IONIC AND BOUND CALCIUM LEVELS IN THE BLOOD OF COWS SUFFERING FROM PARTURIENT PARESIS (MILK FEVER)

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This is to certify that the

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IONIC AND BOUND CALCIUM LEVELS IN THE BLOOD OF COWS SUFFERING FROM PARTURIENT PARESIS (MILK FEVER)

By

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INTRODUCTION

Parturient paresis (Milk Fever) ranks high in economic importance to the dairy industry when consideration is given solely to such diseases as are classified under the heading of metabolic diseases in the bovine.

It has been the general observation of veterinarians and research workers, both in Europe and in America, that the incidence of parturient paresis has increased along with the increased productivity of the dairy cow. This primary acknowledgment based on statistics compiled at various veterinary clinics has been the basis for studies relating to the etiological factor or factors in this disease. Concurrent with this observation statistics have also indicated that the incidence of the disease increases up to the age at which the dairy cow attains the highest level of production and then decreases as the productivity declines with advanced age.

The true underlying cause of the occurrence of this disturbance has not been revealed. The work of investigators has, however, lead to the discovery of a satisfactory therapeutic procedure that can be relied upon to correct the disturbance.

It is with the intent to supplement, and possibly alter in some cases, part of the prevailing data relative to the phase of study that has been undertaken.

REVIEW OF LITERATURE

Introduction

Hutyra and Marek (1922) defined parturient paresis (milk fever) thus: "An afebrile disease which occurs commonly at the termination of parturition and which is manifested by a sudden loss of conciousness and a generalized paretic condition."

Law (1923-24) defined the disease as a nervous disorder which develops in plethoric cows, heavy milkers after calving, and was characterized by loss of sense, of conciousness, and of muscular control: by hypothermia, convulsions, coma, and mellituria.

The disease parturient paresis was first described by German veterinary surgeons at the beginning of the nineteenth century. The time at which the disease first manifested itself corresponded to that period of time in history when dairymen became interested in increasing milk production through generous feeding. As the years passed the efforts on the part of dairymen throughout the world have, for competitive and economic reasons, been intensified to increase the individual production of all cows of all breeds. A better understanding of genetics, nutrition, and animal husbandry has made possible the progressively higher level of production of dairy cattle of all breeds everywhere.

In order to make known the thinking of the eighteenth century veterinarian with respect to this disturbance Ralph (1850) stated that the incidence increased with years and that heavy milkers, after a few calvings, were more liable to this disturbance.

Early Theories

Over the many years that this disease has been investigated several theories have been advanced, many of which time and subsequent research have proven unfounded. In order to provide basis for further study certain of the older theories should be given recognition irrespective of the fact that experimental evidence has, at least in part, failed to support them. Prominent among these theories are the following: cerebral anemia, cerebral congestion, apoplexy, bacterial infection of uterine origin, bacterial infection of mammary origin, anaphylaxis, auto-intoxication, hypoglycemia, magnesium narcosis, and hypocalcemia.

Investigators, as the review of literature bears out, have given serious consideration to certain physiological processes and their progressive adjustments, as gestation progressed, to meet the changing exigencies concurrent with gestation and the ultimate parturition. A lack of a complete understanding of all of the physiological transitions attendant in the female during gestation and parturition compelled them to supplement known facts with theory.

Cerebral Anemia Theory

The theory of cerebral anemia was one of the first to be considered by such workers as Glass (1884), Billings (1884), Frank (1871), Thomassen (1890), McConnell (1890), and more recently by Spiegel (1939). A review of the work of the above men revealed the following fact as the basis for the belief in this theory. Immediately previous to and at the time

of parturition the mammary system undergoes hypertrophy with accentuation of the blood vascular system in preparation for lactation. This was the basis for the theory that the sudden increased demands of the mammary system for blood simultaneously caused a sudden blood deprivation of the brain. The final result was cerebral anemia and a semi-comatose to comatose condition that could, unless checked, lead to death. As late as 1939 this theory had strong adherents and it was believed that the then effective therapeutic procedure of air insufflation of the udder was responsible for the relief of cerebral anemia. The work of Hutyra and Marek (1926) discredited this theory since they were unable to produce the parturient paresis syndrome by removing nearly 50 per cent of the blood from a cow.

Cerebral Congestion Theory

In almost direct contradiction to the cerebral anemia theory was that of cerebral congestion (hyperemia) supported by such investigators as Noquet (1853), Sanson (1854), Ayrault (1857), Gerrard (1879), and Cox (1884). A commonly practiced therapeutic procedure followed shortly before and at this time was that of bleeding as described by Youatt (1830). In order to emphasize the degree of reliance placed upon bleeding as a therapeutic as well as a prophylactic procedure, Youatt (1830), who was a practitioner of veterinary medicine in good repute is quoted, "If the cow is in a high or dangerous state of condition and has been fed on luxuriant pasture, it will be very proper to bleed her and give her a dose of physic and remove her to a field of much shorter bite

a few days before her expected time of calving. I believe that many valuable animals have been saved by this precaution." Stewart (1890), while admitting having been singularly unfortunate in the treatment of this disturbance considered the practice of abstracting large quantities of blood as being therapeutically correct. Rogerson (1840), a contemporary practitioner, reported on venesection for the withdrawal of large quantities, four quarts, of blood as an important part of the therapeutic handling of parturient paresis cases. However, this manner of therapy, applied with the apparent intent to relieve circulatory congestion, was followed without warranted success. During the years in which these contemporary workers were endeavoring to uncover the cause of this mysterious parturient condition a therapeutic procedure that could be depended upon for the relief of the condition had not been found. Consequently a considerable number of subjects died and were presented for autopsy. Festal (1855) maintained that he had found several cases in which blood clots were found under the arachnoid covering of the brain. Sanson (1854) attempted to account for hyperemia of the cerebrum by the fact that physiologically the womb, following parturition, was faced with a sudden departure of blood concurrent with uterine involution. This sudden dimunition of blood to the uterus threw an additional volume of blood upon the general circulatory system more particularly the brain. In consequence of the hyperemia produced there a rupture of the capillaries, it was believed, sometimes occurred. The belief that blood clots in the brain were a common finding in this condition could not be credited for the reason that the presence of the

clots would constitute a pathological state the repair of which, if at all, would be slow and totally inconsistent with the rapid rate of recovery of cases following rational treatment.

Apoplexy Theory

Concurrent with the theory of cerebral congestion was that of apoplexy which had as its supporters Thacker (1841), Festal (1855), Walley (1872), Simonds (1841), Ward (1892-93), and Gerrard (1879). While the etiology of this state was believed to be associated with excessive blood vascular tension or pressure in an indirect way, in that the increased pressure resulted in a disturbance of the spinal and cerebral nervous systems, the phenomenon was related as being primarily nervous in nature. Various physiological abnormalities or, better stated, adjustments that accompany gestation and parturition, it was believed, thrust upon the nervous system shocking disturbances accountable for apoplexy. Emboli, that could very probably have been introduced into the capillaries that supply the uterine caruncles at the time the placental attachments were broken, were believed, in some cases, to be responsible for thrombosis of the cerebral blood supply with resultant apoplexy. Wincop (1868) reported in support of the theory of Simonds (1841) that the disease was centered in the brain and nervous system.

Thus it can be recognized that early investigators were concerned with the role of faulty circulation, and of certain nervous anomalies and their probable association with parturient paresis.

Bacterial Infection of Uterine Origin Theory

It is not surprising that the interest of investigators turned to the uterus as a possible haven for some substance or possible contaminent that could be a contributing factor in parturient paresis. It was less surprising, too, that attention was directed to this organ since it is conspicuously active during and at the close of gestation. Prominent among the workers who studied the bacterial flora of the uterus with intent to determine its probable role in parturient paresis were Lanzilloti-Buonsanti (1890), Zundel (1890), Lyman (1875), Schmidt-Mulheim (1890) (1904), Friedberger and Frohner (1896), Nocard (1896-97), and Guillebeau (1916). It was generally believed by these investigators that bacteria of the anaerobic type, which could already be present in or introduced at the time of parturition into the uterus, could quite probably create toxins which when absorbed could have a depressant effect upon the central, as well as, the vasomotor nervous systems. Thus the uterine infection theory commanded the attention of the above mentioned investigators. Some of these same investigators, however, associated the mammary gland with the onset of parturient paresis.

Bacterial Infection of Mammary Origin Theory

In the udder as in the uterus it was believed possible that certain anaerobic organisms comfortably situated within the gland and vegetating while feeding upon the colostrum could produce toxins that could, as in the uterus, have the effect of a depressant upon the central nervous system. It was this belief shared by Thomassen (1890), Knusel (1903),

and Schmidt (1897) that led to the therapeutic procedure of injecting potassium iodide solution into the udder with intent to destroy the microbes and thus effect relief from the symptoms of central nervous toxemia. Since the anaerobes were the organisms believed by Kunsel (1903) to be responsible for the toxins it was reasoned that the introduction of oxygen into the udder would alter the environment in a way to inhibit the activity of the anaerobes. This belief in anaerobic involvement was abandoned, however, but the fact remained that the mortality rate was lowered following inflation of the udder. The inflation of the udder with air became the standard therapeutic procedure in the treatment of cows affected with parturient paresis. Its application resulted in a marked decrease in the mortality rate in this malady. This surprising fact spurred research workers to seek an answer to the question as to why inflation of the udder with oxygen or sterile air could favorably affect the course of this disease.

Anaphylaxis Theory

About the same time that work with the uterus and the udder and the bacterial invasion thereof was being done certain workers advanced the theory of anaphylaxis. Prominent among these investigators were Hutyra-Marek (1926), and van Goidsenhoven (1927) who reasoned that there could be a protein sentitization following the absorption of milk-casein during the previous lactation. The sensitivity that developed manifested itself in the succeeding lactations when further absorption of milk-casein could take place. This phenomenon was believed to be aided by alexin, a protein antibody substance normally present in the

blood. This hypothesis could not be confirmed by the experimental injection of placental emulsions of milk and of colostrum immediately after parturition.

Auto-intoxication Theory

Menig (1925) theorized that toxic substances likened to ptomaines could be produced in the pregnant uterus which could, when absorbed, exert a depressant effect on the central nervous system and upon the vasomotor center as well. There were two contemporary proponents of the auto-intoxication theory, Menig (1925), and Schmidt-Mihlheim (1890) (1904). The work of these investigators was superseded by that of Schmidt-Kolding (1904). The latter attributed the auto-intoxication phenomona to the absorption from the udder of leucomames which were considered to be the end products from the decomposition of colostrum. Schmidt-Kolding reasoned that the treatment for this difficulty should be directed toward retarding secretion of the udder and at the same time neutralizing the leucomames by the use of some autotoxic agent. He, therefore, advocated the injection of the udder with an aqueous solution of potassium iodide. The immediate decrease in the mortality rate following this therapeutic procedure gave the theory general recognition. This manner of therapy was tried with warranted success by many and in time certain modifications of this therapeutic procedure led to the discovery that the beneficial results obtained was largely, if not wholly, a result of the distention of the udder rather than the autotoxic action of the potassium iodide. To this able investigator

credit was given for the discovery of the inflation method of treatment which was used for many years with a high degree of success in correcting parturient paresis.

Hypoglycemia Theory

Following the advent of the udder inflation method for treating parturient paresis very little was done for a time to expand the general knowledge with respect to the basic cause of this malady.

Neff (1923), reported that a condition of hypoglycemia could be induced by insulin injection. The condition thus produced so nearly resembled parturient paresis that one veterinarian, whose name remains unknown, was stimulated to try the injection of glucose into the blood stream in a case of parturient paresis. It was reported that he effected a cure. This experience led investigators Widmark and Carlens (1925) of Sweden, Auger (1927) of France, and Maguire (1926) of England, to center attention on the hypoglycemia theory. Widmark and Carlens reported the experimental production of hypoglycemia by insulin injections and the description of the symptoms manifested resembled closely those that were commonly observed in parturient paresis including the characteristic attitude of the head turned to rest on the chest. Auger (1927) later duplicated the work of Widmark and Carlens (1925). Thus it was believed for a short time that a significant fact with respect to this disturbance had been made known.

Hayden and Sholl (1923-24) (1924-25) pursued the theory with intent to add further to the data already accumulated by the afore mentioned proponents. The results of these workers brought out the fact that in

the very early stages of parturient paresis there existed a slight hypoglycemia with blood sugar readings of 35 mg per cent compared with the normal mean blood sugar of 51.7 mg per cent. They went further to compile data on the blood sugar levels, making use of the Folin-Wu sugar test, on seven parturient paresis cases. The data based on their work are shown in Table A which clearly indicate the presence of hyperglycemia in all seven cases.

Widmark (1926) attempted to explain the hyperglycemia in parturient paresis as reported by Hayden and Sholl (1923-24) (1924-25) on the basis of absorption of lactose from the mammary gland during gestation. Hayden (1927), however, demonstrated a consistently low blood lactose, which did not support the theory postulated by Widmark.

The work of Schlotthauer (1928), in which the data on the blood sugar levels in eight parturient paresis cases as shown in Table B, supported the findings of Hayden and Sholl (1923-24) (1924-25).

Moussu and Moussu (1926) concluded that parturient paresis is not due to hypoglycemia. This conclusion was based on a mean blood sugar level of 61.0 milligrams per cent for a series of cows suffering from parturient paresis. This conclusion was substantiated by two facts; first, that there is present in this malady a glycosuria which is not consistent with a condition of hypoglycemia, and second, in this condition the secretion of milk is diminished thereby lowering the demand for glucose in the udder for the production of lactose.

Little et al. (1928) determined blood sugar levels on a normal cow at hourly intervals for a period of 17 hours. Their findings,

TABLE A

BLOOD PLASMA GLUCOSE OF COWS WITH PARTURIENT PARESIS
Hayden and Sholl (1923-24) (1924-25)

Date	Case No.	Plasma Glucose: Preinflation mg %	Post-inflation: Time of collecting blood (min.) 10-25 mg %	30-55 mg %	60-85 mg %	105-115 mg %
10/7/26	1	68.7	109.6	137.5	-	-
10/10/26	2	70.7	-	-	-	-
10/14/26	3	62.2	81.3	131.1	-	-
10/23/26	4	45.0	73.3	87.1	-	-
10/29/26	5	77.8	111.5	108.0	99.6	87.0
10/31/26	6	62.7	77.7	113.4	119.4	74.7
1/13/27	7	49.0	60.0	64.0	62.1	Reinflated
	Average	62.3	85.5	106.8	93.7	80.8

TABLE B

BLOOD PLASMA GLUCOSE OF COWS WITH MILK FEVER
Schotthauer (1928b)

Cow No.	Post partum hours after calving	Symptoms	Blood Sugar mg % before treatment	Symptoms 24 hours after treatment	Blood Sugar mg % after treatment
1	8	general paralysis	75.47	normal	54.60
2	12	general paralysis	87.75	normal	72.95
3	3	comatose	107.50	conscious unable to rise	84.50
4	36	comatose	64.50	normal	62.80
5	24	comatose	109.20	normal	57.40
6	74	comatose	109.20	normal	-
7	3	comatose	88.10	normal	62.80
8	24	comatose	156.20	normal	-

illustrated in Table C, showed a variation for individual samples of between 56 and 93 mg per cent. These findings were in accord with those of Hayden (1927), as shown in Table D. Since the data thus obtained indicated a variation of blood sugar levels between fairly wide limits care should be taken when statements of normal or abnormal blood sugar levels are made. The data in Tables A and B indicate that in all but two cases the blood sugar findings in parturient paresis are well above the level at which symptoms of hypoglycemia would be expected to appear.

Further support in opposition to the hypoglycemia theory was provided by the work of Dryerre and Grieg (1928) in which they reported a high blood sugar in eight cases of parturient paresis. These workers stated, "based upon the results of our experiments and upon consideration of all available evidence, we formed the firm opinion that milk fever is not a hypoglycemia."

Subsequent to the work of Dryerre and Greig, Peterson et al. (1931) attempted to reproduce the parturient paresis syndrome, with consideration given to the hypoglycemia theory, by injecting insulin. They succeeded in lowering the blood sugar to 17 mg per cent at which level the subjects were held for prolonged periods of time without producing in any case symptoms suggestive of paralysis or coma. This fact, they believed, should dispel any question as to low blood sugar being a cause of parturient paresis.

Hypocalcemia Theory

Throughout the early part of the nineteenth century, at which time investigators were occupied with the compilation of facts to substantiate

TABLE C

HOURLY BLOOD SUGAR VALUES FROM A NORMAL COW
Little, et al. (1928)

Sample No.	Time	Blood Sugar as per cent of glucose	Food Taken -- General Notes
1	5 P.M.	.056	
2	6 P.M.	.069	7 lbs. hay fed at 5:30 P.M.
3	7 P.M.	.062	chewing cud
4	8 P.M.	.073	
5	9 P.M.	.066	
6	6 A.M.	.066	Taken before feeding
7	7 A.M.	.076	4 lbs. cake given after 6 A.M. test
8	8 A.M.	.090	
9	9 A.M.	.090	
10	10 A.M.	clotted	25 lbs. silage fed after 9 A.M. test
11	11 A.M.	.076	
12	12 Noon	.083	20 lbs. mangels fed at 11:30 A.M.
13	1 P.M.	.093	
14	2 P.M.	.078	
15	3 P.M.	.069	
16	4 P.M.	.079	4 lbs. cake fed at 3:30 P.M.
17	4 days later at 4:30 P.M.	.058	

TABLE D

BLOOD SUGAR VALUES OF NORMAL COWS
Hayden (1927)

Cow No.	Mg of Sugar per 100 ml of Blood		
	Glucose and Lactose	Lactose	Glucose
1	52.2	00.0	52.2
2	45.8	00.0	45.6
3	56.0	00.0	56.0
4	51.45	00.0	51.45
5	46.8	00.0	46.8

or disprove the hypoglycemia theory, certain workers engaged in blood analysis of parturient paresis cases, observed a consistently low blood calcium in all cases. Thus the attention of all workers was directed toward the hypocalcemia theory of parturient paresis. Little and Wright (1925) (1926) were among the first to report on the blood calcium levels of cows suffering from parturient paresis. These men first established the blood calcium levels on normal cows (Table E). They found the levels to range between 9.9 to 10.40 mg per cent.

The work of the above men, directed primarily at the discovery of the cause for the significant fluctuation in the level of blood calcium in normal subjects, failed to account for this variation. Meigs et al. (1919) attempted to account for the variation in calcium levels by stating, "The chief controlling factor is probably the concentration of bicarbonate in the plasma. It is probable that the concentration of calcium tends to vary inversely with that of bicarbonate." Hart et al. (1924) supported this hypothesis. The results of Huffman and Robinson (1926) were not in accord with the above findings. They noted that the day to day fluctuation in blood calcium was not accompanied by any appreciable change in carbon dioxide. Conversely, they observed marked changes in carbon dioxide without any corresponding change in blood calcium. These investigators also reported that there was no indication that calcium and carbon dioxide fluctuate inversely. In a later paper Huffman and Robinson (1926) made known the fact that the carbon dioxide capacity of the blood was lowered in parturient paresis, a fact that was later confirmed by Sampson and Hayden (1935).

TABLE 2

BLOOD CALCIUM LEVELS OF NORMAL COWS
Little, et al. (1915)

Cow No.	Mg per cent (duplicate)	Mg per cent (mean)
13	9.70 10.10	9.9
14	9.80 10.00	9.8
15	10.20 10.00	10.10
16	10.20 9.80	10.00
17	- 9.80	9.80
18	- 10.40	10.40

With a normal mean blood calcium level established, it was possible to correlate the consistently low calcium levels found by the investigators when blood analysis were confined to parturient paresis cases. Blood calcium levels in parturient paresis cases as reported by Little et al. (1925) (1926) are shown in Table F. There was a consistently low blood calcium in all cases with extremely low values appearing in the most severe cases.

Convinced, as most workers were that calcium in the blood plays an important role in parturient paresis attempts were made to duplicate the syndrome experimentally. Symptoms of hypocalcemia were produced by Peterson et al. (1921) by the intravenous injection of a 20 per cent solution of sodium citrate.

Becker (1933) made some observations which appeared to shed some light on one possible prophylactic approach. According to Becker forages grown on acid soils are deficient in calcium, some times, to the extent that animals feeding upon them call upon the skeletal reserve of calcium to meet the needs of other tissues of the body. The addition of lime to soils for forage crops is widely practiced and it is difficult to ascertain whether or not the incidence of parturient paresis would be still greater today with higher producing cows had the soil liming practice not been followed.

Greig and Dryerre (1932) reported on the subcutaneous injection of 30 to 60 grams of calcium gluconate in five per cent solution immediately after parturition as a prophylactic measure. Some authorities believe that prepartum milking in heavy producing cows tends to lower the incidence of the disease. Smith and Blosser (1947) made some

TABLE F

BLOOD PLASMA CALCIUM IN COWS SUFFERING FROM PARTURIENT PARESIS
Little, et al. (1925)

Case No.	Cow Down (duplicate) mg %	Cow Down (mean) mg %	Cow Normal (duplicate) mg %	Cow Normal (mean) mg %	Remarks
1	4.50 -	4.50	9.50 9.70	9.60	Typical Case
2	4.15 4.50	4.35	9.40 9.30	9.35	Typical Case
3	6.00 5.70	5.85	7.95 7.90	7.95	Mild Symptoms
4	5.60 5.10	5.35	10.50 10.30	10.40	Typical Case
5	6.40 6.10	6.25	7.80 7.80	7.80	Mild Symptoms
6	6.90 7.30	7.10	- -	-	Mild Symptoms
7	4.05 4.35	4.20	- -	-	Typical Case
8	4.10 4.05	4.10	10.30 10.60	10.45	Very Severe Case
9	3.60 3.80	3.70	6.70 6.95	6.85	Typical Case
10	5.75 5.95	5.85	7.75 7.85	7.80	Typical Case
11	5.10 5.25	5.20	8.25 8.15	8.10	Typical Case
12	6.30 6.35	6.35	9.00 10.00	9.75	Typical Case

extensive observations which led them to the belief that prepartum milking does not tend to lower the incidence of this disturbance. Along the same line it has been the generally accepted belief that the immediate and complete milking following parturition tends to precipitate an attack. The work of Smith et al. (1948) failed to support this belief.

Parathyroid Deficiency Theory

Coexistent with the hypocalcemia theory is that of parathyroid deficiency which was advanced by Dryerre and Greig (1925) with primary intent to disclose whether or not a failure of the parathyroid could be responsible for the apparent lowered calcium metabolism. The part played by the parathyroid was not completely weighed.

Wilson and Hart (1932), following a study made to determine the reason for the low blood calcium in parturient paresis concluded that it was caused by two possible factors; first, an unusually large demand on the blood for calcium which the calcium regulating mechanism, though properly functioning, is unable to cope with and, second, a failure of the calcium regulating mechanism to take care of the demand for calcium which normally occurs at freshening time.

Dryerre and Greig (1925a) (1925b), during a study in which they studied the possible role of the organs of internal secretion in parturient paresis concluded that there was an increase of guanidine in the blood. Since the work of Paton and Findley (1917) revealed that the parathyroids regulate the metabolism of guanidine in the body it could, therefore, be concluded that the parathyroids play a part in the

disease syndrome. However, the work of Hayden (1929) revealed that the guanidine in the blood of parturient paresis cases differs very little from normal tended to discredit the supposed active part played by the parathyroids in so far as the guanidine hypothesis was concerned. The parathyroids have a definite controlling influence on calcium metabolism and may play a functional role in parturient paresis. The work of Remiovsky and Rubin (1950) indicated that the total calcium level of the blood is probably the result of the interplay of numerous factors which include; calcium intake, calcium absorption, calcium excreation, vitamin D economy, parathyroid gland function, and phosphate, bicarbonate, protein and pH levels. The relations which determine the distribution of the blood calcium between the ionic and the bound forms are largely unknown. Godden and Duckworth (1935) observed that, in addition to a blood serum calcium drop in all animals at the time of parturition, a fall in the calcium complexes accounted for the major part of the fall in total calcium in cases of parturient paresis. It appeared to them that the symptoms of true parturient paresis became acute only when the values for both adsorbable and non-adsorbable calcium were simultaneously at low levels. Duckworth and Godden (1940) were the first investigators to partition the serum calcium during the transient reduction in serum calcium associated with parturition. They found that ionic calcium generally increased and with it were usually found increases in protein bound calcium as parturition approached. Duckworth and Godden (1936) recognized that a close relationship existed between ionic calcium and protein bound calcium. They observed that where the level of ionic calcium is depressed there is an immediate

mobilization of protein bound calcium and conversely when the amount of free calcium ions increases a portion becomes combined with serum protein. Further study by these investigators led to the belief that inorganic phosphorus reduction was accompanied by a reduction in total calcium. Thus it became apparent that inorganic phosphorus could play a part in the parturient paresis syndrome.

Palmer et al. (1930) observed that there is a decrease in inorganic blood phosphorus which is manifested on the day before parturition. The decrease in blood inorganic phosphorus amounted to as much as 3.2 mgs per cent. Wilson and Hart (1932) substantiated this finding. Hayden (1938) brought out the fact that both calcium and phosphorus decline at parturition normally but drop still lower in parturient paresis.

Hypocalcemia coma, as observed in parturient paresis, is explained by Green (1939) as a nervous endocrine disturbance in which hyper function of the anterior pituitary plays the most important part rather than hypofunction of the parathyroids. The generally accepted physiological mechanism for the control of plasma calcium is the parathyroid gland operating in conjunction with the nervous system and drawing on the reserves of calcium in the skeleton. The other endocrine glands, notably the anterior lobe of the pituitary, also play an important part. The inflation of the udder tends to stimulate the sympathetic and the para-sympathetic nervous systems in a way to effect the parathyroid endocrine gland to assert itself and bring about a temporary rise in blood calcium sufficient to cause the pituitary gland and other endocrine glands to resume their normal function. The work of Frei and

Demmel (1932) led them to believe that parturient paresis arises more from disorders of the endocrine system and the vegetative nervous system associated with parturition than from the onset of milk secretion.

The veterinary profession and research workers have mastered the field of clinical diagnosis despite the fact that there are voids to be filled before a clear and unquestioned understanding of this baffling malady can be had. They also have at their command two practical therapeutic procedures that can be applied for the relief of afflicted subjects. They are the air insuflation of the udder, and the intravenous or subcutaneous injection of calcium gluconate. With this knowledge of satisfactory therapy and with it's immediate and proper application very few cases of parturient paresis are lost. The primary concern of the animal husbandryman is a knowledge of some prophylactic measure that can be relied upon to prevent the disturbance.

Vitamin D Administration as Preventative

Hibbs et al. (1946a) (1946b) did some work which indicated that massive doses of vitamin D immediately preceding parturition and shortly thereafter brings about a rise in the level of calcium in the blood. The preliminary work accomplished by the above workers indicated that daily doses of 2,000,000 U.S.P. units of vitamin D in the form of irradiated yeast fed daily for two weeks previous to parturition and continued one week post partum raised the blood calcium and phosphorus twice as much as when 1,000,000 U.S.P. units daily were fed. This fact encourages the supposition that with the feeding of still higher levels of vitamin D a pending attack might be forestalled. The higher levels

of blood calcium and phosphorus thus attained are accomplished at the expense of the skeletal framework.

With respect to the incidence of parturient paresis, both from the standpoint of seasonal occurrence and breed susceptibility, much has been said and the opinions are variable. Metzger and Morrison (1936) reported a higher incidence of parturient paresis in winter than in summer. Similar observations were made by Henderson (1938). However, Hibbs (1946), who studied parturient paresis over a period of four years, found the incidence of parturient paresis from May through September was the same as in the period October through April on a percentage basis. Smith (1947) confirmed the findings of Hibbs. Metzger and Morrison (1936), Henderson (1938), and Hibbs (1946), as well as others not reported in this review, agree that the percentage of parturient paresis is highest in the Jersey Breed.

Magnesium Narcosis Theory

Godden and Duckworth (1935), and Hayden (1936) noted that there was a slight rise in blood magnesium in parturient paresis. Their observations were substantiated by Sjollema and Seekles (1932). These workers were of the opinion, however, that the rise in blood magnesium was not sufficient to account for the coma in parturient paresis as being due to magnesium narcosis as suggested by Klobouk (1932).

Summary of the Review of Literature

Early in the 18th century research workers and veterinarians were plagued by a disturbance in dairy cattle occurring at or immediately

after parturition. A satisfactory therapeutic procedure had not been discovered at this time. Different drugs had been used without success. Various practices, such as blood letting, had been tried with unwarranted success. Veterinarians and research workers grasped at all evidence revealed on post-mortem for a possible clue to it's cause. Theory upon theory was propounded. Prominent among the theories were those of cerebral anemia, cerebral congestion, apoplexy, bacterial infection of uterine origin, bacterial infection of mammary origin, anaphylaxis, auto-intoxication, hypoglycemia, magnesium narcosis, and hypocalcemia. With the expanded knowledge of physiology and endocrinology many of the theories were proven to be unfounded. Nevertheless the painstaking work of the early investigators who propounded these theories laid the foundation for further study that carried over into the 19th century and up to the present time. Little experimental support is offered for any of the theories except the hypoglycemia, and the hypocalcemia theories. Since in recent years several workers have conducted research work which disproved the hypoglycemia theory, investigators have concerned themselves with the theory of hypocalcemia. Mineral metabolism assumed an important position in the basic studies of parturient paresis at this time. The consistently low blood calcium and inorganic phosphorus levels at parturition and during an attack of parturient paresis along with the dramatic way in which affected subjects respond to calcium therapy lends strong support to the hypocalcemia theory.

Prophylactic measures that are entirely satisfactory in forestalling an attack have not been discovered. The more recent work, in which

it was found that massive doses of vitamin D administered immediately before and for a short time following parturition increased the calcium blood level, points the way to a possible prophylactic procedure. To be accepted as practical it must at the same time be found economical. The only recourse to the handling of this disturbance is through therapeutic measures. The udder inflation method which was accepted as satisfactory has now been replaced by the calcium gluconate injection, either subcutaneously or intravenously. The latter is generally preferred.

OBJECT OF THIS STUDY

Since it is an established fact that in parturient paresis there exists a condition of hypocalcemia, and since this metabolic disturbance occurs in well nourished and heavy producing cows, and further since the therapeutic procedure of the intravenous administration of ionic calcium is accepted as being effective in restoring parturient paresis cows to health cows to health concern in this study is, therefore, given to the relationship of bound and ionic calcium in the blood of affected animals.

EXPERIMENTAL PROCEDURE

In order to provide material that could be used both for test trials on the apparatus used (Figure 1) as well as to provide data for comparative purposes blood was collected from normal cows and partitioned to establish the normal range of total blood calcium and the ionic calcium. The cows selected for this purpose were taken at random and represent cows of varying ages and in different stages of lactation.

The cows selected for this study of parturient paresis were those that were presented for treatment to the Farm Veterinary Service at Michigan State College. The subjects (Table I) represented the Holstein, Guernsey, Ayrshire, and Jersey breeds of cattle. Following a careful diagnosis of parturient paresis and immediately preceding therapy a 40 ml sample of blood was obtained from the jugular vein. An anti-coagulant material was not used. Following the collection of the blood sample all cows were treated by the intravenous injection of 500 ml of a 23 per cent solution of calcium gluconate.

The partition of calcium was accomplished by the following procedure: After removing aliquot samples for total serum calcium, inorganic phosphorus, and serum proteins the serum was placed in the ultrafiltration apparatus shown in Figure 1. The apparatus consists of a three fourths inch length of Visking "Nojax" cellulose sausage casing fifteen sixteenth of an inch in diameter closed at the lower end by a number 0 rubber stopper secured tightly by means of a rubber band, and attached at the

APPARATUS FOR RECOVERY OF ULTRA-FILTRATE

Legend

1 - 30/32 inch No Jax Cellulose Casing

2 - Isotonic Saline

3 - Stabilizer

4 - Isotonic Saline

5 - Air

6 - Mercury

7 - Line to Water Pump

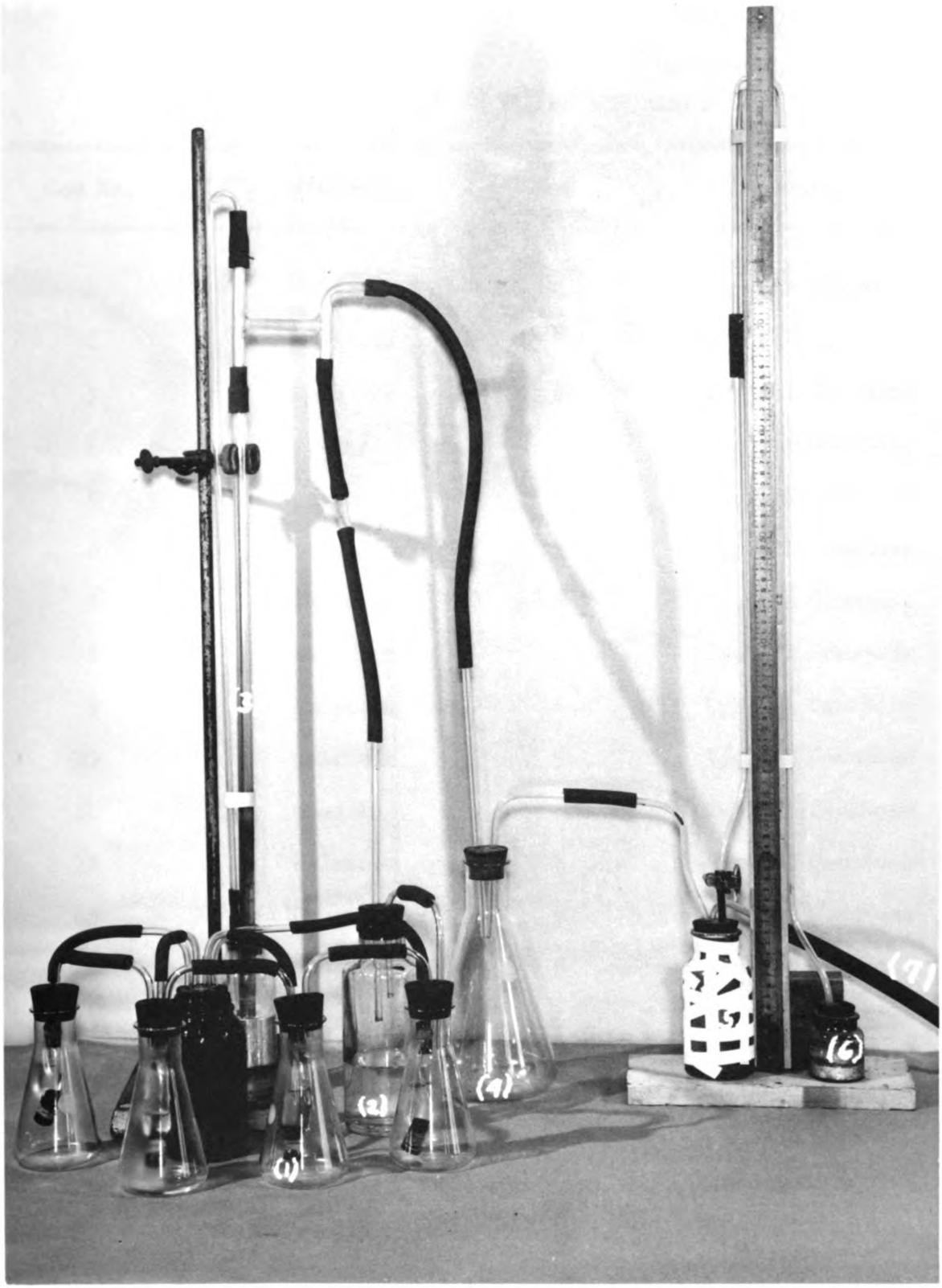
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TABLE I

DESCRIPTION OF COWS WITH PARTURIENT PARESIS

Cow No.	Breed	Age (years)	Remarks
1	Jersey	6	Typical Comatose
2	Holstein	7	Typical Comatose
3	Ayrshire	9	Typical Comatose
4	Jersey	6	Typical Comatose
5	Jersey	6	Typical Comatose
6	Holstein	5	Typical Comatose
7	Jersey	5	Typical Comatose
8	Holstein	6	Typical Comatose
9	Holstein	6	Typical Comatose
10	Holstein	5	Typical Comatose
11	Guernsey	6	Typical Comatose
12	Holstein	8	Typical Comatose
13	Guernsey	8	Typical Comatose
14	Holstein	6	Typical Comatose
15	Jersey	5	Typical Comatose
16	Jersey	4	Typical Comatose
17	Jersey	6	Typical Comatose
18	Holstein	12	Typical Comatose
19	Jersey	4	Typical Comatose

upper end to a short length of thick-walled rubber tubing through which is inserted a three inch length of glass tubing. The latter is mounted in a number 8 rubber stopper which is fitted tightly into a 250 ml wide-mouthed Erlenmeyer flask. Filtration is hastened by applying a partial vacuum to the flask inducing a moderate pressure gradient between the two sides of the membrane. The ultrafiltration sac containing the serum is not left open to the atmosphere, but communicates instead with a flask of physiological saline solution through which air entering the system must pass. The purpose of this arrangement is to prevent concentration by evaporation by making use of the fact that normal saline has approximately the same vapor pressure as the serum. A second glass tube in the stopper leads to the vacuum pump (an aspirator) by way of another container of physiological saline solution, the vacuum release apparatus, described by Erhard and Decherd (1942), and a mercury manometer. Use of the vacuum release apparatus allows for easy regulation of the vacuum applied at a rather constant level.

In most cases only about 12 to 15 ml of serum was available for ultrafiltration. With a vacuum of 200 $\pm$ mm Hg five to six hours were required to obtain enough ultrafiltrate for the calcium determinations.

Biuret tests for protein on the ultrafiltrates were invariably negative. Furthermore, the ultrafiltrates were always water-clear and did not foam on shaking.

Both the total calcium and the ultrafiltrable calcium were determined by the Clark and Callip (1925) method which is a modification of the Kramer and Tisdall (1921) method referred to by Tisdall (1923). In the case of the serum ultrafiltrates it was found desirable to use a four

ml aliquot sample rather than a two ml one because of the very low calcium concentration encountered in sera from cows with parturient paresis. Duplicate analysis were carried out whenever the quantity of serum and it's ultrafiltrate permitted.

It was thought advisable to test the permeability of the Visking cellulose tubing to calcium. Two experiments were performed with this purpose in mind. In the first experiment a solution of calcium chloride adjusted to a pH of approximately six was subjected to the ultrafiltration procedure described above. The ultrafiltrate and the original solution were then analyzed for calcium. In the second experiment a citrated bovine plasma sample was handled in a like manner.

RESULTS

The results for the partition of blood calcium from normal cows is shown in Table II.

The results for the partition of blood calcium from parturient paresis cases are shown in Table III.

The results of the work done to test the permeability of the Viking cellulose tubing to calcium is shown in Table IV.

The average total blood calcium for the normal cows computed from the results shown in Table II was found to be 9.9 mg per cent. The average levels of ionic calcium was 3.85 mg per cent. The average level of inorganic phosphorus was 5.505 mg per cent. The average level of total serum protein was 7.88 mg per cent. The average level of serum albumin was 3.64 mg per cent.

The average total blood serum calcium for the parturient paresis cows shown in Table III was found to be 4.29 mg per cent. The average level of ionic calcium was 2.126 mg per cent. The average level of inorganic phosphorus was 2.75 mg per cent. The average total serum protein was 7.28 mg per cent. The average serum albumin was 4.34 mg per cent.

A comparison of the average determinations taken from both Tables II and III showed a pronounced drop in total calcium, ionic calcium, and inorganic phosphorus in parturient paresis cases. The average total serum protein and serum albumin remained statistically constant both in normal cows and in cows suffering from parturient paresis.

Table IV shows computations that reveal the efficiency of the viking cellulose tubing as an instrument in the partitioning of blood serum calcium. The results of the two trials indicated that both calcium chloride solution, and citrated bovine plasma passed the membrane 100%. This fact justified the assumption that the levels shown in Tables II and III are truly representative.

TABLE II

BLOOD SERUM CALCIUM PARTITION, INORGANIC PHOSPHORUS, TOTAL SERUM PROTEIN, AND SERUM ALBUMIN OF NORMAL COWS

Cow No.	Total Calcium mg %	Ultrafilterable Calcium mg %	Ultrafilterable Calcium % total Calcium	Inorganic Phosphorus mg %	TSP* mg %	Alb.** mg %
1	9.6	3.7	38.5	6.69	7.63	3.68
2	10.9	3.6	33.0	6.46	7.80	3.16
3	9.4	3.9	41.5	4.34	7.99	3.81
4	9.8	5.1	52.4	5.62	6.86	4.36
5	10.1	4.1	40.3	6.91	7.59	4.64
6	10.5	4.3	41.1	5.76	7.75	4.58
7	10.3	4.3	42.1	5.06	7.49	4.04
8	9.7	3.4	35.1	5.32	8.14	3.63
9	10.5	3.7	35.0	5.04	7.94	2.85
10	9.3	3.2	34.7	5.25	9.38	2.42
11	9.0	2.75	30.6	5.66	8.03	3.70
12	10.4	4.0	38.5	4.05	8.08	4.08
13	9.9	4.0	40.4	5.36	7.77	2.43

* TSP = total serum protein
** Alb. = serum albumin

TABLE III

BLOOD SERUM CALCIUM PARTITION, INORGANIC PHOSPHORUS, TOTAL SERUM PROTEIN, AND SERUM ALBUMIN OF PARTURIENT PARESIS COWS

Cow No.	Total Calcium mg %	Ultrafilterable Calcium mg %	Ultrafilterable Calcium % total Calcium	Inorganic Phosphorus mg %	TSP* mg %	Alb.** mg %
1	3.4	1.2	35.3	0.87	6.51	4.38
2	3.55	2.1	58.6	5.12	6.26	4.19
3	4.5	1.8	40.0	2.96	7.16	3.73
4	4.6	1.5	33.3	2.02	7.38	3.93
5	3.6	1.7	46.4	0.52	8.01	4.87
6	4.4	1.95	44.3	1.78	7.16	3.46
7	3.4	1.85	54.4	1.45	6.66	3.96
8	3.4	2.0	58.8	1.38	7.61	4.21
9	5.0	2.5	50.0	2.73	7.68	4.41
10	3.2	1.3	40.6	6.55	7.91	4.51
11	4.1	1.95	47.6	5.65	-	-
12	4.5	2.55	56.7	2.46	7.44	4.11
13	3.5	2.0	57.1	1.23	7.80	5.58
14	6.3	3.15	50.0	1.15	7.84	3.62
15	3.3	1.3	39.4	1.60	5.98	4.04
16	4.2	2.3	54.8	1.69	6.86	4.64
17	4.4	2.1	47.7	3.10	7.03	-
18	4.0	2.15	53.8	4.77	8.05	4.83
19	4.1	2.5	61.0	2.42	-	-

* TSP = total serum protein
** Alb. = serum albumin

TABLE IV

TEST FOR PERMEABILITY OF VIKING CELLULOSE TUBING USED IN THE CALCIUM PARTITION PROCEDURE

Calcium Solutions Tested	Total Calcium mg %	Ultrafiltrable Calcium	
		mg %	% of total calcium
Calcium Chloride	8.9	9.1	102
Citrated bovine plasma	9.4	9.4	100

DISCUSSION

Two significant facts are apparent with regard to parturient paresis. First, there was a consistently low blood calcium in cows stricken with this disturbance. Second, there is an immediate and, in most cases, a dramatic recovery from symptoms following the administration of calcium salts by the intravenous route. The first of these facts is shrouded with mystery primarily because of still another fact which can be answered only in the negative. Why in the case of a strong, vigorous cow, most certainly possessed of a vast store of calcium in it's tissues, does this depletion of the blood calcium occur to the extent that hypocalcemia and the attendant paretic state exist? The second of the above facts is clearly understood only in part. The complete answer there, too, is likewise in the negative. Why does the administration of calcium salts into the blood stream, apart from the fact that it's presence overcomes or corrects the hypocalcemia by the restoration of a normal blood calcium level, activate the physiological processes adequately to maintain the level of calcium without a recurrence of the original pathological state?

The answer to all of the above, not too completely understood phenomona, has been attempted by statements of endocrine gland disfunction or failure. Lack of any specific criteria to explain endocrine disfunction beyond the fact that the parathyroids are known to play an important part in calcium metabolism leads one to ask the following

question. How can tissue disturbance or failure of the endocrine system be responsible in this case when failure of any such consequence by the system has not simultaneously had it's untoward effects upon gestation itself?

The most recent attempt to maintain a high level of calcium in the blood of the administration of massive doses of vitamin D (2,000,000 U.S.P. units or more) immediately preceding parturition having been successful in increasing blood calcium levels leads further to the belief that the endocrine glands responsible for calcium metabolism must be functioning adequately enough. True it can be said that this is all accomplished at the expense of the skeletal framework. But can it not also be said that even this shift of calcium store could not have been effected had the endocrine system not been functioning in a relatively satisfactory capacity?

Any serious disturbance of any of the organs responsible for calcium metabolism can not exist, it is felt, for tissue changes of an extent to modify the normal processes sufficiently to bring on the grave symptoms observed in this disturbance could not be repaired within the brief period of time that recovery takes place following proper and adequate therapy.

The work accomplished in connection with this study brought out the fact that both protein bound calcium and the ionic calcium dropped in parturient paresis. At the same time, it was noted, the ratio between protein bound calcium and ionic calcium remained relatively constant both in health and in parturient paresis. What is the significance of

this relationship? The fact has been demonstrated that there is a continuous compensatory action existing between bound calcium and ionic calcium. This physiological truth deserves consideration.

In parturient paresis there is a definite lack of muscular co-ordination. This loss of normal function involves both the voluntary and involuntary musculature. There must be at least one of two states existing. First, there could be a disturbance of the nervous system. Second, there could be a negative calcium balance within the muscles themselves.

Szent-Gyorgyi (1947) demonstrated a strong affinity between calcium and muscle tissue, and brain tissue. Could not a transitory shift of calcium from the musculature or from the brain tissues be responsible for this syndrome? The above mentioned worker observed that this high adsorptive power toward potassium and cations, including calcium, in general seems to be intimately connected with the function of myosin which is an active component of muscle. Brain tissue, likewise, has a similar affinity for calcium.

It is not without reason to surmise that by the establishment of the normal levels of blood calcium some physiological process hastens to replenish the supply of calcium to the musculature as well as to the brain thereby restoring normal reflexes. The adsorptive power of structural proteins for calcium, and other metals bearing a positive charge is great. The enzyme systems could be functioning in a way to account, either wholly or in part, for such a change. The cortical hormones of the adrenal gland, which have a profound influence on the

development and function of muscle, will also have to be fitted into the picture, Szent-Gyorgyi (1947).

It seems logical to assume that further approach to the study of parturient paresis should stem from the therapeutic knowledge of the disturbance.

SUMMARY

In this study the blood serum samples from 13 normal cows, some in the dry stage and others in various stages of lactation, were partitioned for levels of bound and ionic calcium. The values for the ionic calcium were found to range from 2.75 to 5.1 mg per cent with a mean of 3.85 mg per cent. The bound calcium values ranged from 4.7 to 6.8 mg per cent with a mean of 6.104 mg per cent.

The blood serum samples from 19 parturient paresis cows were likewise partitioned for bound and ionic calcium. The values for ionic calcium ranged from 1.2 to 3.15 mg per cent with a mean of 2.126 mg per cent. The bound calcium ranged from 1.45 to 3.15 mg per cent with a mean of 2.082 mg per cent.

A noteworthy fact revealed in this study is that the ratio between ionic and bound calcium remained relatively constant both in health and in parturient paresis.

REFERENCES

Allcroft, W. M. and H. H. Green. Blood Calcium and Magnesium of the Cow in Health and Disease. Biochem. J. 28: 2220-2226, 1934.

Auger, M. L. Recherches sur la pathogenic de la fievre vitilaire. Rev generale de med. vet. 25: 353-384, 1926. (Translated by P. A. Fish, Cornell Vet. 17: 64-73, 1927).

Ayrault. Cited by Thomassen. A Contribution to the Study of Parturient Paresis of the Cow. J. Comp. Path. and Therap. 3: 1-9, 1890.

Billings, F. S. The Relation of Animal Diseases to Public Health and their Prevention. Am. J. Vet. Med. 8: 86-88, 1864.

Clark, E. P. and J. B. Callip. A Study of the Tisdall Method for the Determination of Blood Serum With a Suggested Modification. J. Biol. Chem. 63: 461, 1925.

Cox, J. H. Parturient Apoplexy in Cattle Commonly Known as Milk Fever. Vet. 57: 193-197, 1864.

Craige, A. H. A Clue to the Cause of Milk Fever in the Metabolism of the Springing Cow. J. Vet. Research 8: 247-256, 1947.

Dryerre, H. and J. R. Greig. The Specific Chemotherapy of Milk Fever by the Parenteral Administration of Ca-boro-Gluconate. Vet. Med. 30: 234-236, 1935.

Dryerre, H. and J. R. Greig. Milk Fever: It's Possible Association with Derangement in the Internal Secretions. Vet. Record 5: 225-231, 1925a.

Dryerre, H. and J. R. Greig. Further Studies on the Etiology of Milk Fever. Vet Record 8:721-726, 1928.

Duckworth, A. and M. Rubin. Proc. Soc. Exp. Biol. and Med. 74: No. 2, 1950.

Duckworth, J. and W. Godden. Studies on the Partition of Serum Calcium. Biochem. J. 30: 1560-1566, 1936.

Erhard, P. S. and Decherd, G. M. A Mercury Vacuum Release For Ultra-filtration and Other Exacuate Systems. J. Lab. Clin. Med. 27: 1206-07, 1942.

Festal, M. Quoted by Gamgee, J. Contemporary Progress in Veterinary Science and Art. Vet. 27: 452, 1855.

Fish, P. A. The Physiology of Milk Fever I. Cornell Vet. 17: 105, 1927.

Fiske, C. H. and Y. Subba Row. Inorganic Phosphate Analysis. J. Biol. Chem. 66: 375, 1925.

Fiske, C. H. and Y. Subba Row. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375-400, 1925.

Frank, C. Parturient Apoplexy. Ann. med. vet. 6: 701, 1871.

Frei, W. and M. Demmel. The Effect of Pregnancy and Lactation on Blood Serum in Cattle: Remarks on Milk Fever. Schweiz Arch. Tierheilk. 74: 525-536, 1932. (Nutrition Abstracts and Revs. 3: 122, 1933-34.)

Friedberger and Frohner. Pathology and Therapeutics of the Domestic Animals. 2nd ed. 1:399, 1896. Published W. T. Koener Co., Chicago.

Gerrard, J. Remarks, Anatomical, Physiological, and Pathological on Parturient Paresis. Vet. 52: 121-146, 1879.

Glass, R. Parturient Apoplexy. Vet. J. 16: 393-398, 1864.

Godden, W. and J. Duckworth. Variations in Serum-Magnesium and in Partition of Serum-Calcium in Normal Parturition and in Milk Fever. Biochem. J. 29: 445, 1935.

Green, H. H. Recent Advances in Our Knowledge of Diseases Associated With Mineral Balance in the Blood of Ruminants. Report of the National Vet. Med. Assoc. of Great Britain and Ireland, London, 1939.

Greig, J. R. and H. Dryerre. Prevention of Milk Fever. Rec. med. vet. de liecole dAlfort, 107: 404-406, 1932.

Guillebeau, A. On the Pathological Anatomy of Milk Fever. Vet. J. 23: 97-102, 1916.

Hart, E. B., H. Steenbock and C. A. Elvehjem. Dietary Factors Influencing Calcium Assimilation V. J. Biol. Chem. 60: 117-131, 1924.

Hayden, C. E. and L. B. Sholl. The Blood and Urine of the Cow in Milk Fever. Report of N. Y. State Vet. College. 91-102, 1923-24.

Hayden, C. E. and L. B. Snoll. The Blood and Urine of the Cow in Milk Fever (Second Report). Report of the N. Y. State Vet. College. 200-205, 1924-25.

Hayden, C. E. The Blood Sugar of the Cow in Milk Fever. Cornell Vet. 17: 121-131, 1927.

Hayden, C. E. The Blood Sugar of the Cow in Milk Fever. Cornell Vet. 17: 123, 1927.

Hayden, C. E. Sugar, Guanidine and Cholesterin in the Blood of the Cow in Milk Fever. Cornell Vet. 19: 285-295, 1929.

Hayden, C. E. Changes in the Blood of the Cow at Parturition. Cornell Vet. 28: 152-160, 1938.

Hayden, C. E., M. G. Fincher and J. Sampson. Acetonemia and Acetonemia With Parturient Paresis. Cornell Vet. 23: 308-378, 1933.

Hayden, C. E. and P. A. Fish. The Normal Blood of Some Domesticated Animals. Cornell Vet. 28: 197-203, 1928.

Henderson, J. A. Dairy Cattle Reproduction Statistics. Cornell Vet. 28: 173-195, 1938.

Hibbs, J. W., W. E. Krauss, C. F. Monroe and T. S. Sutton. Studies on Milk Fever in Dairy Cows. (1) The Possible Role of Vitamin D in Milk Fever. J. Dairy Sci. 29: #9: 617-623, 1946a.

Hibbs, J. W., W. E. Krauss, W. D. Pouden, C. F. Monroe and T. S. Sutton. Studies on Milk Fever in Dairy Cows. (2) The Effect of the Blood Changes in Normal and Milk Fever Cows at Parturition. J. Dairy Sci. 29: #11: 707-781, 1946b.

Hibbs, J. W. Studies on Some of the Nutritional and Physiological Aspects of Milk Fever in Dairy Cows. Abstracts of Doctoral Dissertations. #54, 163-179, 1946-47. Ohio State University.

Howe, P. E. The Determination of Proteins in Blood - A Micro Method. J. Biol. Chem. 49: 109-113, 1921.

Huffman, C. F. and C. S. Robinson. Studies on Chemical Composition of Beef Blood (3). J. Biol. Chem. 69: 101-112, 1926.

Huffman, C. F. and C. S. Robinson. Studies on the Chemical Composition of Beef Blood. J. Biol. Chem. 69: 245-266, 1926.

Hutyra - Marek. Milk Fever or Parturient Paresis. Pathology and Therapeutics of Diseases of Domestic Animals. Vol. 2, p. 848, 1922. Alexander Eger, Chicago, Ill.

Hutyra - Marek. Special Pathology and Therapeutics of Diseases of Domestic Animals. 2rd ed. Vol. 3 p. 514, 1926. Alexander Eger, Chicago, Ill.

Kingsley, G. R. A Rapid Method for the Separation of Serum Albumin and Globulin. J. Biol. Chem. 133: 731-735, 1940.

Klobouk, A. Porodni paresa jako horcikova narkosa (Milk Fever as Magnesium Narcosis). Zverol Rozpravy 6: 13-17, 1932. (Vet. Bull. 2: 567, 1932. Trans E. Pribyl).

Knusel, M. Treatment of Parturient Paresis with Oxygen. Vet. J. 57: 23, 1903.

Kramer, B. and F. F. Tisdall. A Clinical Method for the Quantitative Determinations of Calcium and Magnesium in Small Amounts of Serum or Plasma. Bull. Johns Hopkins Hosp. 32: 44-48, 1921. (Chem. Abstracts 15: 3857, 1921).

Lanzilloti - Buonsanti. Rec. Med. Vet. 1880. Quoted by Thomassen. A Contribution to the Study of Parturient Paresis of the Cow. J. Comp. Path. and Therap. 3: 1-9, 1890.

Law, James. The Blood and Urine of the Cow in Milk Fever. Report N. Y. State Vet. College, 1923-24.

Little, W. L., T. Skene Keith and H. T. Founs. The Blood Sugar of the Cow in Milk Fever and Under Normal Conditions. Vet. J. 84: 284-287, 1928.

Little, W. L. and N. C. Wright. The Aetology of Milk Fever in Cattle. Brit. J. Exptl. Path. 6: 129-134, 1925.

Little, W. L. and N. C. Wright. A Review of Some of the Modern Theories of Milk Fever. Vet. J. 82: 185, 1926.

Little, V. R. and T. H. Blosser. A Review of Some of the Modern Theories of Milk Fever. Vet. J. 82: 185, 1926.

Lyman, R. P. A Theory on the Etiology and Pathology of Milk Fever in Cows. Vet. J. 2: 81-85, 1876.

Maguire, L. C. The Etiology of Milk Fever. Vet. Rec. 6: 52-53, 1926.

Maguire, L. C. The Etiology of Milk Fever. Vet. Rec. 6: 484, 1926.

Meigs, E. Milk Secretion as Related to Diet. Physiol. Reviews. 2: 228, 1922.

Meigs, E. B., N. R. Blatherwick and C. A. Cary. Contributions to the Physiology of Phosphorus and Calcium Metabolism as Related to Milk Secretion. J. Biol. Chem. 37: 1-76, 1919.

Menig, O. Auto-intoxication of Advanced Pregnancy. J. Am. Vet. Med. Assoc. 66: 711-721, 1925.

Metzger, H. J. and H. B. Morrison. The Occurrence of Milk Fever in the Kentucky Station Herd over a Period of Twenty Years. Proc. Am. Soc. Animal Prod. 48-52, 1930.

Moussu, G. and R. Moussu. Glycemie Normal Chez les vaches Laitieres. Compt. rend. 133: 431-433, 1926. (Trans. J. Am. Vet. Med. Assoc. 71: 89-90, 1927).

McConnell, T. M. Parturient Apoplexy. Vet. Record. 3: 2, 1890.

Neefs, E. L'insuline. Ann. med. vet. 68: 475-477, 1923. Cited by Auger [Recherches sur la pathogenic de la fievre vitulaire. Rev. generale de med vet. 25: 353-364, 1926. (Trans. by Fish Cornell Vet. 17: 64-73, 1927)].

Nocard, E. Parturient Paresis. J. Comp. Path. Therap. 9: 82, 1896-97.

Noquet. Cited by Thomassen. A Contribution to the Study of Parturient Fever of the Cow. J. Comp. Path. Therap. 3:1-9, 1890.

Palmer, L. S., W. S. Cunningham and C. H. Eckles. Normal Variations in the Inorganic Phosphorus of the Blood of Dairy Cattle. J. Dairy Sci. 13: 174-195, 1930.

Palmer, L. S. and C. H. Eckles. Normal Variations in the Calcium Content of the Blood of Dairy Cattle. J. Dairy Sci. 13: 351-359, 1930.

Paton, D. N. and L. Findlay. The Parathyroids: Tetania Parathyropriva: It's Nature, Cause and Relations to Idiopathic Tetany. Part I: Introduction, General Methods and Symptoms, Quarterly J. of Exp. Physiology 10: 364, 1917.

Peterson, W. E., E. A. Hewitt, W. L. Boyd and W. R. Brown. Artificially induced Hypoglycemia and Hypocalcemia in the Cow and the Relationship to Parturient Paresis or Milk Fever. J. Am. Vet. Med. Assoc. 79: 217-227, 1931.

Ralph, J. Observations on Puerperal Fever in Cow. Vet. 23: 581, 1850.

Reinvsky, A. and M. Rubin. Experimental Control of Serum Calcium Levels in vivo. Prox. Soc. Exp. Biol. and Med. 74: 415-417, 1950.

Rogerson, J. W. On Puerperal Fever or Paralysis of the Hind Extremities. Vet. 13: 319-322, 1890.

Sampson, J. and C. E. Hayden. The Acid Base Balance in Cows and Ewes during and after Pregnancy, with Special Reference to Milk Fever and Acetonemia. J. Am. Vet. Med. Assoc. 86: 13-23, 1935.

Sanson. J. des veterinaires du medi 57: 1854 (Quoted by Greig, J. R. Calcium Gluconate as a Specific in Milk Fever. Vet. Record 10: 115-120, 1930).

Schlotthauer, C. F. Theories on the Eriology of Milk Fever--Experimental Investigations. Cornell Vet. 18: 217-224, 1928a.

Schlotthauer, C. F. Theories on the Etiology of Milk Fever--Experimental Investigations. Cornell Vet. 18, 223, 1928b.

Schmidt-Mulheim. Rec. Med. Vet. 1880. Quoted by Thomassen. A Contribution to the Study of Parturient Fever of the Cow. J. Comp. Path. Therap. 3: 1-9, 1890.

Schmidt-Mulheim. Milk Fever. U. S. Dept. of Agr. Farmers Bull. #206, 1904.

Schmidt, J. J. Studier og Forsog over Kalvningsfeberans, Aarsag og Behandling. Maanedsskrift fur Dyrlaeger 9: 228, 1897. Cited by Auger [(Recherches sur la pathogenic de la fievre vitulaire. Rev. generale de med. vet. 25: 353-384, 1926) Trans. by P. A. Fish, Cornell Vet. 17: 64-73, 1927].

Schmidt, J. - Kolding. Milk Fever. U. S. Dept. of Agr. Farmers Bull. #206, 1904.

Simonds, B. J. Parturient Apoplexy. Vet. 14: 161, 1841.

Sjollema, B. and L. Seekles, Magnesium of Blood-Especially in Tetany (Translated) Klin. Wochsehr 11: 989-990, 1932. (Nutrition Abstracts and Reviews 2: 291, 1932-33.)

Smith, V. R. and T. H. Blosser. Parturient Paresis. I. The Incidence of Parturient Paresis and Changes in the Total Blood Serum Calcium at Parturition in Prepartum Milked Cows. J. Dairy Sci. 30:861-866, 1947.

Smith, V. R., R. P. Niedermeier and R. G. Hassen. Parturient Paresis. II. The Effect of Partial Versus Complete Milking Upon the Total Blood Serum Calcium of Dairy Cows at Parturition. J. Dairy Sci. 31: 173-177, 1948.

Spiegel, E. A. and M. A. Spiegel. Physico-chemical Mechanisms in Convulsive Activities. J. Nervous and Mental Diseases 90: 188-209, 1939.

Stewart, C. Parturient Apoplexy. Vet. 63: 417, 1890.

Szent-Gyorgyi. Chemistry of Muscular Contractions. 1947. Academic Press Inc., Publishers, New York City.

Szent-Gyorgyi. Nature of Life. 1948. Academic Press Inc., Publishers, New York City.

Thacker, W. A Case of Puerperal Fever. Vet. 14: 283-285, 1841.

Thomassen. A Contribution to the Study of Parturient Fever of the Cow. J. Comp. Path. Therap. 3: 1-9, 1890.

Tisdall, F. F. A Note on the Kramer-Tisdall method for the determination of calcium in small amounts of serum. J. Biol. Chem. 56: 439-441, 1923.

Van Goldsenhoven. Anaphylaxie et fievre vitulaire. Ann. med. vet. 56:139-202-264, 1911. Quoted by Auger, M. L. Rechierches sur la pathogenic de la fievre vitulaire. Rev. generale de med. vet. 25: 353-384, 1926.

Walley, T. Parturient Apoplexy. Vet. 45: 869-890, 1872.

Ward, C. Parturient Paresis. Vet. Record 5: 426, 1892-93.

Whincop, G. G. A Practical Treatise on Parturient Apoplexy or Milk Fever in the Cow. Vet. 41: 705-706, 1868.

Widmark, E. and O. Carlens. Parturient Paresis: A Hypoglycemia Coma. Svensk. Veterinartidskrift 30: Oct. 1925 (Trans. by Nelson, C. A., Am. Vet. 6: 28, 1925).

Widmark, E. Uber Ursache und Behandlung der Geburtsparese. Berlin. tierarztl Wochschr 42: 537, 1926. (Trans. by Udall, Cornell Vet. 16: 304, 1926.)

Wilson, L. T. and E. B. Hart. The Chemistry of the Blood of Dairy Cows Before and After Parturition and it's Relation to Milk Fever. J. Dairy Sci. 15: 116-131, 1932.

Youatt, W. On Milk Fever in Cattle or Dropping after Calving. Vet. 3: 451-457, 1830.

Zundel. Quoted by Thomassen, A Contribution to the Study of Parturient Fever of the Cow. J. Comp. Path. Therap. 3: 1-9, 1890.