. Dairy Sci. 25, 537.

Ward, G.M., Blosser, T.H., & Adams, M.F. (1952). J. Dairy Sci. 35, 587.

Widmark, E. (1926). Berl. tierartzl. Wschr. 42, 537. (Trans. by Udall, Cornell Vet. 16, 304, 1926.).

Widmark, E., & Carlens, O. (1925a). Svensk Vet.Tidskr. 30. (Cited by Hibbs, 1950).

Widmark, E. & Carlens, O. (1925b). Biochem. Z. 154, 454.

Widmark, E. & Carlens, O. (1925c). Biochem. Z. 158, 3.

Widmark, E. & Carlens, O. (1925d). Biochem. Z. 158, 81.

Wilson, L.T., & Hart, E.B. (1932). J. Dairy Sci. 15, 116.

ACKNOWLEDGEMENTS.

This work has been done under the direction of Dr. Alex. Robertson, to whom I am indebted for much helpful advice and guidance.

I wish to thank Mr. W. M. Mitchell, Director of Veterinary Education, University of Edinburgh, for permission to carry out the work.

My thanks are also due to Dr. H. Paver for his advice on the statistical analysis and presentation of the data.

APPENDIX I.

TABLE I

Serum Calcium in Normal Parturient Cows.

First Calf Animals.

Calcium mgm. per cent.

Time from Calving.	Cow Number.							Mean.
	2	8	9	12	17	57	67	
14 days	-	-	12.00	-	-	10.00	10.60	10.86
12 days	-	-	11.17	-	-	10.90	-	11.04
11 days	-	-	-	-	-	10.20	10.20	10.20
10 days	-	-	-	-	12.08	10.15	-	11.12
9 days	-	-	-	-	-	10.25	10.40	10.32
8 days	-	-	-	-	12.19	10.30	-	11.26
7 days	-	-	11.26	-	-	10.65	11.20	11.04
6 days	-	-	-	11.87	-	10.70	-	11.29
5 days	-	10.60	9.93	10.02	10.60	10.00	10.70	10.31
4 days	-	-	10.70	10.25	-	10.50	-	10.48
3 days	-	11.08	10.50	10.70	11.77	10.40	11.40	10.98
2 days	-	-	10.50	10.91	12.35	9.70	-	10.87
1 day	10.20	-	-	10.42	12.45'	9.90	11.15	10.82
12 hours	-	-	11.20	11.18	12.72	10.45'	-	11.39
4 hours	-	-	11.20	-	-	8.80	11.20	10.40
1 hour	-	-	10.20	-	-	9.50	10.60	10.10
Calving	8.00	10.10	-	10.97	12.19	9.80	10.40	10.24
2 hours	-	-	10.67	-	11.79	-	10.40	10.95
4 hours	8.77	-	-	12.77	-	9.60	10.45	10.39
8 hours	-	-	10.30	-	-	10.00	-	10.15
12 hours	-	10.85	-	10.18	12.29	9.50	9.75	10.51
16 hours	-	-	-	-	-	9.35	9.90	9.63
20 hours	9.25	-	-	-	11.77	8.35	-	9.79
24 hours	-	-	-	11.83	-	-	9.30'	10.56
32 hours	-	-	11.02	-	-	8.95	9.15	9.71
40 hours	-	-	-	11.02	11.13	9.00	9.20	9.77
2 days	-	10.00	-	10.86	-	9.00	9.32"	9.78
3-4 days	10.70	-	-	-	12.58	9.65	10.60	10.88
5-7 days	-	-	10.81	10.60	-	10.30	10.75	10.62
10-20 days	10.92	10.55	12.29	11.87	10.40	10.15	10.80	10.98

' indicates the mean of two samples.

" indicates the mean of three samples.

TABLE 2.

Serum Calcium in Normal Parturient Cows.

Second Calf Animals.

Calcium mgm. per cent.

Time from Calving	Cow Number.								Mean.
	54	4	5	16	24	53	3	59	
14 days	-	-	-	-	-	-	-	10.00	10.00
12 days	-	-	-	10.70	-	10.35	-	9.60	10.22
11 days	-	10.81	-	10.09	-	-	-	9.90	10.27
10 days	-	-	-	-	-	-	-	10.10	10.10
9 days	-	10.57	-	-	-	-	-	10.35	10.46
8 days	-	-	-	11.45	10.56	-	-	-	11.00
7 days	10.65	11.55	-	-	-	10.65	-	9.75	10.65
6 days	-	11.42	-	-	11.28	9.80	-	9.90	10.60
5 days	10.50	9.93	-	11.13	-	10.35	11.60	10.00	10.59
4 days	10.65	10.25	9.98	11.29	11.13	9.95	-	10.60	10.55
3 days	10.45	-	-	11.29	10.56	9.85	11.23	9.60	10.50
2 days	10.07	10.50	11.78	12.08	10.71	10.25	10.82	-	10.88
1 day	11.10	10.82	-	12.61	10.01'	10.00	-	9.90	10.74
12 hours	10.20	10.00	-	12.51	-	10.00	9.49'	-	10.44
4 hours	-	9.05	-	11.98	-	10.00	9.52	9.60	10.03
1 hour	10.05	9.86	-	-	9.93	9.85	9.52	-	9.84
Calving	10.05	10.07	10.52	11.71	8.89	9.90	-	9.10	9.99
2 hours	-	-	8.80	11.93	9.99	9.90	10.09	8.10	9.79
4 hours	10.35	9.40	-	-	10.09	9.70	9.56	9.10	9.70
8 hours	10.50	-	-	11.93	10.29	9.35	9.14	9.10	10.05
12 hours	9.45	-	-	12.35	9.57	-	8.52	8.15	9.61
16 hours	9.40	-	8.97	-	-	9.70	-	-	9.36
20 hours	-	-	-	-	9.57	8.90	-	-	9.24
24 hours	-	9.25	-	11.36	-	8.25	8.79	8.20'	9.27
32 hours	8.90	-	-	11.10	-	8.55	-	7.85	9.10
40 hours	8.40	-	-	-	9.72	-	-	-	9.06
2 days	9.90	9.12	-	11.27	9.57	8.90'	8.37	7.72'	9.26
3-4 days	11.00	10.51	10.62	11.37	9.83	10.00	9.26	8.75	10.17
5-7 days	9.25	-	-	-	-	9.80	8.99	9.00	9.26
10-20 days	9.00	10.49	9.66	11.08	10.56	9.90	10.29	9.20	10.02

' indicates the mean of two samples.

TABLE 3.

Serum Calcium in Normal Parturient Cows.

Third Calf Animals.

Calcium mgm. per cent.

Time from Calving	Cow Number.									Mean.
	6	10	11	13	14	15	22	27	55	
14 days	10.60	-	-	-	-	10.49	-	-	-	10.54
12 days	10.60	10.60	-	-	-	-	-	-	-	10.60
11 days	-	-	11.18	-	-	10.54	-	-	-	10.86
10 days	-	-	-	-	-	12.08	-	-	-	12.08
9 days	-	-	11.13	-	-	-	-	10.09	10.00	10.41
8 days	-	-	-	-	-	12.82	-	-	-	12.82
7 days	-	-	11.61'	-	-	11.29	10.50	10.82	10.80	11.00
6 days	-	-	-	10.86	10.42	-	-	9.83	10.75	10.47
5 days	-	-	10.87	-	11.07	11.77	11.54	10.24	10.30	10.97
4 days	-	-	10.02	10.46	-	12.24	-	11.34	10.40	10.89
3 days	-	-	-	11.29	8.58	12.35	10.71	11.02	10.30	11.15
2 days	10.97	11.03	-	11.39	9.54	11.55	11.18	10.82	10.75	10.90
1 day	-	10.97	10.81	-	-	-	10.71	9.83	10.40	10.54
12 hours	-	-	-	10.34	-	12.08	-	9.83	10.50	10.69
4 hours	10.18	-	-	-	10.76	-	-	-	8.75	9.89
1 hour	8.58	10.70	11.61	10.39	10.33	-	-	-	8.55	10.03
Calving	-	11.18	11.29	-	8.59	10.23	10.41	8.89	8.00	9.79
2 hours	9.20	8.33	11.29	10.07	-	10.12	9.72	8.94	8.70	9.55
4 hours	8.16	-	10.92	9.12	8.69	10.17	9.72	8.63	7.70	9.12
8 hours	6.94	8.82	10.60	-	-	-	10.29	7.96	8.65	8.88
12 hours	-	-	-	9.92	-	9.72	-	7.96	9.10	9.18
16 hours	7.30	-	11.45	8.85	8.11	-	-	-	8.50	8.84
20 hours	-	9.73	-	-	-	-	8.99	8.42	7.50	8.66
24 hours	7.26	-	9.38	-	8.59	9.96	9.83	8.94	-	8.99
32 hours	-	-	-	-	-	-	-	-	8.00	8.00
40 hours	9.12	-	9.22	10.49	8.70	-	-	-	8.65	9.24
2 days	10.04	9.81	10.20'	8.85	9.69	12.19	8.99	9.40'	8.40	9.73
3-4 days	10.09	-	-	9.98	-	-	9.20	9.46'	9.10'	9.56
5-7 days	-	-	-	-	10.28	-	9.88	10.56	9.20	9.98
10-20 days	10.70	11.60	11.13	12.08	11.87	10.87	10.87	10.61	9.95	11.08

' indicates the mean of two samples.

TABLE 4.

Serum Calcium in Normal Parturient Cows.

Fourth *Calf Animals.

Calcium mgm. per cent.

Time from Calving	Cow Number.								Mean.
	18	19	26	30	20	23	21	28	
14 days	-	-	12.89	-	-	-	-	-	12.89
12 days	11.82	-	10.45	-	-	-	-	-	11.14
11 days	-	-	-	-	-	-	-	-	-
10 days	-	-	10.71	-	-	-	-	10.82	10.77
9 days	-	-	-	-	11.65	10.82	-	-	11.24
8 days	11.61	-	-	-	-	-	11.13	-	11.37
7 days	-	-	10.24	10.15	-	-	-	-	10.19
6 days	11.98	-	-	-	11.23	10.50	-	10.93'	11.16
5 days	10.97	-	10.71	-	-	-	10.88	-	10.85
4 days	-	-	-	-	9.15	10.92	-	11.75	10.61
3 days	12.83	-	10.56	-	8.79	-	11.23	10.60	10.80
2 days	12.67	10.81	10.35	10.90	11.59	11.13	11.18	10.87	11.19
1 day	11.63	10.14	-	-	12.48	11.65	11.18	9.57	11.10
12 hours	10.97	-	10.87	-	10.19	-	11.23	8.84	10.42
4 hours	-	10.05	-	-	-	-	-	-	10.05
1 hour	-	-	8.32	-	-	9.98	-	-	9.15
Calving	9.88	8.48	7.93'	-	7.07	-	8.63	8.42	8.40
2 hours	12.62	8.32	-	-	-	8.74	9.25	-	9.73
4 hours	11.13	9.49	6.55	5.65	8.74	9.62	9.15	8.22	8.57
8 hours	-	9.65	6.86	7.25	8.58	10.04	9.52	8.32	8.60
12 hours	9.86	9.33	8.42	-	8.37	-	9.41	8.99	9.25
16 hours	10.23	10.72	-	7.45	8.22	9.88	8.94	-	9.24
20 hours	-	-	9.10	-	8.68	-	-	7.39	8.39
24 hours	-	-	-	7.35	-	-	-	-	7.35
32 hours	10.14	-	8.84	-	9.98	9.62	10.19	8.84	9.60
40 hours	-	10.02	9.67	-	-	-	-	-	9.85
2 days	10.14	10.87	9.88'	-	10.22'	8.89	9.78	-	9.96
3-4 days	-	12.45	10.55'	-	9.46	9.41	10.50	7.80	10.03
5-7 days	-	-	10.11'	-	10.29	-	10.40	10.40	10.30
10-20 days	9.90	11.08	10.30	-	-	-	10.66	11.07	10.60

' indicates the mean of two samples.

* Fourth indicates fourth and over.

TABLE 5.

Serum Calcium in Normal Parturient Cows.

Comparison of the 2 day prepartum level compared with the lowest value in the period 12 hours prepartum to 3 days postpartum.

Calcium mgm. per cent.

Cow Number	2 days Prepartum	Lowest Value	Difference.	%age Fall.
First Calf Animal.				
2	10.20	8.00	2.20	21.56
8	11.08	10.00	1.08	9.75
9	10.50	10.20	0.30	2.85
12	10.91	10.18	0.73	6.69
17	12.35	11.13	1.22	9.88
57	9.70	8.35	1.35	13.92
67	11.15	9.15	2.00	17.94
Second Calf Animal.				
3	10.82	8.37	2.45	22.64
4	10.50	9.05	1.45	13.81
5	11.72	8.80	2.92	24.91
16	12.08	11.10	0.98	8.11
24	10.71	8.89	1.82	16.99
53	10.25	8.25	2.00	19.51
54	10.07	8.40	1.67	16.58
59	9.60	7.72	1.88	19.58
Third Calf Animal.				
6	10.97	6.94	4.03	36.74
10	11.03	8.33	2.70	24.47
11	10.81	9.22	1.59	14.71
13	11.39	8.85	2.54	22.30
14	9.54	8.11	1.43	14.99
15	11.55	9.73	1.83	15.84
22	11.18	8.99	2.19	19.59
27	10.82	7.96	2.86	26.42
55	10.75	7.50	3.25	30.23
Fourth* Calf Animal.				
18	12.67	9.86	2.81	22.18
19	10.81	8.32	2.49	23.03
20	11.59	7.07	4.52	38.99
21	11.18	8.63	2.55	22.81
23	11.13	8.74	2.39	21.47
26	10.35	6.55	3.80	36.71
28	10.87	7.39	3.48	32.01
30	10.90	5.65	5.25	48.16

* Fourth means fourth or subsequent calf.

TABLE 6.

Serum Magnesium in Normal Parturient Cows.

First Calf Animals.

Magnesium mgm. per cent.

Time from Calfing.	Cow Number.							Mean.
	2	8	9	12	17	57	67	
14 days	-	-	2.45	-	-	2.85	3.10	2.80
12 days	-	-	2.31	-	-	2.80	-	2.55
11 days	-	-	-	-	-	3.05	2.90	2.97
10 days	-	-	-	-	2.32	2.80	-	2.56
9 days	-	-	-	-	-	3.25	2.85	3.05
8 days	-	-	-	-	-	3.25	-	3.25
7 days	-	-	2.55	-	-	3.10	3.10	2.92
6 days	-	-	-	2.59	-	2.85	-	2.72
5 days	-	2.45	2.17	2.91	2.78	3.55	3.05	2.82
4 days	-	-	2.34	2.85	-	3.65	-	2.95
3 days	-	2.45	3.25	-	2.92	3.10	3.10	2.92
2 days	-	-	3.30	2.55	2.56	3.20	-	2.90
1 day	2.90	-	-	2.50	2.82'	3.15	3.25	2.92
12 hours	-	-	3.40	2.40	2.89	2.90'	-	2.90
4 hours	-	-	3.00	-	-	3.15	3.05	3.06
1 hour	-	-	2.75	-	-	2.85	3.15	2.92
Calving	2.85	2.45	-	2.55	3.12	2.90	3.30	2.86
2 hours	-	-	2.90	-	3.13	-	3.20	3.08
4 hours	2.65	2.60	-	2.95	-	3.10	3.15	2.89
8 hours	-	2.80	2.70	-	-	2.80	-	2.75
12 hours	-	2.80	-	2.59	2.94	3.05	3.00	2.88
16 hours	-	-	-	-	-	2.95	3.05	3.00
20 hours	-	2.80	2.55	-	2.87	3.05	-	2.82
24 hours	-	-	-	1.95	-	-	2.97'	2.78
32 hours	-	-	2.55	-	-	2.90	2.90	2.78
40 hours	-	-	-	2.06	2.86	2.95	2.90	2.69
2 days	-	2.40	-	1.96	-	2.85	3.03"	2.56
3-4 days	2.90	-	-	-	2.76	2.90	3.05	2.90
5-7 days	-	-	2.65	-	-	2.95	3.20	2.93
10-20 days	2.55	2.51	3.00	2.91	2.52	2.75	3.15	2.77

' indicates the mean of two samples.

" indicates the mean of three samples.

TABLE 7.

Serum Magnesium in Normal Parturient Cows.

Second Calf Animals.

Magnesium mgm. per cent.

Time from Calving.	Cow Number.								Mean.
	54	4	5	16	24	53	3	59	
14 days	-	-	-	-	-	-	-	3.05	3.05
12 days	-	-	-	2.08	-	3.20	-	3.05	2.78
11 days	-	2.20	-	2.21	-	-	-	2.95	2.45
10 days	-	-	-	-	-	-	-	2.90	2.90
9 days	-	2.69	-	-	-	-	-	2.90	2.70
8 days	-	-	-	2.47	2.77	-	-	-	2.62
7 ddys	3.05	2.60	-	-	-	2.95	-	2.90	2.87
6 days	-	2.50	-	-	2.39	2.95	-	2.75	2.65
5 days	3.40	2.85	-	2.85	-	3.10	2.59	2.95	2.96
4 days	3.40	2.70	2.65	2.45	2.32	3.30	-	2.80	2.80
3 days	3.40	-	-	2.94	2.61	3.15	2.63	2.90	2.94
2 days	3.30	2.35	2.62	2.76	2.25	3.40	2.64	-	2.76
1 day	3.25	2.75	-	2.70	2.53'	3.40	-	3.25	2.98
12 hours	3.55	2.82	-	3.00	-	3.50	2.80'	-	3.11
4 hours	-	-	-	2.98	-	3.60	3.27	3.40	3.31
1 hour	3.90	3.00	-	-	2.51	3.30	3.34	-	3.21
Calving	3.75	3.20	2.90	2.85	3.34	3.35	-	3.70	3.29
2 hours	-	-	2.75	2.94	2.89	3.15	2.98	3.50	3.04
4 hours	3.95	2.80	-	-	2.63	3.55	3.00	3.65	3.26
8 hours	3.55	-	-	2.81	2.47	3.65	3.01	3.05	3.09
12 hours	3.55	-	-	2.76	2.55	-	3.07	3.45	3.08
16 hours	3.70	-	3.35	-	-	3.05	-	-	3.37
20 hours	-	-	-	-	2.81	3.20	-	-	3.01
24 hours	-	2.90	-	2.52	-	3.10	2.92	3.50'	2.99
32 hours	3.40	-	-	2.91	-	3.00	-	3.50	3.20
40 hours	3.55	-	-	-	3.13	-	-	-	3.34
2 days	3.95	3.00	-	2.82	3.37	3.12'	2.21	3.47'	3.13
3-4 days	3.25	2.50	3.15	2.62	2.86	2.95	2.81	2.85	2.87
5-7 days	3.05	-	-	-	-	2.90	2.73	3.20	2.97
10-20 days	2.90	2.85	3.00	2.60	2.50	3.05	2.77	3.25	2.86

' indicates the mean of two samples.

TABLE 8.

Serum Magnesium in Normal Parturient Cows.

Third Calf Animals.

Magnesium mgm. per cent.

Time from Calving.	Cow Number.									Mean
	10	6	11	13	14	15	22	27	55	
14 days	-	2.40	-	-	-	2.48	-	-	-	2.44
12 days	2.50	2.59	-	-	-	-	-	-	-	2.45
11 days	-	-	2.40	-	-	2.68	-	-	-	2.54
10 days	-	-	-	-	-	2.18	-	-	-	2.18
9 days	-	-	-	-	-	-	-	2.50	3.10	2.80
8 days	-	-	-	-	-	-	-	-	-	-
7 days	-	-	2.24'	-	-	2.24	2.41	3.03	3.35	2.65
6 days	-	-	-	2.53	2.64	-	-	3.13	3.05	2.84
5 days	-	-	-	-	2.65	2.38	2.56	2.68	2.95	2.64
4 days	-	-	2.35	2.31	-	2.21	-	2.41	3.20	2.50
3 days	-	-	-	2.50	2.50	2.25	2.25	2.80	3.25	2.59
2 days	2.30	2.59	2.25	2.36	-	2.40	2.51	3.08	3.30	2.59
1 day	2.14	-	1.88	-	-	-	2.76	3.45	3.25	2.69
12 hours	-	-	-	2.17	-	2.58	-	3.09	3.10	2.74
4 hours	-	2.92	-	-	2.75	-	-	-	3.60	3.09
1 hour	2.40	3.40	2.46	2.56	2.87	-	-	-	4.25	2.99
Calving	3.20	-	2.55	3.05	3.14	2.86	3.02	3.37	3.40	3.07
2 hours	1.95	3.10	3.15	2.76	-	3.03	3.15	3.39	4.50	3.13
4 hours	-	-	3.00	2.94	3.00	2.98	2.89	3.29	4.40	3.21
8 hours	2.56	3.90	2.92	-	-	-	2.92	3.56	4.60	3.41
12 hours	-	-	-	2.55	-	2.70	-	3.46	4.30	3.25
16 hours	-	3.50	2.70	2.94	2.59	-	-	-	4.65	3.28
20 hours	2.51	-	-	-	-	-	2.58	3.21	4.65	3.24
24 hours	-	4.00	3.06	-	2.66	2.56	3.26	2.89	-	3.07
32 hours	-	-	-	-	-	-	-	-	3.90	3.90
40 hours	-	3.20	3.20	2.92	2.59	-	-	-	3.95	3.17
2 days	2.55	3.12	2.54'	2.44	2.64	2.56	3.26	2.95'	3.65	2.86
3-4 days	-	2.51	-	2.70	-	2.56	3.49	2.59'	3.20'	2.84
5-7 days	-	-	-	-	2.81	-	2.63	2.70	2.90	2.76
10-20 days	2.55	2.62	2.96	2.96	2.94	2.62	2.81	3.10	3.10	2.82

' indicates the mean of two samples.

TABLE 9.

Serum Magnesium in Normal Parturient Cows.

Fourth* Calf Animals.

Magnesium mgm.per cent.

Time from Calving	Cow Number.								Mean
	18	19	26	30	23	20	21	28	
14 days	-	-	2.89	-	-	-	-	-	2.89
12 days	2.17	-	2.90	-	-	-	-	-	2.54
11 days	-	-	-	-	-	-	-	-	-
10 days	-	-	2.80	-	-	-	-	2.44	2.62
9 days	-	-	-	-	2.54	2.58	-	-	2.56
8 days	2.38	-	-	-	-	-	2.59	-	2.49
7 days	-	-	2.85	3.50	-	-	-	-	3.17
6 days	2.76	-	-	-	2.61	3.07	-	3.08'	2.88
5 days	2.47	-	2.90	-	-	-	2.77	-	2.71
4 days	-	-	-	-	2.74	3.13	-	2.92	2.93
3 days	2.47	-	3.41	-	-	3.37	2.85	2.70	2.96
2 days	2.24	2.98	3.20	3.05	2.35	2.61	2.82	2.55	2.73
1 day	2.65'	3.23	-	-	2.33	2.56	2.59	2.90	2.71
12 hours	2.53	-	2.38	-	-	2.77	2.74	3.50	2.78
4 hours	-	3.04	-	-	-	-	-	-	3.04
1 hour	-	-	2.99	-	2.36	-	-	-	2.68
Calving	3.03	3.60	2.88'	-	-	3.45	3.14	2.75	3.31
2 hours	3.03	3.88	-	-	2.25	-	3.36	-	3.13
4 hours	2.73	4.13	3.45	3.10	3.21	3.23	3.72	3.40	3.37
8 hours	-	4.10	3.45	3.70	2.29	3.79	3.65	3.55	3.50
12 hours	3.04	4.34	3.62	-	-	3.45	3.10	3.40	3.49
16 hours	3.18	4.20	-	3.70	2.72	3.34	3.39	-	3.42
20 hours	-	-	3.70	-	-	3.22	-	3.15	3.36
24 hours	-	-	-	3.50	-	-	-	-	3.50
32 hours	2.86	-	3.16	-	3.34	3.15	3.46	3.10	3.18
40 hours	-	4.00	3.08	-	-	-	-	-	3.54
2 days	2.41	4.00	2.70'	-	3.48	2.65'	2.87	-	3.02
3-4 days	-	2.45	2.39'	-	3.02	2.16	2.55	3.17	2.62
5-7 days	-	-	2.87'	-	-	2.68	2.51	2.60	2.67
10-20 days	2.72	3.52	2.62	-	-	-	2.68	3.08	2.92

' indicates the mean of two samples.

* Fourth means fourth and over.

TABLE 10.

Serum Magnesium in Normal Parturient Cows.

Comparison of the 2 day prepartum level compared with the highest value in the period 12 hours prepartum to 3 days postpartum.

Serum Magnesium mgm. per cent.

Cow Number	2 days prepartum	Highest value	Difference	%age rise
First Calf Animal.				
2	2.90	2.90	0.00	0.00
8	2.45	2.80	0.35	14.29
9	3.30	3.40	0.10	3.03
12	2.55	2.95	0.40	15.69
17	2.56	3.13	0.57	22.26
57	3.20	3.15	-0.05	-1.56
67	3.10	3.30	0.20	6.45
Second Calf Animal.				
3	2.64	3.34	0.70	26.51
4	2.35	3.20	0.85	36.17
5	2.62	3.35	0.73	27.86
16	2.76	3.00	0.24	8.69
24	2.25	3.37	1.12	49.77
53	3.40	3.65	0.25	7.35
54	3.30	3.95	0.65	19.70
59	2.90	3.70	0.80	27.58
Third Calf Animals.				
6	2.59	4.00	1.41	54.44
10	2.30	3.20	0.90	39.13
11	2.25	3.20	0.95	42.22
13	2.36	3.05	0.69	29.24
14	2.50	3.14	0.64	25.60
15	2.40	3.03	0.63	26.25
22	2.51	3.49	0.98	39.05
27	3.08	3.54	0.46	14.94
55	3.30	4.65	1.35	40.91
Fourth Calf Animals.**				
18	2.24	3.18	0.96	42.86
19	2.98	4.34	1.36	45.64
20	2.61	3.79	1.18	45.21
21	2.82	3.72	0.90	31.91
23	2.35	3.48	1.13	48.09
26	3.20	3.70	0.50	15.63
28	2.55	3.75	1.20	47.06
30	3.05	3.70	0.65	21.31

** Fourth means fourth or subsequent calf.

TABLE 11.

Plasma Inorganic Phosphate in Normal Parturient Cows.

First Calf Animals.

Inorganic Phosphate mgm. per cent.

Time from calving.	Cow Number.							Mean.
	2	8	9	12	17	57	67	
14 days	-	-	6.65	-	-	6.00	6.00	6.22
12 days	-	-	7.65	-	-	6.80	-	7.28
11 days	-	-	-	-	-	5.40	5.70	5.55
10 days	-	-	-	-	3.90	6.25	-	5.08
9 days	-	-	-	-	-	5.60	6.10	5.85
8 days	-	-	6.50	-	5.50	5.95	-	5.98
7 days	-	-	6.80	5.00	-	6.50	5.20	5.90
6 days	-	-	-	5.40	-	5.60	-	5.50
5 days	-	5.35	6.10	5.50	4.60	6.45	5.80	5.62
4 days	-	-	4.20	6.50	-	6.35	-	5.68
3 days	-	7.25	4.00	6.12	6.40	7.00	5.85	6.10
2 days	-	-	4.85	5.20	6.40	5.95	-	5.60
1 day	4.80	-	-	6.05	5.30'	5.40	4.80	5.27
12 hours	-	-	6.15	5.20	5.10	5.55'	-	5.50
4 hours	-	-	5.50	6.50	-	5.60	4.30	5.48
1 hour	3.30	-	6.20	-	-	5.00	3.40	4.48
Calving	2.70	5.80	-	4.35	4.60	5.50	4.10	4.51
2 hours	-	5.05	4.90	-	4.40	-	5.35	4.92
4 hours	5.80	8.15	-	7.25	-	6.95	5.60	6.75
8 hours	-	-	6.80	-	-	7.45	-	7.13
12 hours	-	-	-	7.20	5.70	7.30	5.45	6.41
16 hours	-	-	6.25	-	-	6.90	4.75	5.97
20 hours	7.50	-	-	-	4.80	6.70	-	6.33
1 day	-	-	-	6.15	-	-	4.65'	5.40
32 hours	-	-	6.10	4.35	3.50	5.90	4.85	4.94
40 hours	-	-	-	-	-	5.15	3.70	4.42
2 days	-	6.45	-	5.35	-	3.15	4.30"	4.81
3-4 days	6.00	-	-	-	3.00	3.65	4.95	4.40
5-7 days	-	-	6.75	5.10	-	5.65	4.40	5.48
10-20 days	5.90	6.10	6.00	6.50	6.00	5.85	5.30	5.98

' indicates the mean of 2 samples.

" indicates the mean of 3 samples.

TABLE 12.

Plasma Inorganic Phosphate in Normal Parturient Cows.

Second Calf Animals.

Time from Calving	Inorganic Phosphate mgm. per cent. Cow Number.								Mean.
	54	4	5	16	24	53	3	59	
14 days	-	-	-	-	-	-	-	6.40	6.40
12 days	-	-	-	5.50	-	7.30	-	6.10	6.30
11 days	-	5.70	-	6.80	-	-	-	6.10	6.20
10 days	-	-	-	-	-	-	-	5.05	5.05
9 days	-	7.00	-	-	-	-	-	5.90	6.45
8 days	-	-	-	5.45	6.10	-	-	-	5.78
7 days	6.65	6.80	-	-	-	6.00	-	5.90	6.34
6 days	-	6.35	-	-	4.90	7.40	-	5.75	6.10
5 days	5.85	7.40	-	4.70	-	5.40	6.70	6.80	6.14
4 days	4.50	5.40	4.75	5.70	5.40	6.45	-	6.65	5.55
3 days4	5.55	-	-	6.50	6.20	5.60	5.20	6.60	5.94
2 days	6.35	6.10	6.75	5.90	6.20	6.30	5.30	-	6.13
1 day	6.25	6.80'	-	5.40	5.25'	6.40	-	6.60	6.12
12 hours	6.80	5.60	-	5.20	-	5.30	5.65'	-	5.71
4 hours	-	4.60	-	4.60	-	6.10	4.15	5.25	4.94
1 hour	4.85	3.45	-	-	4.20	4.90	5.70	-	4.62
Calving	4.15	3.80	4.50	3.20	2.90'	5.60	-	3.40	3.94
2 hours	-	-	5.05	3.50	-	5.80	5.00	3.25	4.46
4 hours	6.15	6.15	-	-	5.00	6.75	5.90	5.25	5.87
8 hours	5.65	-	-	6.00	4.70	7.15	6.80	4.80	5.85
12 hours	6.05	-	-	6.50	5.00	-	6.40	5.70	5.93
16 hours	5.40	-	5.85	-	-	6.80	-	-	6.02
20 hours	-	-	-	-	5.20	6.30	-	-	5.75
24 hours	-	7.05	-	5.80	-	5.90	5.90	4.95'	5.92
32 hours	5.90	-	-	6.15	-	6.90	-	4.65	5.90
40 hours	5.85	-	-	-	4.90	-	-	-	5.38
2 days	4.65	5.85	-	6.20	5.50	5.65'	4.20	4.90'	5.28
3-4 days	5.05	6.45	5.80	5.00	5.50	6.00	5.00	6.70	5.69
5-7 days	6.50	-	-	-	-	5.40	6.30	6.10	6.08
10-20 days	4.80	6.55	6.12	8.20	5.60	4.95	5.80	5.70	5.72

' indicates the mean of two samples.

TABLE 13

Plasma Inorganic Phosphate in Normal Parturient Cows.

Third Calf Animals

Inorganic Phosphate mgm. per cent.

time from Calving.	Cow Number.									Mean.
	10	6	11	13	14	15	22	27	55	
14 days	-	5.20	-	-	-	6.70	-	-	-	5.95
12 days	4.40	6.05	-	-	-	-	-	-	-	5.33
11 days	-	-	5.30	-	-	5.10	-	-	-	5.20
10 days	-	-	-	-	-	6.20	-	-	-	6.20
9 days	-	-	5.35	-	-	-	-	6.00	5.30	5.72
8 days	-	-	5.80	-	-	5.80	-	-	-	5.80
7 days	-	-	5.80'	-	-	4.90	3.50	5.35	5.50	5.82
6 days	-	-	5.75	6.00	7.00	-	-	5.25	5.35	5.87
5 days	-	-	5.50	-	5.90	6.55	6.20	4.55	5.75	5.74
4 days	-	-	5.40	5.50	-	6.30	-	4.80	6.25	5.65
3 days	7.15	-	-	5.70	6.30	6.70	4.50	4.20	6.20	5.82
2 days	6.30	5.85	5.50'	5.00	5.20	6.00	4.80	4.90	6.95	5.60
1 day	6.95	-	5.35	-	-	-	6.20	4.70	6.20	5.88
12 hours	-	-	-	5.50	-	6.20	-	4.85	5.40	5.49
4 hours	-	4.15	-	-	4.55	-	-	-	4.90	4.53
1 hour	4.25	3.20	4.20	3.50	3.80	-	-	-	3.20	3.69
Calving	3.40	-	3.85	3.60	3.25	3.00	3.50	3.60	3.10	3.41
2 hours	3.65	3.15	3.55	3.85	-	3.10	3.80	2.75	5.40	3.43
4 hours	-	4.75	5.85	5.25	4.60	4.05	4.90	2.20	5.00	4.58
8 hours	6.40	3.75	6.20	-	-	-	5.60	2.20	4.40	4.88
12 hours	-	-	-	4.80	-	6.50	-	2.50	4.90	4.68
16 hours	-	3.10	5.30	4.90	3.50	-	-	-	4.45	4.25
20 hours	6.20	-	-	-	-	-	5.20	5.20	4.10	5.18
24 hours	-	3.00	4.40	-	3.10	5.40	4.60	4.30	-	4.13
32 hours	-	-	-	-	-	-	-	-	3.50	3.50
40 hours	-	2.65	4.45	3.95	3.00	-	-	-	4.00	3.65
2 days	7.12	4.15	5.30'	5.40	3.90	5.70	5.00	4.05'	2.00	4.78
3-4 days	-	7.10	-	7.00	-	5.80	4.70	4.10'	3.95'	5.44
5-7 days	-	-	-	-	6.30	-	5.10	3.90	4.45	4.99
10-20 days	5.00	7.05	5.10	6.60	7.30	7.00	7.40	5.70	5.20	6.26

' indicates the mean of two samples.

TABLE 14.

Plasma Inorganic Phosphate in Normal Parturient Cows.

Fourth* Calf Animals.

Inorganic Phosphate mgm. per cent.

Time from Calving.	Cow Number.								Mean.
	18	19	26	30	23	20	21	28	
14 days	-	-	4.60	-	-	-	-	-	4.60
12 days	5.70	-	5.20	-	-	-	-	-	5.45
11 days	-	-	-	-	-	-	-	-	-
10 days	-	-	5.80	-	-	-	-	4.90	5.35
9 days	-	-	-	-	4.70	6.05	-	-	5.38
8 days	4.45	-	-	-	-	-	6.35	-	5.40
7 days	-	-	5.90	-	-	-	-	-	5.90
6 days	4.75	-	-	5.25	4.10	8.10	-	5.55'	5.55
5 days	4.60	-	4.60	-	-	-	6.20	-	5.13
4 days	-	-	-	-	4.40	8.80	-	4.55	5.92
3 days	4.15	-	5.25	-	-	9.40	5.80	4.65	5.84
2 days	4.60	3.80	5.15	7.20	6.70	6.50	6.60	4.55	5.62
1 day	4.70'	4.50	-	-	7.00	5.50	5.55	4.80	5.34
12 hours	4.60	-	4.50	-	-	5.40	5.30	3.90	4.72
4 hours	-	4.20	-	-	-	-	-	-	4.20
1 hour	-	-	1.65	-	5.70	-	-	-	3.68
Calving	3.90	2.60	1.55'	-	-	2.20	4.10	2.40	2.79
2 hours	4.30	2.30	-	-	5.00	-	4.90	2.30	3.76
4 hours	3.50	4.10	2.20	2.55	6.00	3.70	5.90	2.30	3.78
8 hours	-	-	3.70	2.95	6.60	4.60	6.20	3.75	4.63
12 hours	4.00	2.90	3.10	-	-	4.50	4.70	-	3.84
16 hours	4.40	3.45	-	4.70	6.60	4.70	4.80	-	4.78
20 hours	-	-	3.40	-	-	5.20	-	-	4.30
24 hours	-	-	-	4.00	-	-	-	4.45	4.23
32 hours	4.60	-	4.10	-	5.70	5.90	4.50	5.00	4.97
40 hours	-	5.60	4.80	-	-	-	-	-	5.20
2 days	4.18	4.25	4.50'	-	5.30	4.65'	4.00	-	4.48
3-4 days	-	6.60	4.60'	-	5.30	5.30	5.60	5.65	5.51
5-7 days	-	-	5.40'	-	-	6.00	6.20	5.00	5.65
10-20 days	6.80	4.60	4.90	-	-	-	6.20	6.20	5.74

' indicates the mean of two samples.

* Fourth means fourth and over.

TABLE 15

Plasma Inorganic Phosphate in Normal Parturient Cows.

Comparison of the 2 day prepartum level compared with the lowest value in the period 12 hours prepartum to 3 days postpartum.

Plasma Inorganic Phosphate mgm. per cent.

Cow Number	2 days prepartum	Lowest value.	Difference.	%age Fall.
First Calf Animals.				
2	4.80	2.70	2.10	43.75
8	7.25	5.05	2.20	30.34
9	4.85	4.90	0.05	+1.00
12	5.20	4.35	0.85	16.35
17	6.40	3.00	3.40	53.13
57	5.95	3.15	2.80	47.06
67	5.85	3.40	2.45	41.88
Second Calf Animals.				
3	5.30	4.15	1.15	21.69
4	6.10	3.45	2.65	43.44
5	6.75	4.50	2.25	33.33
16	5.90	3.20	2.70	45.76
24	6.20	2.90	3.30	53.22
53	6.30	4.90	1.40	22.22
54	6.35	4.15	2.20	34.65
59	6.60	3.25	3.35	50.76
Third Calf Animals.				
6	5.85	2.85	3.00	51.28
10	6.20	3.40	2.80	45.16
11	5.50	3.55	1.95	35.44
13	5.00	3.50	1.50	30.00
14	5.20	3.00	2.20	42.31
15	6.00	3.00	3.00	50.00
22	4.80	3.50	1.30	27.08
27	4.90	2.20	2.70	55.10
55	6.95	2.00	4.95	71.08
Fourth[x] Calf Animals.				
18	4.60	3.50	1.10	23.91
19	3.80	2.30	1.50	39.46
20	6.50	2.20	4.30	66.16
21	6.60	4.00	2.60	39.39
23	6.70	5.00	1.70	25.37
26	5.15	1.55	3.60	69.99
28	4.55	2.30	2.25	49.45
30	7.20	2.55	4.65	64.58

x Fourth means fourth or subsequent Calf.

TABLE 16.

"BORDERLINE" COWS.

Mgm. per cent.

Time from Calving	Calcium.		Magnesium.		Inorganic Phosphate.	
	Cow 7	Cow 56	Cow 7	Cow 56	Cow 7	Cow 56
8 days	11.00	10.50	2.50	3.35	6.20	6.20
7 days	-	10.60	-	2.85	-	6.15
6 days	10.65	9.85	2.30	3.05	7.15	6.60
5 days	10.50	10.70	2.70	3.15	7.95	6.45
4 days	-	10.25	-	3.10	-	8.50
3 days	10.21	9.80	2.85	3.20	8.80	5.95
2 days	10.35	10.20	3.00	3.50	5.50	7.45
1 day	9.72	10.40'	3.08	4.07'	5.45	5.95'
16 hours	-	9.30	-	4.60	-	5.20
12 hours	-	8.45	-	4.40	-	4.65
8 hours	-	8.00	-	4.35	-	3.60
2 hours	7.87	7.05	3.20	6.00	2.60	3.35
Calving	7.65	6.50	4.10	4.80	2.20	3.20
4 hours	6.15	6.60	3.91	4.85	1.70	2.95
8 hours	5.42	6.10	3.23	4.45	2.50	4.20
12 hours	-	6.40	-	4.25	-	4.20
18 hours	6.80	6.30	-	4.10	4.25	3.75
24 hours	-	6.00	-	3.95	-	3.25
30 hours	6.70	6.30	3.42	4.05	3.75	2.75
34 hours	-	7.00	-	3.85	-	3.20
2 days	8.80	7.95'	3.08	3.25'	3.30	5.17'
3 days	9.70	9.75	2.90	2.55	4.25	5.85
4 days	-	9.20	-	3.20	-	7.70
5-7 days	10.35	9.00	2.65	3.45	5.15	5.20
14 days	9.33	10.40	2.85	3.30	6.80	7.05

' indicates the mean of two samples.

TABLE 17.

Summary of Maximum Blood Changes

in Normal Parturient Cows.

	First Calf (7)	Second Calf (8)	Third Calf. (9)	Fourth Calf (8)
Serum Magnesium.				
2 days prepartum	2.87	2.78	2.59	2.73
Highest value	3.09	3.45	3.48	3.71
Difference	0.22	0.67	0.89	0.98
%age Rise.	7.67	24.10	34.36	35.90
Serum Calcium.				
2 days prepartum	10.84	10.72	10.89	11.18
Lowest value.	9.57	8.82	8.40	7.78
Difference	1.27	1.89	2.49	3.41
%age Fall	11.71	17.63	22.87	30.58
Plasma Inorganic Phosphate				
2 days prepartum	5.76	6.19	5.60	5.64
Lowest value	3.79	3.81	3.00	2.93
Difference	1.95	2.38	2.60	2.71
%age fall.	33.85	38.45	46.42	48.05

TABLE 18.

Blood Changes in Prepartum Milked Parturient Cows.

Mgm. per cent.

Time from Calving.	Cow 1.			Cow 2.		
	Ca.	Mg.	I.P.	Ca.	Mg.	I.P.
12 days	9.88	3.25	5.40	10.70	3.20	6.50
11 days	9.88	3.15	5.50	-	-	-
10 days	9.88	2.99	5.60	-	-	-
9 days	10.76	2.96	5.45	10.35	3.25	5.05
8 days	9.90	3.20	5.40	-	-	-
7 days	9.44	3.43	4.40	10.10	3.05	4.40
6 days	9.49	3.41	3.90	10.00	3.05	4.65
5 days	9.54	3.50	3.60	9.90	3.20	4.00
4 days	8.74	3.09	4.40	8.85	3.25	4.00
3 days	8.86	2.96	4.90	-	-	-
2 days	8.88	3.08	4.60	8.45	3.05	3.15
1 day	7.72	3.03	3.70	8.60	3.50	4.20
Calving	7.77	3.40	2.70	8.50	3.50	2.95
2 hours	7.72	3.07	2.50	7.50	3.50	3.02
8 hours	7.97	3.17	2.30	7.80	3.50	4.50
12 hours	-	-	-	7.60	3.25	4.05
16 hours	7.76	3.17	2.80	7.00	3.25	2.32
20 hours	7.97	3.13	2.90	6.80	3.20	3.00
24 hours	8.53	3.15	3.30	7.20	3.45	3.85
36 hours	8.79	3.20	3.40	7.45'	3.30'	3.65'
48 hours	8.84	3.22	3.35	6.98"	3.02"	3.12"
3 days	8.99	3.36	3.60	8.55	2.85	3.30
4 days	9.14	3.11	3.30	8.00	3.20	4.40
5 days	10.05	2.78	3.80	9.35	3.35	6.50
7 days	11.51	2.68	3.70	8.50	3.00	4.65
14 days	9.44	2.81	4.30	9.05	3.40	6.10

' indicates the mean of 2 samples.

" indicates the mean of 3 samples.

TABLE 19.

Total Milk Secretion of Calcium, Magnesium and Phosphate in Prepartum Milked Cows during the Prepartum Period.

Days before Calving	Cow 1. Total Yield kg.	Cow 1. Total Ca. g.	Cow 1. Total Mg. g.	Cow 1. Total P. g.	Cow 2. Total Yield kg.	Cow 2. Total Ca. g.	Cow 2. Total Mg. g.	Cow 2. Total P. g.
12	.97	1.00	0.14	0.67	.06	0.02	0.00	0.02
11	1.15	0.93	0.17	0.69	.10	0.07	0.02	0.04
10	1.75	1.27	0.32	0.93	.14	0.12	0.03	0.08
9	3.34	2.47	0.58	2.14	.30	0.35	0.07	0.27
8	4.73	3.61	0.78	3.38	1.12	1.68	0.30	1.47
7	6.88	8.20	1.64	7.46	.75	1.19	0.20	1.00
6	8.07	10.97	1.96	10.10	3.08	4.93	0.70	4.34
5	9.60	13.40	2.11	12.83	4.20	6.80	0.90	6.17
4	10.88	14.69	2.25	14.25	7.26	11.62	1.38	10.92
3	12.68	17.97	2.15	15.77	9.76	16.01	1.38	14.55
2	8.82	20.20	2.25	15.70	10.78	17.68	2.05	13.95
1	13.85	20.65	2.34	16.57	9.31	15.26	1.77	11.89
Totals	82.72	114.96	16.69	109.49	46.86	75.77	8.80	64.70

TABLE 20.

Blood Changes in Parturient and Mastectomised Cows.

Mgm. per cent.

Time from Calving	Calcium.			Magnesium.			Inorganic Phosphate.		
	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3
14 days	10.70	9.15	10.30	2.70	2.20	2.55	5.40	4.92	5.33
13 days	-	9.80	10.30	-	2.10	2.90	-	4.42	5.64
11 days	-	-	10.65	-	-	2.90	-	-	5.52
10 days	-	-	10.60	-	-	2.92	-	-	5.27
9 days	10.70	-	10.40	2.65	-	2.80	4.42	-	5.18
8 days	10.90	-	10.45	2.70	-	2.85	3.54	-	6.05
7 days	10.80	-	10.60	2.70	-	2.80	4.05	-	5.53
6 days	10.90	-	10.10	2.75	-	2.75	4.65	-	5.53
5 days	10.60	9.55	10.48	2.70	1.85	2.80	3.90	5.18	5.78
4 days	10.40	-	10.45	2.70	-	2.80	4.84	-	6.22
3 days	11.60	-	-	2.65	-	-	4.33	-	-
2 days	11.70	9.45	10.55	2.60	2.10	2.60	4.54	5.06	7.54
1 day	11.00	-	9.85	2.80	-	2.70	3.91	-	6.58
8 hours	10.60	-	9.50	2.70	-	2.75	3.74	-	6.22
2 hours	10.40	-	-	3.20	-	-	2.24	-	-
1 hour	10.50	-	-	3.00	-	-	3.06	-	-
Calving	10.10	9.20	9.30	2.80	2.40	2.75	2.62	4.94	3.97
2 hours	10.45	9.30	-	2.95	2.40	-	3.38	4.64	-
4 hours	10.60	9.20	9.50	2.95	2.40	2.80	3.80	4.08	4.24
8 hours	10.90	9.45	10.30	3.10	2.50	2.65	4.71	6.84	5.18
12 hours	10.70	9.70	10.60	3.00	2.35	2.60	5.16	6.81	4.23
16 hours	10.80	9.55	10.30	2.95	2.25	2.40	5.12	5.50	5.40
20 hours	11.15	9.40	10.20	2.80	2.25	2.35	5.36	6.40	4.84
24 hours	11.10	9.70	10.20	2.85	2.10	2.40	5.70	6.51	4.75
30 hours	10.70	10.30	10.10	2.90	2.20	2.40	5.70	6.49	3.97
36 hours	10.70	-	10.10	2.85	-	2.40	5.92	-	5.31
42 hours	-	9.90	10.35	-	2.25	2.30	-	6.83	5.88
2 days	10.30	10.45	10.45	2.80	2.40	2.40	5.59	5.80	6.18
3 days	9.85	10.50	10.30	2.85	2.25	2.70	6.09	6.49	7.18
4 days	10.30	10.55	10.50	2.90	2.35	2.75	5.88	6.53	6.49
5 days	10.10	10.40	10.30	2.95	2.30	2.85	6.16	6.37	6.66
6 days	10.35	10.25	10.15	2.90	2.55	2.80	6.79	5.88	6.92
14 days	9.80	10.55	11.00	2.70	2.40	2.70	5.78	6.05	3.99

APPENDIX 2.

TABLE 21

The Effect of Intravenous Injection of Four Ounces Calcium Borogluconate on the Serum Calcium in Normal Parturient and Non-Parturient Cows.

Mgm. Calcium per cent.

Cow Number	Time from Injection.							
	Pre-Injection	15 Mins.	30 Mins.	1 Hour	2 Hours	4 Hours	6 Hours	10 Hours
	Parturient Cows.							
C 1	9.80	19.20	17.60	15.80	13.50	11.50	10.30	10.05
C 2	10.70	17.80	15.60	14.60	13.10	11.70	10.80	9.60
C 3	8.30	12.70	12.00	11.50	11.00	11.00	9.80	8.70
C 4	10.80	19.90	18.30	16.80	16.00	13.10	11.70	10.85
C 5	9.65	17.35	15.60	14.40	13.50	11.90	10.40	9.00
C 6	8.90	17.80	17.05	15.70	12.80	11.60	10.60	9.45
Mean	9.69	17.46	16.03	14.80	13.32	11.80	10.60	9.61
	Non-Parturient Cows							
C 7	10.90	20.50	18.20	15.50	13.50	11.90	11.50	11.00
C 8	10.45	19.80	17.35	16.20	13.30	11.60	10.40	10.60
C 9	11.20	18.20	16.00	13.80	12.60	10.80	11.00	10.30
C 10	11.70	20.70	18.10	15.80	13.30	11.70	10.55	10.60
C 11	10.70	18.00	16.70	14.75	13.65	12.20	11.40	10.80
C 12	11.45	16.00	15.40	14.60	14.00	13.70	12.50	12.40
Mean	11.07	18.73	19.96	15.11	13.39	11.98	11.23	10.95

TABLE 22.

The Effect of Intravenous Injection of Four Ounces Calcium Borogluconate on the Serum Magnesium in Normal Parturient and Non-Parturient Cows.

Mgm. Magnesium per cent.

Cow Number	Time from Injection.							
	Pre-Injection	15 Mins.	30 Mins.	1 hour	2 Hours	4 Hours	6 Hours	10 Hours
		Parturient Cows.						
C 1	2.70	2.45	2.50	2.45	2.35	2.40	2.30	2.20
C 2	1.91	1.70	1.93	1.78	1.74	1.98	1.85	1.75
C 3	2.81	2.85	2.71	2.78	2.66	2.65	2.44	2.43
C 4	4.30	4.25	3.95	4.25	4.40	4.45	4.25	4.40
C 5	4.10	4.20	4.10	4.10	3.25	3.50	3.50	3.50
C 6	3.25	3.40	3.10	3.30	3.30	2.90	3.00	3.55
Mean	3.18	3.14	3.05	3.11	2.95	2.98	2.89	2.97
	Non-Parturient Cows.							
C 7	4.40	4.20	4.40	4.00	3.40	3.55	3.90	4.05
C 8	4.10	3.55	3.75	3.65	3.70	3.90	3.50	3.70
C 9	3.95	4.00	3.70	4.20	3.95	4.05	4.30	3.95
C 10	3.40	3.50	3.10	3.00	2.95	2.80	2.85	3.30
C 11	4.15	3.80	3.75	3.60	3.40	3.80	3.80	3.65
C 12	3.20	2.90	3.20	2.85	2.60	2.65	2.90	2.50
Mean	3.87	3.66	3.65	3.55	3.33	3.30	3.54	3.52

TABLE 23

The Effect of Intravenous Injection of Four Ounces Calcium Borogluconate on the Plasma Inorganic Phosphate in Normal Parturient and Non-Parturient Cows.

Mgm. Inorganic Phosphate per cent.

Cow Number	Time for Injection.							
	Pre-Injection	15 Mins.	30 Mins.	1 Hour	2 Hours	4 Hours	6 Hours	10 Hours
	Parturient Cows.							
C 1	3.54	3.92	4.06	4.66	5.13	3.41	4.00	3.10
C 2	3.38	4.75	5.22	5.47	3.13	3.10	2.32	1.24
C 3	2.21	2.90	2.62	3.86	4.00	5.40	3.04	2.35
C 4	1.29	3.97	4.56	2.10	2.80	2.36	3.07	3.41
C 5	3.56	4.25	4.95	5.31	5.81	5.04	4.09	3.45
C 6	6.14	7.62	8.26	8.77	6.92	5.42	4.66	5.02
Mean	3.35	4.57	4.98	5.03	4.63	4.12	3.53	3.09
	Non-Parturient Cows.							
C 7	4.26	5.12	5.72	6.17	5.11	3.75	4.03	5.62
C 8	6.03	6.00	6.03	5.70	4.81	4.84	4.58	5.00
C 9	3.92	4.06	5.53	5.31	3.71	3.92	4.16	3.97
C 10	4.79	5.75	5.37	5.09	4.90	3.71	5.62	5.68
C 11	3.04	2.10	1.49	3.54	4.56	3.92	2.18	5.13
C 12	1.23	1.55	1.36	1.40	3.30	3.50	3.07	1.65
Mean	3.88	4.10	4.25	4.53	4.39	3.94	3.94	4.51

TABLE 24.

The Effect of Intravenous Injection of Two Ounces Acid Sodium Phosphate on the Plasma Inorganic Phosphate in Normal Parturient and Non-Parturient Cows.

Mgm. Inorganic Phosphate per cent.

Cow Number	Time for Injection.							
	Pre-Injection	15 Mins.	30 Mins.	1 Hour	2 Hours	3 Hours	6 Hours	10 Hours
	Parturient Cows.							
P 1	3.07	11.65	10.45	8.90	6.14	5.28	2.96	3.80
P 2	3.72	12.80	11.95	10.75	7.84	6.84	6.05	5.82
P 3	4.20	15.13	13.50	10.36	9.50	7.09	6.22	4.55
P 4	3.71	14.25	12.60	11.00	8.05	5.48	5.48	5.82
P 5	4.77	14.68	13.33	11.25	8.99	7.45	6.72	6.14
P 6	1.55	8.77	8.14	6.22	4.28	3.68	3.66	3.58
Mean	3.50	12.88	11.66	9.75	7.[illegible]	5.97	5.52	4.95
	Non-Parturient Cows.							
P 7	5.09	17.50	14.05	11.24	7.62	6.92	6.79	5.48
P 8	5.88	15.15	14.25	12.10	9.32	7.18	6.24	5.22
P 9	5.66	14.70	11.93	8.78	6.66	6.62	5.44	5.70
P 10	5.18	15.13	13.83	10.54	7.79	6.35	5.70	5.03
P 11	5.07	13.35	10.45	7.80	4.75	4.87	3.70	4.48
P 12	4.15	14.00	11.75	9.63	7.54	6.18	6.44	7.27
Mean	5.17	14.97	12.71	10.01	7.28	6.35	5.72	5.53

TABLE 25

The Effect of Intravenous Injection of Two Ounces Acid Sodium Phosphate on the Serum Calcium in Normal Parturient and Non-parturient Cows.

Mgm. Calcium per cent.

Cow Number	Time from Injection.							
	Pre-Injection	15 Mins.	30 Mins.	1 Hour	2 Hours	4 Hours	6 Hours	10 Hours
	Parturient Cows.							
P 1	8.80	8.30	7.80	8.15	7.80	7.50	7.95	8.10
P 2	10.10	9.55	9.60	9.30	9.00	9.20	9.10	9.00
P 3	10.30	9.30	9.00	9.25	8.95	8.50	8.10	8.00
P 4	8.60	7.10	6.80	7.30	6.90	7.25	7.20	6.80
P 5	8.85	8.00	7.95	7.55	8.30	8.30	8.90	8.65
P 6	8.30	7.20	6.90	6.50	6.30	6.65	6.50	6.60
Mean	9.16	8.24	8.01	8.01	7.88	7.90	7.96	7.86
	Non-Parturient Cows.							
P 7	10.75	9.00	8.95	8.40	8.50	9.30	8.95	9.20
P 8	11.30	10.70	10.70	10.25	10.50	10.40	10.20	10.20
P 9	10.90	10.35	9.95	9.70	9.40	9.20	10.20	10.60
P 10	10.60	9.10	9.00	9.00	9.10	9.50	9.95	9.90
P 11	11.30	10.10	9.90	9.50	9.60	9.80	9.20	9.95
P 12	10.90	9.50	9.70	9.70	9.70	9.60	9.60	10.50
Mean	10.96	9.79	9.70	9.43	9.47	9.63	9.68	10.06

TABLE 26.

The Effect of Intravenous Injection of Two Ounces Acid Sodium Phosphate on the Serum Magnesium in Normal Parturient and Non-Parturient Cows.

Mgm. Magnesium per cent.

Cow Number	Time from Injection.							
	Injection	15 Mins.	30 Mins.	1 Hour	2 Hours	4 Hours	6 Hours	10 Hours
	Parturient Cows.							
P 1	3.25	3.30	3.35	3.35	3.30	3.45	3.45	3.45
P 2	3.10	3.00	2.95	3.15	3.20	2.95	2.85	3.00
P 3	3.30	3.00	2.95	3.20	3.35	3.50	3.50	3.65
P 4	3.40	3.45	3.40	3.30	3.20	3.20	3.20	3.40
P 5	2.70	2.60	2.50	2.45	2.40	2.60	2.60	2.60
P 6	3.75	3.75	3.70	3.70	3.65	3.75	3.60	3.80
Mean	3.25	3.18	3.13	3.19	3.20	3.22	3.20	3.32
	Non-Parturient Cows.							
P 7	2.70	2.90	3.00	2.95	3.10	3.00	2.90	3.15
P 8	2.90	3.10	3.25	3.20	2.85	2.65	2.60	2.65
P 9	3.40	3.45	3.45	3.45	3.70	3.50	3.45	3.45
P 10	2.70	2.50	2.65	2.60	2.80	2.90	2.85	2.90
P 11	2.75	3.15	2.90	2.75	2.80	3.25	2.75	3.00
P 12	3.70	4.05	4.20	4.00	4.00	4.30	4.25	4.20
Mean	3.03	3.19	3.24	3.16	3.21	3.27	3.13	3.23

TABLE 27

The Effect of Udder Inflation on the Serum Calcium, Serum Magnesium and Plasma Inorganic Phosphate in Normal Parturient Cows.

Mgm. per cent.

Cow Number	Time from Injection.							
	Pre-Inflation	15 Mins.	30 Mins.	1 Hour	2 Hours	4 Hours	6 Hours	10 Hours
	Serum Calcium							
U 1	9.90	10.50	10.25	10.50	10.95	10.90	10.95	10.90
U 2	8.90	9.00	9.65	9.80	9.00	9.80	10.00	10.00
U 3	7.60	9.30	9.50	9.80	9.90	9.80	10.25	9.40
U 4	8.60	9.10	9.40	9.10	10.35	9.65	10.80	10.10
U 5	7.80	8.50	9.80	10.00	10.45	9.75	10.20	9.00
U 6	9.70	10.55	10.80	11.20	11.15	10.75	11.55	10.10
Mean	8.75	9.49	9.90	10.07	10.30	10.11	10.63	9.92
	Serum Magnesium.							
U 1	3.80	4.25	3.65	3.65	3.50	3.30	3.50	3.30
U 2	3.65	3.80	3.80	4.05	4.00	4.00	3.95	3.80
U 3	3.10	3.10	3.45	3.35	3.40	3.10	3.00	3.10
U 4	2.60	2.60	2.60	2.70	2.75	2.75	2.85	2.70
U 5	3.40	3.40	3.70	3.70	3.65	3.10	3.60	3.40
U 6	3.15	3.25	3.40	3.55	3.30	3.30	3.20	3.00
Mean	3.28	3.40	3.43	3.50	3.43	3.26	3.35	3.22
	Plasma Inorganic Phosphate.							
U 1	0.12	2.60	0.46	2.01	1.48	1.69	3.41	2.14
U 2	2.41	2.90	3.02	3.46	3.77	3.86	5.18	4.42
U 3	1.52	4.45	1.24	1.89	1.23	0.23	2.60	2.06
U 4	1.99	2.44	3.94	4.09	4.33	4.58	4.39	1.73
U 5	2.71	3.77	4.10	5.12	5.70	6.61	7.58	8.73
U 6	2.23	4.19	4.10	4.55	5.22	5.65	5.99	6.54
Mean	1.83	3.39	2.81	3.52	3.62	3.77	4.86	4.27

TABLE 28.

The Effect of Udder Inflation on the Serum Calcium in Normal Non-parturient Cows.

Mgm. Calcium per cent.

Cow Number	Time from Inflation. Pre-Inflation	15 Mins.	30 Mins	1 Hour	2 Hours	4 Hours	6 Hours	10 Hours
	Lactating-milked							
U 7	11.10	11.35	10.70	10.60	10.85	10.50	10.55	10.40
U 8	9.80	10.10	10.70	10.35	10.40	9.35	8.80	10.20
U 9	10.35	10.80	10.90	11.40	10.30	9.30	10.05	10.10
U 10	9.50	10.00	10.05	10.00	9.90	9.60	9.95	10.05
Mean	10.19	10.36	10.59	10.59	10.36	9.69	10.09	10.19
Lactating-not milked.								
U 11	10.40	11.20	10.65	9.90	9.80	9.70	9.75	10.00
U 12	9.80	10.90	11.00	11.50	11.00	11.35	9.95	9.35
U 13	10.30	10.30	10.70	10.70	10.30	10.25	9.80	10.15
U 14	10.10	10.95	10.80	10.85	11.15	10.70	10.90	10.70
Mean	10.15	10.84	10.79	10.74	10.56	10.50	10.10	10.02
Non-lactating.								
U 15	9.50	9.90	9.80	8.80	8.90	8.90	8.85	9.70
U 16	10.00	9.80	9.65	9.40	9.25	9.00	8.95	9.25
U 17	10.70	-	10.00	8.50	9.40	9.50	9.60	9.25
U 18	11.65	11.15	11.30	10.10	10.00	9.50	9.05	9.70
Mean	10.46	10.28	10.19	9.20	9.39	9.22	9.11	9.47

TABLE 29.

The Effect of Udder Inflation on the Serum Magnesium

in Normal Non-parturient Cows.

Mgm. Magnesium per cent.

Cow Number	Time from Inflation.							
	Pre-Inflation	15 Mins.	30 Mins.	1 Hour	2 Hours	4 Hours	6 Hours	10 Hours
	Lactating-milked.							
U 7	2.35	2.60	2.30	2.20	2.35	2.65	2.80	2.80
U 8	2.80	2.55	2.65	2.60	2.50	2.40	2.70	2.90
U 9	2.90	2.90	2.70	2.70	2.70	2.60	2.50	2.40
U 10	2.50	2.75	2.65	2.50	2.35	2.65	2.50	2.65
Mean	2.64	2.70	2.57	2.50	2.47	2.57	2.62	2.69
	Lactating-not milked.							
U 11	2.70	2.70	2.60	2.70	2.60	2.70	2.60	2.80
U 12	2.90	3.15	3.25	3.30	3.35	3.40	3.15	3.20
U 13	2.90	2.70	2.80	2.85	2.75	2.60	2.70	2.70
U 14	3.20	3.00	3.10	3.05	2.95	3.00	3.25	3.10
Mean	2.92	2.89	2.94	2.97	2.91	2.92	2.92	2.82
	Non-lactating.							
U 15	2.60	2.80	2.70	2.70	2.70	2.90	2.90	2.75
U 16	2.85	2.90	2.70	2.70	2.75	2.65	2.75	2.80
U 17	3.10	-	3.15	3.20	3.10	3.20	3.30	3.40
U 18	3.25	3.35	2.70	2.60	2.60	2.55	2.70	2.80
Mean	2.95	3.02	2.81	2.83	2.79	2.78	2.91	2.94

TABLE 30.

The Effect of Udder Inflation on the Plasma Inorganic Phosphate in Normal Non-parturient Cows.

Cow Number	Mgm. Inorganic Phosphate per cent.							
	Pre-Inflation	Time from Inflation.						
		15 Mins.	30 Mins.	1 Hour	2 Hours	4 Hours	6 Hours	10 Hours
	Lactating-milked.							
17	6.79	6.88	6.26	6.58	6.05	5.44	5.76	6.14
18	4.50	5.58	5.62	5.56	6.12	4.96	4.48	4.92
19	5.85	5.53	5.58	6.40	6.22	6.76	6.75	6.14
110	4.33	5.22	5.44	5.96	6.05	5.82	5.43	3.84
Mean	5.37	5.80	5.75	6.12	6.11	5.77	5.65	5.26
	Lactating-not milked.							
111	6.35	7.14	6.75	6.49	7.00	6.64	6.78	6.22
112	6.96	7.96	7.83	8.39	7.70	7.70	8.90	9.16
113	3.97	5.21	6.22	5.57	5.44	6.09	6.49	7.27
114	5.02	6.49	6.29	6.79	6.52	6.30	6.66	7.88
Mean	5.57	6.70	6.77	6.81	6.66	6.68	7.21	7.63
	Non-lactating.							
115	5.90	6.40	6.38	5.78	5.27	4.78	5.20	5.58
116	5.48	6.25	7.04	6.66	6.58	5.78	5.92	5.57
117	5.78	5.88	5.70	5.70	5.40	4.63	5.04	5.53
118	5.06	5.88	6.22	6.22	6.22	5.36	4.06	5.08
Mean	5.55	6.10	6.33	6.09	5.87	5.14	5.05	5.44

APPENDIX 3.

TABLE 31

Pre-treatment Levels of Serum Calcium, Serum Magnesium and Plasma Inorganic Phosphate in Milk Fever Cases, with Time between Calving and First Treatment, Clinical Type, Milk Fever Occurred.

Cow Number	Pre-Treatment			Time from Calving	Clinical Type	Treatment	Calving Number
	Ca.	Mg.	I.P.				
29	3.27	5.58	0.60	32h.	Mod.	S.	4
31 (1)	3.64	3.00	1.15	12h.	Mod.	U.	8
(2)	4.57	3.90	2.05		Mild		
32 (1)	2.43	3.20	0.50	1h.	Sev.	U.	4
(2)	4.68	3.00	0.45		Mod.		
(3)	7.85	2.55	0.90		Mod.		
33	4.16	4.80	2.20	60h.	Mod.	S.	3.
34 (1)	6.24	3.35	0.80	4h.	Mild	U.	4
(2)	-	3.50	1.40		Mild		
36	3.69	4.10	1.60	48h.	Mod.	S.	2
38 (1)	4.24	3.97	1.20	4h.	Mod.	U.	3
(2)	4.04	4.00	1.10		Mod.		
42	6.21	2.95	2.50	20h.	Mild	S.	5+
43	5.96	3.15	2.50	16h.	Mild	S.	5+
44 (1)	5.15	3.50	1.00	12h.	Mild	U.	5
(2)	6.46	3.34	3.70		Mod.		
(3)	5.05	3.45	0.90		Mild		
(4)	2.93	2.70	0.95		Mild		
45	5.25	3.40	0.80	6h.	Sev.	S.	5
46	4.30	3.40	1.00	4h.	Mod.	S.	6
47 (1)	8.13	2.80	2.35	-2h.	Mod.	U.	4+
(2)	9.70	2.80	4.15		Mild		
48 (1)	4.25	3.20	0.58	7h.	Mild	U.	4
(2)	6.90	3.20	1.15		Mild		
49	5.20	3.25	1.40	12h.	Mod.	S.	4
50	3.10	3.85	0.80	26h.	Mod.	S.	4
51 (1)	4.30	4.45	0.45	7h.	Mod.	U.	5+
(2)	3.00	4.60	0.65		Sev.		
60 (1)	4.60	2.85	0.80	12h.	Mod.	U.	5
(2)	6.20	2.75	1.20		Mod.		
61	3.50	3.80	0.60	18h.	Mod.	S.	5
62 (1)	5.70	3.50	0.85	8h.	Mild	U.	3
(2)	7.50	3.40	2.45		Mild		
63	4.40	3.30	0.45	14h.	Mod.	S.	4
64	2.80	3.80	1.10	-2h.	Mild	S.	4
65 (1)	5.20	2.90	1.95	48h.	Mod.	U.	4+
(2)	7.30	3.40	2.50		Mild		
66	3.80	2.60	0.70	33h.	Mod.	S.	7
68	5.30	3.10	1.80	30h.	Mild	S.	7
69	7.30	4.65	1.85	36h.	Mild	S.	5
70	6.50	3.30	2.40	20h.	Mild	S.	4+
74	4.80	4.25	2.46	4h.	Mod.	S.	4
76	4.35	4.50	2.16	20h.	Mild	S.	4
78	5.40	3.50	0.53	6h.	Mild	S.	4
79	4.80	3.90	1.45	6h.	Mild	S.	4
80	6.50	2.25	1.25	7h.	Mild	S.	3

...........contd.

TABLE 31 (Contd.)

Cow Number	Pre-Treatment Ca.	Mg.	I.P.	Time from Calving.	Clinical Type	Treatment	Calving Number
81 (1)	5.20	2.73	2.25	-3h.	Mod.	U.	6
(2)	4.60	2.23	1.09		Sev.		
82	3.80	3.10	2.12	-3h.	Mod.	U.'	7
83	4.80	3.50	0.96	22h.	Mod.	S.	3
84 (1)	4.15	4.65	0.82	3h.	Mod.	U.	6
(2)	7.80	4.40	2.60		Mild		
85 (1)	5.40	4.05	1.75	6h.	Mod.	U.	3
(2)	4.40	3.06	1.11		Mod.		
(3)	6.20	2.97	2.00		Mild		
87	4.20	4.60	0.06	?	?	S.	6
88	3.10	4.65	0.29	30h.	Mod.	S.	6
89 (1)	4.50	-	0.94	-6h.	Mod.	U.	4
(2)	3.80	-	0.19		Sev.		
90 (1)	4.90	3.00	0.86	-8h.	Mod	U.	6
(2)	5.70	4.00	2.43		Mild		
91	4.30	3.10	1.46	36h.	Sev.	S.	3
92 (1)	4.70	2.90	0.77	-10h.	Mild	U.	4
(2)	5.60	3.05	2.99		Mild		
(3)	4.85	2.30	2.16		Mod.		
93 (1)	5.55	3.30	2.71	8h.	Mild	U.	3
(2)	5.50	3.40	2.69		Mild		
94	3.10	3.00	2.71	48h.	Mod.	S.	3
96	3.90	3.60	1.94	36h.	Mild	S.	5
98 (1)	4.80	3.77	1.15	16h.	Mod.	U.	5
(2)	4.40	3.75	2.49		Mod.		
99 (1)	4.70	3.10	0.55	8h.	Mild	U.	4
(2)	8.20	3.15	0.83		Mild		
100	4.50	2.85	1.62	4mo.	Mild	S.	4
101	4.20	2.60	1.03	96h.	Sev.	S.	4+
102 (1)	3.90	2.90	0.74	12h.	Mod.	U.	4
(2)	4.00	3.25	2.08		Mild		
103	2.50	2.20	2.42	18h.	Mod.	S.	3
104	3.50	3.15	0.45	12h.	Sev.	S.	4
105 (1)	4.85	2.90	1.33	-6h.	Mod.	U.	3+
(2)	7.80	2.65	1.67		Mild		
106	4.30	2.65	0.61	15h.	Mod.	S.	4
111	3.50	3.65	0.95	16h.	Sev.	S.	7
112	4.30	5.50	0.76	36h.	Sev.	S.	5
113	3.50	3.40	1.11	27h.	?	S.	3.
114	7.00	2.60	0.75	12h.	?	S.	3
115	3.90	2.40	0.58	23h.	?	S.	4
117	5.40	2.46	1.92	15h.	Mod.	S.	4+
118	5.00	2.84	5.52	13h.	?	S.	4
119	4.95	3.75	2.58	1h.	?	S.	3
120 (1)	4.60	2.82	2.70	8h.	Mod.	U.	3+
(2)	4.50	2.24	2.07		Mod.		
121 (1)	4.40	3.20	1.00	26h.	Mod.	U.	5+
(2)	3.80	2.95	1.00		Mod.		
122 (1)	3.60	3.45	1.89	20h.	Mod.	U.	4
(2)	4.00	3.00	2.06		Mod.		
123 (1)	4.80	2.74	0.84	-3h.	Sev.	U.	7
(2)	7.80	2.83	1.73		Mild		
124 (1)	4.20	3.91	2.89	5h.	Mod.	U.	6
(2)	5.00	4.33	2.34		Mod.		

..........contd.

TABLE 31 (Contd.)

Cow Number	Pre-Treatment Ca.	Mg.	I.P.	Time from Calving.	Clinical Type	Response to Treatment	Calving Number.
125	4.50	3.30	0.59	?	?	?	4
126	6.80	1.95	1.55	7d.	Mod.	?	4*
127	3.00	3.47	1.68	20h.	Sev.	?	3
128	6.20	2.29	1.50	25h.	Mild	?	4*
129	4.20	2.36	1.75	3w.	Mod.	?	5
130	5.80	2.53	1.65	17h.	Mod.	?	4*
131	5.60	2.10	2.34	5h.	Mild	?	3
132	5.20	2.44	2.06	-1h.	?	?	3*
133	6.40	2.20	5.97	-14d.	Mild	?	?
134	6.60	2.28	2.49	11h.	Mild	?	?
135	4.20	3.00	2.46	2h.	?	?	7
136	7.60	2.98	2.26	2h.	Mild	?	5
137	4.20	3.37	0.63	20h.	Mod.	?	5
138	4.20	2.50	2.30	2h.	Mild	?	4
140	5.10	1.50	0.60	5d.	Mod.	?	4
141	6.00	3.23	2.64	28h.	Mild	?	6
142	3.80	2.20	1.00	16h.	Sev.	?	3
143	5.10	3.55	1.20	13h.	Mod.	?	4.
144	5.40	1.70	1.82	24h.	Mod.	?	4
145	3.48	4.00	2.80	48h.	Sev,	?	6
146	5.00	4.15	1.34	6h.	Mod.	?	4
147	6.60	4.70	2.05	30h.	Mild	?	5
148	6.00	1.40	2.69	96h.※	Mild	?	3
149	4.70	3.38	0.80	?	?	?	?
150	4.80	2.73	1.10	10d."	?	?	?
151	5.00	2.57	2.05	14d.	?	?	?
152	6.00	1.50	2.80	6d.	?	?	?
153	8.00	3.27	2.76	48h.	?	?	?

' Cow relapsed but no samples were obtained before 2nd Treatment.

※ after abeshion at 6 months.

" after abortion at 7 months.

Mod = moderate symptoms of milk fever.

Mild = Mild symptoms of milk fever.

Sev. = severe symptoms of milk fever.

S = satisfactory response to first treatment.

U = unsatisfactory response to first treatment.

Figures in parenthesis indicate the number of the treatment in cases which relapsed.

TABLE 32.

Milk Fever Cases Treated with Calcium Borogluconate.

Satisfactory Response to Treatment.

Serum Calcium

Mgm. per cent.

Cow Number	Pre-Treatment	Time after Treatment. 15 Mins.	30 Mins.	1 hour	2 hours	4 hours	8 hours	12 hours
29	3.27	10.40	8.00	7.90	-	6.34	5.87	5.92
31 (2)	4.57	11.65	-	-	10.87	9.84	-	4.41
33	2.16	13.73	-	-	-	-	8.00	-
36	3.69	17.05	12.58	11.13	-	7.63	-	7.49
42	6.21	13.13	-	-	-	-	-	-
43	5.96	12.73	-	-	-	-	-	-
45	5.25	18.88	-	-	-	-	10.50	-
46	4.30	11.30	-	-	-	-	7.20	7.00
47 (2)	9.69	17.42	-	-	-	-	-	-
48 (2)	6.90	15.35	-	-	-	9.55	8.10	7.15
50	3.10	9.05	-	-	-	-	5.60	-
61	3.50	11.70	-	8.90	8.10	6.05	5.30	4.80
62 (2)	7.50	-	-	-	10.80	-	-	7.20
63	4.40	11.00	-	9.50	-	7.40	6.00	6.10
64	2.80	13.50	-	-	-	6.50	-	-
66	3.85	10.95	-	9.90	8.90	7.70	7.00	7.20
68	5.30	10.70	-	-	-	9.00	-	-
69	7.80	14.50	-	-	-	10.80	-	-
76	4.35	11.80	-	-	-	-	-	-
78	5.40	12.60	-	-	-	-	-	-
79	4.80	13.90	-	-	-	-	-	-
84 (2)	7.80	14.80	-	-	-	10.00	-	-
85 (3)	6.20	-	14.20	-	-	9.90	8.40	6.90
91	4.30	11.05	-	-	-	-	-	7.20
92 (3)	4.85	11.10	-	10.10	-	7.30	6.80	7.00
93 (2)	5.50	15.50	-	-	-	-	-	-
94	3.10	9.30	8.70	7.40	5.80	4.80	5.10	4.40
96	3.90	10.70	10.30	8.90	8.20	7.00	6.65	5.80
98 (2)	4.40	12.70	-	-	-	-	-	-
103	2.50	11.60	-	-	-	-	-	-
106	4.30	12.60	-	-	-	-	-	-
111	3.50	13.90	11.50	9.65	8.45	6.35	5.80	5.70
113	3.50	16.60	-	-	-	-	-	-
115	3.90	13.60	-	-	-	-	-	-
Mean	4.84	12.96	10.88	9.26	8.73	7.89	6.86	6.28

TABLE 33.

Milk Fever Cases Treated with Calcium Borogluconate.

Satisfactory Response to Treatment.

Plasma Inorganic Phosphate.

Cow Number	Pre Treatment	Time after Treatment.						
		15 Mins.	30 Mins.	1 hour	2 hours	4 hours	8 hours	12 hours
29	0.60	1.55	1.45	1.75	-	2.95	2.30	3.80
31 (2)	2.05	3.20	-	-	3.30	4.20	-	5.10
33	2.20	3.30	-	-	-	-	4.80	-
36	1.60	3.20	3.35	4.20	-	2.80	-	4.50
42	2.50	3.00	-	-	-	-	-	-
43	2.50	3.60	-	-	-	-	-	-
45	0.80	2.00	-	-	-	-	4.55	-
46	1.00	1.35	-	-	-	-	3.30	4.00
47 (2)	4.15	5.10	-	-	-	-	-	-
48 (2)	1.15	1.20	-	-	-	1.70	3.20	3.20
50	0.80	1.35	-	-	-	-	2.70	-
61	0.60	1.65	-	3.60	3.65	3.85	3.90	4.00
62 (2)	2.45	-	-	-	3.50	-	-	3.10
63	0.45	1.85	-	2.70	-	2.60	2.30	2.10
64	1.10	1.95	-	-	-	2.60	-	-
66	1.55	2.25	-	2.65	3.10	2.65	2.35	3.80
68	1.80	2.20	-	-	-	3.70	-	-
69	1.85	2.30	-	-	-	2.80	-	-
76	2.16	3.04	-	-	-	-	-	-
78	0.53	0.91	-	-	-	-	-	-
79	1.45	2.50	-	-	-	-	-	-
84 (2)	2.68	3.68	-	-	-	4.49	-	-
85 (3)	2.00	-	4.69	-	-	4.77	0.95	4.33
91	1.46	2.20	-	-	-	-	-	3.02
92 (3)	2.30	2.20	-	2.40	-	2.40	2.30	2.00
93 (2)	2.69	4.33	-	-	-	-	-	-
94	1.35	2.40	2.31	2.03	1.89	1.86	2.81	3.17
96	1.94	3.19	3.53	3.88	4.50	4.60	3.28	4.62
98 (2)	2.49	3.30	-	-	-	-	-	-
103	2.42	3.22	-	-	-	-	-	-
106	0.61	1.09	-	-	-	-	-	-
111	0.95	2.07	2.25	2.00	1.20	2.07	1.73	2.82
113	1.11	2.02	-	-	-	-	-	-
115	0.58	1.64	-	-	-	-	-	-
Mean	1.64	2.46	2.93	2.80	3.02	3.25	2.93	3.58

TABLE 34.

Milk Fever Cases Treated with Calcium Borogluconate.

Unsatisfactory Response to Treatment.

Serum Calcium.

Mgm.per cent.

Cow Number	Pre-Treatment	Time after Treatment.						
		15 Mins.	30 Mins.	1 hour	2 hours	4 hours	8 hours	12 hours
31	3.64	17.73	12.38	11.65	10.24	7.90	7.28	4.58
32 (1)	2.43	18.35	12.65	10.61	9.93	-	6.66	4.68
32 (2)	4.68	11.90	10.04	9.75	-	-	7.85	-
34	6.24	9.05	-	-	-	-	-	-
44 (1)	5.15	10.40	-	8.03	-	6.46	5.05	-
44 (3)	6.46	-	10.10	-	-	7.82	-	2.93
47	8.13	15.04	-	-	-	9.69	-	-
48	4.25	12.33	-	-	-	6.90	-	-
60	4.60	10.50	-	-	-	-	-	6.20
62	5.70	9.90	-	8.70	-	-	7.50	-
65	5.20	15.90	-	-	-	-	8.00	7.30
81	5.20	12.70	12.00	11.00	9.30	8.00	7.20	4.60
84	4.15	13.10	11.10	9.80	8.30	6.90	6.95	7.15
85 (1)	5.40	14.90	-	-	10.40	8.10	6.20	4.90
85 (2)	4.40	-	-	12.20	-	-	-	-
89	4.50	13.70	13.50	10.20	-	8.00	5.10	3.80
92 (1)	4.70	11.05	10.00	9.15	8.00	6.80	6.60	6.20
92 (2)	5.60	-	12.90	-	-	8.50	-	-
93	5.55	10.90	-	-	-	-	-	-
98	4.80	11.00	-	-	-	-	-	-
99	4.70	11.70	-	-	-	-	8.20	-
102	3.90	12.00	-	-	-	-	-	-
105	4.85	9.20	-	-	-	7.80	-	-
Mean	4.97	12.58	11.63	10.11	9.36	7.74	6.88	5.23

TABLE 35.

Milk Fever Cases Treated with Calcium Borogluconate.

Unsatisfactory Response to Treatment.

Plasma Inorganic Phosphate

Mgm. per cent.

Cow Number	Pre-Treatment	15 Mins.	30 Mins.	1 hour	2 hours	4 hours	8 hours	hours
31	1.15	1.50	1.70	2.40	3.30	2.45	2.40	1.85
32 (1)	0.50	0.85	0.85	0.90	1.00	-	0.40	0.45
32 (2)	0.45	0.65	0.75	1.11	-	-	0.90	-
34	0.80	1.15	-	1.40	-	-	-	-
44 (1)	1.00	1.30	-	1.50	1.60	1.00	0.90	-
44 (3)	3.70	-	4.25	-	-	4.10	-	0.95
47	2.35	3.10	-	-	-	4.15	-	-
48	0.50	1.20	-	-	-	1.15	-	-
60	0.80	1.62	-	-	-	-	-	1.20
62	0.85	1.20	-	1.65	-	-	2.45	-
65	1.95	2.20	-	-	-	-	2.25	2.50
81	2.25	2.60	2.08	3.50	4.18	2.55	2.63	1.07
84	0.82	2.00	1.93	2.63	1.78	1.90	2.32	1.02
85 (1)	1.75	2.63	-	-	2.76	1.39	1.67	0.55
85 (2)	1.11	-	-	2.08	-	-	-	-
89	0.94	1.88	2.16	1.43	-	2.00	1.27	0.19
92 (1)	0.77	0.76	1.16	1.38	1.80	1.56	1.68	2.38
92 (2)	2.99	-	3.92	-	-	3.05	-	-
93	2.71	4.26	-	-	-	-	-	-
98	1.15	1.50	-	-	-	-	-	-
99	0.55	0.92	-	-	-	-	0.83	-
102	0.74	0.83	-	-	-	-	-	-
105	1.33	1.50	-	-	-	1.67	-	-
Mean	1.35	1.68	2.09	1.82	2.20	2.25	1.64	1.22

TABLE 36.

Milk Fever Cases.

The Effect of Udder Inflation on the Serum Calcium, Serum Magnesium and Plasma Inorganic Phosphate.

Mgm. per cent.

Cow Number	Pre-Inflation.	Time after Inflation. 15 mins.	30 mins.	1 hour	2 hours	4 hours	6 hours	10 hours	12-24 hours	3-7 days
Serum Calcium.										
34 (2)	- ※	10.03	-	-	-	-	10.18"	-	10.24	-
44 (2)	5.05	5.90	7.32	-	7.87	-	7.77"	-	6.46	-
44 (4)	2.93	4.29	5.10	5.96	-	-	7.17	7.17"	6.72	10.20
49 (1)	5.20	6.80	-	- "	-	-	8.05	-	-	-
81 (2)	4.60	5.20	-	5.60	5.90"	-	-	7.00	6.50	-
99 (2)	8.20	9.50	-	-	- "	-	-	-	-	-
32 (3)	7.85	8.27	9.14	9.05	-	9.41	9.46	8.00"	8.32	8.99
80 (1)	6.50	7.60"	8.10	7.80	8.10	8.30	8.80	9.20	9.60	-
89 (2)	3.80	4.90	6.10	8.20"	8.20	8.15	7.30	6.40	5.10	8.00
104 (1)	3.50	4.75	5.00	5.90"	6.80	6.80	7.05	7.45	-	-
90 (1)	4.90	5.10	5.20	5.80	5.90	6.05	-	5.70'	-	-
Mean	5.26	6.23	6.57	6.90	7.13	7.74	7.66	7.27	7.28	9.06
Serum Magnesium.										
34 (2)	3.50	3.45	-	-	-	-	3.00"	-	2.64	-
44 (2)	3.45	3.75	3.85	-	3.90	-	3.85"	-	3.34	-
44 (4)	2.70	2.75	2.85	3.20	-	-	3.40	3.65"	2.70	1.75
49 (1)	3.25	3.55	-	- "	-	-	3.60	-	-	-
81 (2)	2.23	2.35	-	2.75	3.04"	-	-	2.96	2.83	-
99 (2)	3.15	3.50	-	-	- "	-	-	-	-	-
32 (3)	2.55	2.80	3.05	3.05	-	3.00	3.07	3.45"	3.08	2.50
80 (1)	2.25	2.36"	2.39	2.45	2.50	2.55	2.40	2.35	2.15	2.10
104 (1)	3.15	3.30	3.55	3.70"	3.70	3.80	3.70	3.57	-	-
90 (1)	3.00	3.00	3.35	3.50	3.80	3.80	-	4.00'	-	-
Mean	2.92	3.08	3.17	3.11	3.39	3.04	3.29	3.33	2.79	2.12
Plasma Inorganic Phosphate.										
34 (2)	1.40	2.10	-	-	-	-	4.00"	-	4.60	-
44 (2)	0.90	1.20	1.40	-	3.05	-	2.80"	-	3.70	-
44 (4)	0.95	2.20	2.10	3.00	-	-	2.00	3.50"	4.25	4.40
49 (1)	1.40	2.10	-	- "	-	-	5.30	-	-	-
81 (2)	1.07	0.78	-	1.91	2.84"	-	-	4.10	4.10	-
99 (2)	0.83	1.39	-	-	- "	-	-	-	-	-
32 (3)	0.90	1.25	1.75	2.30	-	3.00	3.20	4.50"	3.80	3.10
80 (1)	1.28	2.25"	2.11	2.28	2.82	3.76	3.24	2.44	1.23	-
89 (2)	0.19	1.15	2.20	0.04"	3.26	5.96	1.50	6.58	2.02	4.54
104 (1)	0.45	0.79	1.11	1.61"	2.26	2.72	3.58	4.19	-	-
90 (1)	0.86	1.34	1.65	2.86	3.54	3.84	2.67	2.43'	-	-
Mean	0.93	1.50	1.76	2.00	2.96	3.86	3.14	3.96	3.39	4.01

" indicates time at which cow rose to her feet.

' cow died after further treatment.

※ The calcium figures for case 34 (2) have been omitted from the calculation of the means as no pre-treatment value was available for this case.