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DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
William Edward Julien, B.S., M.S.

* * * * *

The Ohio State University
1976

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CHAPTER 1

NUTRITION AND HEALTH IN THE PARTURIENT DAIRY COW

Breeding and feeding for higher levels of milk production in dairy cows have produced animals which often verge on abnormal physiological states. Consequently it is in the dairy cow where the metabolic diseases, particularly those diseases etiologically related directly to nutrition are of greatest importance.

The incidence of metabolic disturbances in dairy cows is highest in the period commencing with parturition and extending until the peak of lactation. This is a period marked by significant physiological changes within the cow as indicated by rapid exchange of nutrients and sudden variations in intake. These variations are influenced by variations in ingestion, digestion and absorption. These factors interact and are potential mediators of damaging changes within the internal environment. In essence, they favor a reduction in the metabolic stability of the animal by challenging the homeostatic mechanisms, thus increasing individual susceptibility to diseases of stressed metabolism. This fact is of considerable concern to the dairy industry due to the increasing prevalence and high morbidity and mortality rate associated with metabolic disease.
Parturition encompasses the various physiological processes involved in the birth of young. Garm (2) in considering parturition in light of Selye's theory of general adaptation, proposed that metabolic diseases are diseases of adaptation. He states: "Adaptation to gestation decreases resistance to other forms of stress. This decrease in resistance is most pronounced towards the end of pregnancy, culminating at parturition. Extra demands placed upon the organism are eliciting factors and give rise to these adaptative lesions". The primary eliciting factor continually referred to by Garm was nutrition.

It has long been recognized by dairymen that nutrition during the preparturient period has a significant influence on the reaction of the cow to parturition and onset of lactation. It is common husbandry practice to attempt to provide the dam with an adequate, properly balanced ration to meet these ends. Within recent years, however, the nutritional management of the preparturient cow has been dictated more by economics than by physiological need and common sense. Subsequently, during the same period, the incidence of metabolic disease has risen steadily. This unfortunate correlation has its basis in the fact that the feeding of dairy cattle for high milk yields is etiologically related to the diseases of metabolism so common in these animals. Emphasis in dry cow nutrition has shifted towards meeting anticipated rather than actual nutrient need. This is confounded by attempting to accomplish such a program in what appears to be the most economical fashion. Consequently, these animals are subjected to nutritional
inputs which range from deficiency to overconsumption, both of which frequently occur simultaneously.

This work was undertaken under the premise that metabolic diseases of the parturient cow are directly attributable both in their etiology and prophylaxis to preparturient nutrition. Of the common metabolic disorders of the parturient dairy cow as listed by Blood et al. (1), three were chosen for study on the basis of their relative significance to the dairy industry in general, and the Ohio dairy industry in particular, as well as the need for additional scientific insight into their respective etiologies.

Parturient paresis (Chapter 2), a disease of abnormal calcium homeostasis was chosen on the basis of the preponderance of conflicting evidence surrounding its prophylactic treatment as well as a need for reexamination of calcium and phosphorus nutrition in cows on the basis of new discoveries in the area of calcium metabolism. It also represents a distinct type of metabolic disease: the nutrient imbalance.

Retained placenta (Chapter 4) is not commonly regarded as a metabolic disease. However, its incidence has been acknowledged by authors to be influenced by preparturient nutrition. Our working hypothesis is that this disease, when not induced mechanically, or pathogenically, is a metabolic disturbance that may be responsive to selenium and vitamin E supplementation. Furthermore, it was theorized that retained placenta is a clinical expression of selenium deficiency in the mature dairy cow and thus represents a deficiency metabolic disease.
The downer cow syndrome (Chapter 3), an undefined metabolic disease of the parturient cow is of extreme interest due to the lack of any scientific basis for its etiology, prevention, or therapy. Due to the serious nature of this disease, research need in this area is urgent. It represents yet another type of metabolic disturbance: the complex syndrome which often encompasses the previous two categories. Another metabolic disease not studied in these experiments but of equal importance is displaced abomasum.

The hypotheses of this author were based on the premise that proper nutrition and good health in the parturient cow are highly correlated. It was the intention of this work to collect experimental information to test this premise, as well as provide new information which may be beneficial for handling problems specifically studied as well as to the problems of metabolic disturbance in dairy cattle in general.
LIST OF REFERENCES


CHAPTER 2

CALCIUM, PHOSPHORUS, AND VITAMIN D₃
INTERRELATIONSHIPS IN PARTURIENT COWS

Parturient paresis (milk fever) is a metabolic disorder which occurs most frequently at or near parturition and the subsequent onset of lactation in older multiparous dairy cows. Clinically, it is a derangement of Ca and P metabolism that expresses itself as an impairment of neuromuscular function (75, 15).

A 1970 survey indicated that 10% of all cows calving in the state of Wisconsin were affected by this disease during that year (75). Parturient paresis has been estimated to reduce the productive life of dairy cattle by 3.5 years (122). In terms of economic loss, this disease costs the American Dairy Industry approximately 10.5 million dollars per year (87) although this figure is believed to be a conservative estimate. Thus, milk fever is a problem that has considerable economic importance to the dairy industry.

The most desirable way to reduce the losses from parturient paresis is to prevent its occurrence on a herd basis. Two of the more significant methods currently being studied to achieve this end are the dietary manipulation of Ca and P, and the pharmacologic use of vitamin D₃ and its metabolites. Those nutrients have been shown to be
of importance in Ca homeostasis. It has also been suggested that they significantly interact with each other and that these interactions can either be supportive or detrimental to the animal’s maintaining Ca homeostasis at parturition. This study was initiated to examine these interactions and establish criteria whereby they could effectively be utilized in the prevention of parturient paresis.

2.1 Etiology of Parturient Paresis

Parturient paresis is a metabolic disorder which occurs most frequently at or near parturition and the subsequent onset of lactation (75). Clinically, it is a derangement in Ca and P metabolism, the expression of which is dependent upon those physiological responses which are related to normal Ca and P metabolism for their activity. Particularly important is the impairment of neuromuscular function (15) associated with this occurrence.

A cow with parturient paresis may be categorized as hypocalcemic, hypophosphatemic, and hypermagnesemic (84). Affected animals tend to be hyperglycemic with no serum mean increases in insulin. A several fold increase in serum hydrocortisone levels is also noted (87).

Onset of the disease usually occurs within a 12 to 24 hr postpartum period, although prepartum attacks are reported. Midlactational hypocalcemia also results in similar symptoms and is treated similarly to parturient paresis. Initially, animals appear restless and show signs of incoordination and unsteadiness and anorexia. Within a few hours, muscle tremors involving the larger muscle masses occur. This is then followed by ataxia, labored
breathing, anorexia, and alimentary stasis. Animals assume a characteristic pose, the animal being on her sternum with her head bent back into the flank and resting on the shoulder. The eyes appear dull and staring, the pupils dilated and the muzzle dry. The pulse is usually 50-80, with the body temperature being usually subnormal. The animal eventually becomes laterally recumbent and comatose. If left untreated, the coma will deepen and death will ensue (87, 154).

The initial biochemical change which occurs in paretic cows is the drop in serum Ca and P. Ca depletion is usually noted shortly before calving and reaches its lowest point within 24 hrs postpartum. Hibbs et al. (71) reported that animals which eventually developed parturient paresis had serum Ca values of 7.5 mg% by 12 hrs postpartum. Blood Ca levels as low as 1.5 mg% have been reported, but usually paretic cows average 4.5 mg%. Serum inorganic P declines over a 24 hr prepartum period and reaches its lowest point at parturition (100, 68). Cows with parturient paresis usually have a mean serum P level of 2.1 at the time of treatment (87). Shortly after the initial decline in Ca is observed, serum Mg levels begin to rise (87, 105). Average values for serum Mg of affected cows is around 3.0 mg% (87).

The reported rise in hydrocortisone is a normal parturient response (23), and associated with the hyperglycemia noted during this period. Hydrocortisone elicits this primarily by its glucogenic effect but also it has been known to inhibit insulin response at target tissues. There is also an apparent interference with insulin
secretion which occurs in the hypocalcemic state (87). This defect in insulin response and the diabetic-like state produced by the increased adrenal secretion does not appear to be pathological, but it is believed to contribute to the overall pathogenesis of the disease (87).

Several excellent reviews (64, 13, 75) as to the history, theories, treatments, and preventives of this disease have been presented in the literature. All generally agree with the observation of Stott (144), who proposed that parturient paresis was primarily a disease of nutritional origin but dependent upon physiological factors for expression.

2.2 Calcium and Phosphorus Metabolism in the Parturient Cow

The physiological changes in the cow during gestation and at parturition have a profound effect upon the maintenance of Ca and P homeostasis. As gestation approaches termination, the turnover rates of plasma Ca and inorganic P increase significantly (149). Ca and P regulation must adjust to increases in Ca and P outflow which peak during this period. A fetal drain of 5.3 grams of Ca and 2.0 grams of P per day have been shown during the last third of gestation. The act of parturition itself and the subsequent initiation of milk synthesis increase average outflow to 12.6 grams of Ca and 10.8 grams of P per day. These losses are often greater than the total Ca and P that is readily available to the cow from tissue storage and intestinal absorption (75).

In measuring the homeostatic response to gestation and lactation in dairy cows, workers (157, 149) found that most normal cows vary
from a borderline state to a positive state of Ca and P balance during late gestation. Prepartal cows range from 8.5 to 11.4 mg% in total plasma Ca and from 3.1 to 6.0 mg% in total plasma inorganic P during this period. Parturition, however, invariably affects the animal's ability to maintain homeostasis. Drops in plasma Ca and P of 1 to 2 mg% would appear to be a normal consequence during the period of parturition (139, 127, 58).

Ramberg (131) could find no other important cause of Ca loss aside from milk secretion. However both Neidermier (105) and Robertson (134) have reported serum drops of .5 mg% of Ca and 1 to 2 mg% of P in parturient, surgically mastectomized cows. Negative Ca and P balances have also been documented for as long as 2 weeks prior to parturition in dairy cows (157). Although the importance of milk synthesis to Ca and P blood changes cannot be denied, these data would suggest that there are other changes in the parturient cow which challenge body homeostasis mechanisms, or make ionic regulation more difficult.

The control of Ca and P metabolism is elicited through exchange between blood and bone, soft tissue and other body fluids, plus intestinal absorption and endogenous intestinal secretion. The relative importance of endogenous Ca and P exchange and exogenous Ca and P absorption to regulation has been shown to vary with physiologic condition (130). Endogenous Ca and P has been shown to be in a dynamic state; turnover being dependent upon dietary, endocrine and physiological influence. The major part of endogenous Ca and inorganic P is stored as labile and cancellous bone (131). In the
parturient cow, the availability of bone mineral appears to be limited for several reasons. Kleiber et al. (77) concluded that in the mature cow, readily exchangeable bone pools of Ca and P amounted to only 2% and 1% respectively of total bone Ca and P content. Several workers (144, 78) have postulated that the endocrine changes during gestation and parturition inhibit resorption of bone mineral, thus limiting available Ca and P even further. Payne (122), in an earlier study, reported bone mobilization rates in parturient cows of only .5 gm/hr and readily available Ca reserves of only 6.36 grams as compared to the 7.57 gram reserves in dry nonpregnant cows. These data tend to support the theory of Ramberg (130) that endogenous Ca (and therefore P) contribute little to homeostatic control in the parturient animal.

The parturient cow appears to be greatly dependent upon exogenous inflow of Ca and P in order to maintain Ca and P balance. Changes in serum Ca and P in relation to changes in dry matter intake have been reported by several other workers (126, 101). The importance of dietary input to the bovine was demonstrated by Halse (51) and later by Robertson (135), who correlated depression in serum Ca and P with a fasting state in lactating cows. Ramberg (130) concluded that intestinal absorption alone is the sole source of homeostatic control in the nonlactating cow.

Parturition frequently is preceded by a period of reduced feed intake, the degree of which varies from animal to animal (101). There is also reduced fecal output, loss of rumen sounds and a general stasis of the gastrointestinal tract (101). Payne (122) inhibited gastric motility with the drug hyocine, and reported a corresponding
drop in serum Ca in dry cows late in gestation. It was concluded that reduced motility produced a decline in Ca and P availability as well as a decreased absorption due to reduced dry matter intake. The natural anorexia of some parturient animals conceivably could produce this same effect. The inappetence has been associated (101, 40) with hormonal changes occurring during this period, specifically with the reported surge of estrogen. Henricks et al. (59) found that total serum estrogen rose dramatically in a 2 week prepartum period, peaked at parturition and declined rapidly within 12 hours postpartum. During the peak period of estrogenic activity, gastrointestinal motility was minimal (40). Muir (103) significantly reduced voluntary intake of mature non-pregnant cows by injection of physiologic levels of estrogen. Similar results were obtained by Bargeloh (4), using estradiol. In Muir's study, a corresponding drop in plasma ionic Ca occurred while Bargeloh demonstrated a reduced net absorption of Ca from the gastrointestinal tract. It was concluded that the estrogenic role in Ca and P homeostasis was primarily through its effect upon feed intake and that changes in serum Ca and inorganic P resulted from reduced absorption and availability.

In addition to observable changes in the gonadotropic hormones, parturition is thought to affect several other endocrine systems, either directly or indirectly which are cited as affecting Ca and P homeostasis. Changes in parathormone (PTH), thyrocalcitonin (TCT), the glucocorticoids, prostaglandin, prolactin, growth hormone and mineralocorticoids at parturition have been reported in the bovine (23). Of these, only the normal regulatory hormones of Ca (and P)
metabolism; PTH and TCT, the glucocorticoids, ACTH, and prostaglandin have been documented as eliciting changes in serum Ca and P (102, 153).

The importance of PTH to Ca and P metabolism is well documented (132, 24). PTH through cyclic AMP is able to increase bone resorption rates of Ca and indirectly, P, and thus increase the concentration of serum Ca and P. PTH also causes an increased renal reabsorption of Ca and prevents renal reabsorption of P. The circulatory titer of PTH is inversely proportional to plasma ionic Ca, but is unaffected by ionic P. Under normal physiological conditions, the cow is strongly dependent upon these mechanisms for maintenance of plasma ionic Ca and P. Parturition, however, somewhat negates this dependence. Hibbs et al. (70), working under the assumption that parturient hypocalcemia is actually the clinical expression of hypoparathyroidism, injected multiparous Jersey cows with 3000 to 5000 USP units of PTH but was unable to prevent the drop in serum Ca or cure the milk fever. Histological examination has recently revealed that the parathyroid glands of hypocalcemic cows are actually functioning (18). It was also reported that an inverse relationship actually existed between circulatory levels of Ca and PTH; the more severe the hypocalcemia, the higher the circulatory levels of PTH (98). This apparent unresponsiveness to PTH in the parturient cow is thought to be a result of certain other endocrine responses to parturition. Cluess et al. (21) demonstrated an enhancement of phospholipid synthesis in rat bone tissue as a result of elevated estrogen levels. This was associated with a depression in serum Ca due to inhibition of PTH induced bone resorption. In sheep, elevated levels of prostaglandin
appeared to interfere with PTH action on adenyl cyclase, thus limiting Ca bone turnover. The availability of bone Ca and P tends to decrease with increasing age (77, 122, 130). This may also be of significance in the lack of response to PTH in the parturient cow of these minerals.

The second major hormone involved directly with Ca and therefore indirectly with P homeostasis is TCT, a peptide secreted by the C cells of the thyroid (24). TCT reduces elevated plasma Ca by inhibiting PTH directed bone resorption (49, 156). It is believed by some workers (18) that TCT is actually released at parturition and that this release is primarily responsible for drops in serum Ca. Ultrastructural examinations of bovine thyroid have shown them to be apparently active at parturition (18). However, failure to produce a drop in serum Ca when TCT was infused immediately before or after parturition, as well as a lack of documentation of any other stimuli for TCT release except hypercalcemia, a condition not normally associated with parturition, leaves the importance of TCT in parturient animals open to question.

Elevation in circulatory levels of ACTH, the glucocorticoids and prostoglandin are recognized as being commensurate with parturition (24). The ability of these hormones to depress circulating levels of Ca and P has been postulated to be produced through interferences with PTH action at bone reception sites (153, 102). The importance of these hormones, however, to changes in ionic homeostasis is not well documented.
2.3 Magnesium Metabolism of the Parturient Cow

The changes associated with Ca and P homeostasis that are elicited through physiological reactions to parturition apparently are not seen in Mg metabolism. Although there is limited documentation of actual metabolic turnover rate for Mg in late gestation and early lactation, the fact that plasma Mg balance tends to remain positive or actually increases at parturition (105, 4) strongly indicates that Mg is independent of the other macroelements in parturient response.

Mg turnover rate as affected by fetal drain has not been extensively studied. O'Kelly et al. (114) demonstrated a net dietary Mg requirement of 8.5, 7.0, and 9 mg a day for beef cattle at 155, 200, and 255 days gestation in order to maintain Mg blood levels of 2 mg%. These workers indicate an increased requirement of 2 to 6 grams per day over maintenance for pregnancy based upon NRC requirements. This increase in dietary need can be taken to be an expression of the magnitude of fetal demand. The ability of the animal to meet this demand is apparently adequate as a tendency towards a negative Mg balance is usually not seen until an animal is several weeks into lactation (25, 154).

The initiation of lactation has also been shown to increase the Mg requirement. In order to maintain serum Mg levels of 2 mg% in lactating cows, dietary Mg requirements were found to be increased to 18 to 22 grams per day for lactating beef cattle (113). Smith estimated that the dietary requirement for a lactating dairy cow producing 30 kg per day amounted to 26.4 grams per day. Net loss of
Mg in milk amounts to .112 g/kg or .01% of the total solids content (17, 143). This loss becomes a significant drain on plasma Mg over a period of time as indicated by Mg balance studies (103, 4).

The control of Mg metabolism is similar to other ions in that it is a product of endogenous and exogenous inflow and excretory outflow. Mg differs from Ca and P in that no definitive hormonal regulatory mechanisms have been demonstrated to exist for its homeostatic control (165). Also, the readily available endogenous pools of Mg are small when compared to those of Ca and P and contribute minimally towards maintaining homeostasis, even under normal conditions (165). Therefore it is thought by most workers that the animal is highly dependent upon intestinal absorption and possibly upon renal reabsorption for maintenance (165).

Underwood estimates that less than 33% of the total dietary Mg ingested is actually absorbed for metabolic use. This absorption is believed to be passive in nature; the amount absorbed generally being dependent upon the total available Mg in the diet (154).

Renal control of plasma Mg homeostasis has been suggested by workers to be important in several species (165). Balance studies indicate that cows on low Mg diets tend to excrete Mg at a much slower rate than animals on a diet adequate in Mg (143). A linear relationship was found to exist between urinary loss of Mg and serum Mg concentration for serum values above 1.5 mg%. Below this, Mg urinary loss ceased. Barker et al. (5) concluded that in man, urinary excretion is by a filtration reabsorption mechanism in which tubular reabsorption normally is operating at or near a maximum rate, and at
least partly separate from that of other ions. Thus, Mg is apparently a threshold substance and appears in the urine only when filtration load exceeds the maximum tubular reabsorption rate. This value for cattle was estimated by Wilson (164) to be around 2 mg%.

A major route of plasma Mg ion loss aside from urinary excretion is via intestinal secretion. Balance studies have shown a net loss of 200 mg per day or 4 to 5 mg/kg of body weight of Mg lost in digestive secretion per day (142, 37). This is equal to the total extracellular Mg content of the adult bovine. This loss is less affected by depression in serum Mg as compared to urinary loss as reductions in secretory loss only are expressed after urinary loss has been depressed for several days (143).

Considering the apparent dependence upon exogenous sources of Mg for maintenance, the reduced feed intake of the parturient cow (4, 101), would place the animal in a hazardous position in regard to her ability to maintain positive balance. The negative Ca and P balance, which occur at this period, have been directly related to this phenomenon (103, 4). The ability of the parturient cow to remain in positive balance, as has been shown (105), is inconsistent with this assumption. There is very little information currently in the literature to suggest a plausible explanation for this occurrence. Bargeloh (4) suggests that Mg is being neither utilized or excreted during the parturient period. This hypothesis has merit for several reasons. Of the changes in the parturient cow, directly related to mineral metabolism, the elevation of PTH as an attempt to maintain Ca homeostasis is well documented (98, 18). The possibility of Mg
release from bone as a result of PTH stimulation for Ca release does exist. MacIntyre et al. (91) found that administration of bovine PTH to rats and wether lambs decreased renal excretion of Ca and Mg, elevating serum values. Bargeloh (4) found that the Ca and Mg urinary excretion of the parturient cow was also depressed; Ca resorption being a function of PTH renal stimulation. Thus PTH mediated changes in normal Mg metabolism are likely.

Parturition is a period of peak estrogenic activity (59). By injecting lactating cows with estrogen at a level comparable to that seen at 250 days of gestation, Bargeloh (4) was able to significantly reduce Mg content of the milk. Thus estrogen at its peak activity may more dramatically affect Mg loss in colostrum.

The apparent loss of alimentary activity at parturition (122), although probably reducing the availability of dietary Mg as in the case with Ca (103), may also indirectly contribute to parturient Mg metabolism. The significant loss of ionic Mg in alimentary secretions >200 mg per day may be reduced in conjunction with the temporary reductions in gut activity.

2.4 Influence of Dietary Calcium and Phosphorus in Parturient Paresis

The relationship of prepartal Ca and P intake to the incidence of parturient paresis has been well documented in the literature (12, 144, 75). Recent progress in the area of Ca and P metabolism and their respective regulatory physiological mechanisms (3, 28) has provided information which allows for a new perspective into the physiological importance of their proper dietary management.
Early workers, recognizing the problem of parturient paresis as one of hypocalcemia, believed that it was simply an expression of a Ca deficiency and advised increasing the amounts of Ca in the dry cow ration (38, 84). Numerous workers have examined this problem. A summary of their data by Jorgensen (75) showed that high Ca intakes, over 100 grams per day, increased the incidence of parturient paresis rather than decreasing it, thus indicating that the problem is of a more complex nature than a simple lack of relative input of Ca to the system.

Due to the inconclusive results obtained through additional Ca supplemented in the diet, a second theory based upon the work of Baumann et al. (7) and others (29, 50) was proposed by Boda et al. (12), who suggested that parturient paresis was a nutritionally induced hypoparathyroidism. This condition could be prevented and corrected through manipulation of the ratio of Ca and P in the diet. These workers found that by feeding a ratio of 1.3:3 Ca/P to dry cows, the incidence of parturient paresis was significantly reduced as compared to controls on a high Ca intake. This ratio was believed to lower blood Ca through a reduced Ca availability and thus stimulate PTH mediated bone mobilization. This theory has been given much attention by several laboratories. Jorgensen (75) reports that ratios ranging from 0.10:1 to 6:1 Ca/P have been tested with inconclusive results. The recent discovery (18, 98) that a hypoparathyroid state does not normally exist in the paretic cow questions the hypoparathyroid theory and the supposed inducement of this condition through nutritional manipulation. However, the
susceptibility of cows to paretic attack has been correlated with certain dietary ratios of Ca and P by several laboratories.

Vipperman et al. (155) reported that in growing swine, the optimum Ca to P ratio varied with the level of Ca and the level of P in the diet. These workers concluded that Ca to P ratios were not as important in the utilization of these two elements as the dietary levels per se. Jorgensen (75) in summarizing the data reported by a number of workers, reached a similar conclusion.

The absorption of Ca and P has been shown to be affected by the relative concentration of both elements in the diet. O'dell et al. (112) reported a reduced Ca absorption in guinea pigs on high P diets. Hibbs et al. (66) found a reduced Ca and P absorption rate in dry cows on diets low in P. This could be reversed by P supplementation. Low levels of dietary P have been found to reduce the amounts of Ca that were absorbed in swine (155). Stott (144) was able to show an adaptation to a high Ca intake in dry cows provided that the P in the diet did not exceed .2% of the total dry matter intake. Other workers (35, 137) have shown a similar adaptation to low Ca intakes in several species but this response was P dependent and required the presence of vitamin D. Stott (144) concluded that it is the availability and metabolic needs of P, provided that there is adequate biologically available Ca, that determines whether Ca is absorbed, stored or excreted and that the effect is probably at the intestinal level.
The absorption of P is generally believed to be passive (141), the degree of which is a linear function of phosphate concentration in the duodenum (166, 90). Although recent evidence has suggested the existence of an active transport system mediated by vitamin D (160), the literature would indicate that P metabolism lacks the fine homeostatic control observed in Ca regulation. Renal excretion of inorganic P is PTH mediated and thus a function of serum Ca concentration (3). Recently thyrocalcitonin was suggested as having a more potent excretory effect upon P than Ca (160).

Ca metabolism has been recognized as an active process (141, 116). Uptake occurs at the brush border of the epithelial cell of the intestinal lumen and requires the cellular expenditure of energy, is carrier mediated and involves the translocation of Ca against an electrochemical gradient (116). The formation of the Ca binding protein carrier can occur only in the presence of the 1,25 hydroxylated form of vitamin D (116, 108). Plasma Ca as opposed to inorganic P is under a finely regulated homeostatic control mediated through the hormones of the parathyroid and thyroid (3); parathormone and thyrocalcitonin.

The interrelationship of Ca and P at the intestinal level is not entirely clear. In monogastric species the formation of phytate and oxalate salts of Ca have been shown to inhibit absorption. In the ruminant, these substances are largely destroyed in the rumen and are of minor importance (141). Precipitation of Ca phosphate has been shown to occur in the small intestine of ruminating and nonruminating calves (141) but only with the pH above 6.5; this does not normally
occur in the middle and upper small intestines which is the major site of Ca and P absorption. Recently, Tanaka et al. (151) were able to demonstrate that diets low in inorganic P resulted in increased synthesis of the 1,25 form of vitamin D; the metabolite that has been shown essential for the absorption of Ca. These workers found that in thyro-parathyroidectomized rats under a variety of dietary Ca and P intake levels, the ability to synthesize the 25, (OH) D₃ to 1,25 (OH)₂ D correlates with serum inorganic P values below 7-8 mg% while the synthesis of the less active 24,25 (OH)₂ D₃ correlate with serum values above 7-8 mg%. There is, in addition, a close correlation between reduced kidney cortex inorganic P levels and synthesis of 1,25 (OH)₂ D₃, which suggest that the renal tubular cell inorganic P level underlies the regulation of synthesis of 1,25 (OH)₂ D₃ in the kidney and that PTH and calcitonin regulate 1,25 (OH)₂ D₃ synthesis via their effects on renal cell inorganic P levels. Other workers, however, have failed to establish a similar correlation between renal cell inorganic P concentration and 1,25 (OH)₂ D₃ synthesis (110).

2.5 Therapeutic Value of Vitamin D

In studying the interrelationships of induced rickets and tetany in Ca deficient rats, Hess et al. (62) found they could increase the concentration of serum inorganic Ca and P by administration of irradiated ergosterol. This and similar data prompted Grieg (47) and later Sjollema (140) to propose that vitamin D might be effective in milk fever prevention and therapy.

The findings of Little et al. (85) and Hess et al. (61) showing that the oral supplementation of vitamin D or its derivatives causes
increases in serum inorganic Ca and P in several species, including
the bovine, was cited by Hibbs et al. (67) as evidence that vitamin D
was of therapeutic value in parturient paresis. Hibbs et al. (67)
initiated a series of experiments to test this theory. One million
units of irradiated ergosterol in the form of yeast was fed for a
four week perpartum and one week postpartum period to a group of
mature preparturient cows. No reduction in milk fever was observed in
the treatment group. In comparing the one million unit treatment to
an increased dosage of two million units, Hibbs et al. (68) reported
a transitory increase in serum Ca and P in animals fed the higher
dosage but no increases in the one million unit group. This change
in the two million group was insufficient to prevent milk fever,
however. Dosage (71) was increased to five million units, and the
length of the treatment was decreased to two weeks. A greater serum
Ca response was observed but this was also transitory and proved
unsuccesful in prevention.

Failure to produce a positive response in treated animals caused
Hibbs et al. (69) to increase the dosage and to further decrease the
treatment time. It was assumed that their previous failure could be
explained by the work of Wilder (162) and Campbell et al. (17), who
found that prolonged feeding of vitamin D led to suppression of para-
thyroid function. The importance of parathormone to Ca metabolism is
well documented. As milk fever is primarily a failure of the blood
Ca regulatory mechanism to mobilize enough Ca, any further inhibition
apparently caused by prolonged vitamin D supplementation would be
detrimental. Therefore a higher dose for a shorter duration seemed
to be indicated. Four groups of mature Jersey cows were fed 5, 10, 20, and 30 million units respectively of vitamin D per day for a period of 3 to 8 days prepartum. It was found that protection from milk fever was provided by all groups but that the 20 and 30 million unit animals had the highest level of protection (80%). Blood Ca and P were elevated throughout the critical period for the 10, 20 and 30 million unit groups. Histological sections of parathyroid tissue taken from representatives of each group indicated a low level of parathyroid activity, and it was suggested that vitamin D supplementation caused a parathyroid replacement action which masked the hypoparathyroid condition.

Dell et al. (27) were able to reduce the incidence of milk fever in Jersey cattle from 61 to 23% by feeding 30 million units of vitamin D for 3 to 7 days prepartum. Similar results were reported by Weighton (161). Hibbs et al. (65), found that major protection from feeding 30 million units of vitamin D could not be expected until it had been fed for 3 days. This protection dropped off rapidly one day after the termination of supplementation.

Manston et al. (92) examined the possibility of intramuscular injection of a vitamin D preparation to obtain protection. Their work reported a net increase of 50 grams and 40 grams of Ca and P respectively when 10 million units of vitamin D were injected into pregnant cows. Injection of 40 million units and 2.5 million units were also made with net increases of 30 grams and 20 grams at 40 million units and 18 grams and 35 grams at 2.5 million units. A clinical toxicity manifested by anorexia, uremia, and gastrointestinal
stasis was seen in the 40 million unit treatment group. Upon slaughter, examination of the 40 and 10 million unit group showed extensive lesions of the heart, endothelial and arterial systems, renal calculi and general calciferous deposition in the kidney. These lesions were most apparent in the 40 million unit treatment animals. Further work by Payne et al. (123) and Payne (122) concluded that the occurrence of metastatic lesions in animals treated with 10 million units of vitamin D₃ were rare and advised that an intramuscular injection of this magnitude administered not less than 24 hours but not more than 8 days prepartum was a safe and effective method of milk fever prevention, provided the diet contained adequate P and Mg.

The management of prepartum feeding and intramuscular injection of vitamin D presents a problem in that they are dependent upon the accurate prediction of parturition to ensure a maximal effect. Hibbs et al. (66) proposed that continuous feeding of vitamin D might help to maintain available calcium stores at a high level and thus prevent milk fever. Vitamin D was fed continuously in the grain ration to mature cows at a rate of 32,000 units per pound of grain consumed and a minimum of 96,000 units per day where no grain was fed. This helped reduce the incidence among treated animals with a previous history but it increased the incidence of milk fever somewhat in non-parturient cows. It was concluded that although this type of vitamin D supplementation will protect cows with previous histories, such levels would tend to increase rather than decrease the herd.
incidence of milk fever if fed to all cows in a herd under normal management conditions.

The recent discovery of Lund and DeLuca, an active intermediate metabolite of vitamin D₃, 25-OH D₃, was proposed by the Wisconsin workers as a method of milk fever prevention. 200 µg of 25-OH+D₃ was given in an intravenous injection at least 24 hours prepartum to a group of paretic prone cows. Injections were given not more than 3 days prior to calving. This dosage prevented parturient paresis. An oral dosage was also tested. 250 µg of 25 OH D₃ was encapsulated and given at least 18 hours but not more than 3 days prepartum. The amount of milk fever was decreased by 35% in treatment animals in one herd and 27% in another. Bringe (16), Jorgensen (74), and Olsen (115) found that a 2 mg injection of 25-OH-D₃ was ineffective in milk fever prevention. Administration of 4 to 8 mg of 25-OH-D₃ 72 hours to 10 days precalving resulted in a 0 incidence in treated cows. The authors conclude that 25-OH-D is effective in reducing the incidence of milk fever.

2.6 Vitamin D Metabolism

In 1919, Mellanby (97) discovered an antirickitic substance in cod liver oil. This substance, designated vitamin D, was documented as having a catalytic role in Ca metabolism and was found to be essential for the prevention of several physiological diseases of metabolic origin (55, 60, 43, 96).

Cruickshank et al. (26) were the first to suggest that vitamin D does not function in an unaltered state. The demonstration of a lag time of 4 to 12 hours in the rat by Harrison (54) and 4 to 16 hours in
the chick by Sallis et al. (130), between administration of vitamin D and visible physiological effects led to the hypotheses that vitamin D must be metabolized to a biologically active form before it functions. Norman et al. (108) was able to detect 5 metabolic end products from radiochemically pure H3 vitamin D3 administered to rats. Of these, four were found to have biological activity. Lund et al. (90) were able to isolate three metabolites of radio labeled vitamin D3 one of which had activity comparable to the parent vitamin. This was characterized by Blunt et al. (9) as 25-OH-D3 and shown (10) to be 40% more biologically active than vitamin D3. Ponchar (124) determined the greatest concentrations of this metabolite to be in liver tissue and proposed this organ as the site of synthesis. The work of Hasting et al. (57) offered conclusive evidence to this effect, through characterization of the liver enzyme system responsible for the hydroxylation reaction.

Hanssler (53) and later Lawson et al. (83) isolated a second metabolite of vitamin D in intestinal mucosa of D deficient chicks, which was found to be more polar than the 25 form and possessed intestinal Ca transport activity. It was also shown that the 1α position of the molecule was modified before it initiated any biological response. Fraser et al. (41) were able to first isolate the kidney as the origin of this metabolite. This was confirmed by Norman et al. (110) and Gray et al. (46). Norman et al. (110) identified this kidney tissue metabolite as identical to that isolated earlier in chick mucosa. This metabolite was found to be hydroxylated in both the 1 and 25 position and were designated as 1,25 OHD3
Several laboratories (104, 118, 129) were all able to show the 1,25 form to be 80 to 100 times more rapid in biological activity than the 25 form. This suggests that it is the 1,25 OHD₃ rather than the 25 form which is the active form. Tanaka et al. (150) were successful in blocking the hydroxylation of 25 to 1,25 by administering Actinomycin D to weanling rats. Frolik et al. (42) could find no evidence of further structural changes after hydroxylation at the 1 and 25 positions. Their data bear out the earlier conclusion of the Wisconsin workers as to the biological importance of the 1,25 form.

The initial work done in the Wisconsin laboratory disclosed two additional metabolites of vitamin D₃. Suda et al. (148) isolated from the plasma of pigs given several large doses of D₃ a metabolite which was .5X as active as vitamin D₃ in curing rickets in the rat. This was initially identified as 21,25 OHD₃. It was found to be less efficient in promoting gut absorption but more efficient in Ca mobilization from bone than vitamin D₃. More complete characterization showed the metabolite to actually be 24,25-OHD₃. Omadahl et al. (117) showed the 24,25 form to be synthesized in the kidney in the presence of high Ca intake or when there is no net synthesis of 1,25 OHD₃. It has variable biological activity being able to only slightly stimulate intestinal Ca absorption but it shows more activity on a stimulation of bone Ca mobilization.

Suda (148) demonstrated the presence of another metabolite, 25,25 OHD₃. It has little biological activity although it has shown some intestinal stimulatory action. It was proposed that this could
conceivably be an intermediate in final cleavage sections before elimination from the body.

2.7 Vitamin D<sub>2</sub> Metabolism

Suda et al. (146) after feeding 500,000 IU of vitamin D<sub>2</sub> to pigs for 26 days, isolated a polar, biologically active metabolite: 25-OHD<sub>2</sub>. It was concluded that vitamin D<sub>2</sub> must be metabolized identically to vitamin D<sub>3</sub>.

2.8 Mode of Action of Vitamin D

Vitamin D has long been delegated a catalytic role in Ca metabolism. It has been shown to be essential for the normal function of most of the physiologic mechanisms associated with Ca metabolism. Generally these may be thought of as those mechanisms employed in the absorption of dietary Ca and possibly P and those associated with the replenishment and mobilization of endogenous Ca stores, i.e. bone.

Martin and DeLuca (93) demonstrated that the initial velocity for the uptake of Ca by epithelial tissue of the intestine of the rat was two times greater when vitamin D was supplemented than when no vitamin D was fed. The recent discovery of a calcium-binding protein (159) located in the mucosal goblet cells of the intestinal lumen (14) and the correlation between its appearance in tissue, the administration of vitamin D, and the increased absorption of Ca has led workers to conclude that vitamin D is essential for the absorption of dietary Ca (116, 110).

Workers generally believe that vitamin D in the form of the renal hydroxylated form, 1,25(OH)<sub>2</sub>D must undergo a two step binding process within the intestinal epithelial cell before it can produce a
biologically measurable response. The $1,25(OH)_{2}D$ is first associated with a binding protein in the cytoplasm of the cell. Thus bound, it penetrates the internal nucleus of the cell as it is associated with a chromatin. This association results in the synthesis of new RNA which appears to be coded for the formation of the calcium-binding protein. This particular protein is believed to be responsible for the initial influx of Ca into the cell. In addition, two enzymes, a calcium ATPase and a brush border alkaline phosphatase are also synthesized. These enzymes appear to facilitate the influx of Ca into the cell from the brush border of the epithelial cell on the lumenal side as well as mediating release on the serosal side via a "Ca-Na pump". Modifications in the phospholipid and cholesterol esters of the entire cell membrane have also been reported on a vitamin D induced response but the significance of these changes to Ca metabolism are not currently understood.

Wasserman et al. (159) have suggested that a vitamin D mediated active transport system that is independent of that of Ca exists for P. Elevation in serum P (116) has been shown to occur after the administration of either the 25 or 1,25 form of $D_{3}$. However, in nephrectomized rats, the response to the 25(OH)D is lost, suggesting that $1,25 (OH)_{2}D$ may be essential for P transport as well.

Carlson (19) demonstrated that vitamin D is able to bring about the mobilization of Ca from previously formed bone. Harrison et al. (54) concluded that vitamin D was essential for this process to occur. It was found that parathormone, which functions in the body's control of Ca through mobilization of Ca from bone (3) cannot function in the
absence of vitamin D. This would indicate that vitamin D in either of its metabolic forms acts synergistically with parathormone to promote bone mobilization; however, the mechanism involved is not clearly understood.

Workers (116) have shown that vitamin D is also essential for the normal calcification of bone. This particular function, however, is believed to be a consequence of the stimulating effect vitamin D has upon alimentary absorption of Ca and perhaps P.

Recent reviews covering all aspects of vitamin D and Ca metabolism have been published by Omadahl et al. (116), Norman (107), and Borle (14).

2.9 Experimental Procedures

The use of the field trial permits a large number of observations to be made under varying conditions in a comparatively short period of time. This type of experiment is particularly useful in establishing the efficacy of prophylactic agents in disease control. This experiment involved 17 dairy operations centered primarily in north-eastern Ohio. Four major dairy breeds; Holstein, Jersey, Guernsey and Ayrshire were represented in the sampling. The total number of observations made was 364, 182 of which were in the treatment group.

The primary objectives of this experiment were:

1) To test the therapeutic value of intramuscular injections of vitamin D in the prevention of parturient paresis.

2) To determine the possible effects of daily dietary intakes of calcium and particularly phosphorus on the efficacy of the injected vitamin D.
3) To examine the role of dietary calcium and phosphorus on the incidence of parturient paresis.

Seventeen dairy farms located in the counties of Wayne, Medina, Portage, Mahoning, and Columbiana in northeastern Ohio, and Franklin in central Ohio cooperated in the study (Appendix Table 1). Herds were chosen on the basis of past incidence of parturient paresis as indicated by the records of local veterinarians. Data were collected on the nutritional, reproductive and health management of each herd.

Only animals entering their third lactation or older were considered for the experiment. The age, lactation number, lifetime productive average and past health history were recorded for each animal. Animals were then randomly assigned to either the treatment or control group.

Twenty-five percent of the animals involved in each herd were bled from either the jugular or tail vein after group assignment. Approximately 20 ml of whole blood was collected at this time in heparinized tubes for subsequent plasma analysis of inorganic Ca, P and Mg.

Treatment animals received an intramuscular injection of 10 million units of vitamin D₃ which was supplied by the experimenter to the cooperators. Vitamin D₃, in the form of cholicalciferol resin, was supplied by Dawes Laboratories and emulsified at the Ohio Agricultural Research and Development Center in an ethanol vehicle at a concentration of 2 million units per cc. Injections were given by the herdsman in the area of the flank, not less than 24 hours but not more than 192 hours prior to parturition. An injection schedule was
designed for the 7-day interval. A specific day of each week was designated as day 0. At this time all treatment animals due to freshen for the next 7 days received 5 cc or 10 million units of D₃. If an animal failed to calve during this time, she was reinjected on day 0 of the following week. A maximum of three injections was allowed. If an animal still had not freshened after the third injection she was withdrawn from the experiment. Records were kept on the date or dates of injection, the date of calving, the incidence of parturient paresis and response to therapy, if necessary.

To determine the amount of dietary Ca and P consumed by each animal, the components of the rations fed were collected and relative amounts consumed recorded and submitted to the Ohio Ration Evaluation Laboratory for analysis.

2.10 General Methods

Preparation of vitamin D₃

Approximately 500 g of cholicalciferol resin assaying 22.8 million units per g was supplied by Dawe's Laboratories. 43.9 grams of this resin were added to 300 ml of absolute ethanol and mixed vigorously until it had entered into solution. This was then brought up to volume (500 ml) with additional absolute ethanol. D₃ activity of this mixture was calculated at 2 million units per cc. The preparation was then dispensed into 30 cc serum vials and stored under refrigeration until needed.

Analytical procedures

Plasma Ca and Mg determinations were accomplished by atomic absorption spectroscopy using the procedures of Willis (163) with
certain modifications (103). Plasma samples were diluted 1:50 with
0.1 N HCl and lanthanum chloride solution. Plasma phosphorus
determinations were done by colorimetric analysis using a modified
AOAC procedure (125).

Mineral content of feed samples was determined by spectrographic
analysis with an emission spectrograph.

Nitrogen determinations were done by AOAC procedures. Net
energy values were estimated by methods outlined in OARDC Bulletin
554 (128).

2.11 Results

The intramuscular injection of 10 million units of vitamin D₃
reduced the overall incidence of parturient paresis by 65% in animals
having a previous history of this disorder. Actual incidence in the
treated, previous history cows was 27% as compared to 41% for the
control animals. This was in contrast to the results in the non-
previous history animals, where no significant reduction in incidence
was noted between treated (22.8% incidence) and untreated (23%
incidence) groups (Table 1).

The efficacy of vitamin D was found to vary significantly with
the total grams of Ca and P consumed per head per day. Among the 17
management units involved in the study, three different Ca and P
intake groups were evident. The first of these, was a consistently
high intake of both Ca (>0.5%) and P (>0.25%) throughout the dry period.
The second was a reduced Ca (<0.5%) fed with an elevated (>0.25%) P. In
the third Ca and P intake was within the recommendations of the
National Research Council (NRC) (111) for the pregnant, mature,
TABLE 1. Incidence of parturient paresis in previous history and no previous history cows as affected by treatments with 10 million units of vitamin D₃.

<table>
<thead>
<tr>
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<th>Previous history (%)</th>
<th>No previous history</th>
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<tbody>
<tr>
<td>Treated</td>
<td>27.0</td>
<td>22.8</td>
</tr>
<tr>
<td>Control</td>
<td>41.0</td>
<td>23.0</td>
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TABLE 2. Effect of dietary calcium and phosphorus on efficiency of treatment with 10 million units of vitamin D₃ for the prevention of parturient paresis.

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<th>Group No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>NRC Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;.5% Ca, &gt;.25% P</td>
<td>&lt;.5% Ca, &gt;.25% P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence</td>
<td>31.9</td>
<td>27.6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Efficacy</td>
<td>68.1</td>
<td>72.4</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>
non-lactating dairy cow; .5% of the total dry matter consumed as Ca and .25% as P. It was found that the total incidence of parturient paresis, for treated animals, both previous and non-previous history, was 31.9%, with an efficacy value of 68.1% in the first group. Total incidence of both treated previous and non-previous history cows in the second group was 27.6% and an efficacy value of 72.4%. In the third group, treated previous and non-previous history cattle had an incidence of only 2%, with an efficacy value of 98% (Table 2).

Efficacy of vitamin D₃ treatment in previous history animals was significantly less in both the group 1 (62.2%) and group 2 (68.2%) cows as compared to those in group 3 (100%) (Table 3). Significant reductions in incidence, however, were seen only in group 1 and group 3 where incidence was reduced from 60% to 37.8% and 10% to 0% respectively. No significant reduction was noted in the low Ca, high P cows (Table 4).

In non-previous history cows, treatment was 72% effective in group 1, 76% effective in group 2 and 95% effective in the recommended level group (Table 3). No significant reduction in incidence over controls was noted in any group of treated cows. On the elevated Ca and P diets, treated animals had an incidence of 28% as compared to 31% for controls. Treated cows in group 2 had an incidence of 24% which was not significantly different from the 14.2% observed in controls. Incidence at recommended intakes was reduced, but insignificantly, from 9.3% in controls to 5.0% in treated cows (Table 4).
TABLE 3. Effect of dietary calcium and phosphorus on efficacy of treatment with 10 million units of vitamin D₃ for the prevention of parturient paresis in previous history and no previous history cows.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;.5% Ca, &gt;.25% P</td>
<td>&lt;.5% Ca, &gt;.25% P</td>
<td>NRC Recommendations</td>
</tr>
<tr>
<td>Efficacy</td>
<td>62.2</td>
<td>68.2</td>
<td>100</td>
</tr>
<tr>
<td>previous</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Efficacy</td>
<td>72.0</td>
<td>76.0</td>
<td>95</td>
</tr>
<tr>
<td>no previous history</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4. Effect of dietary calcium and phosphorus on incidence of parturient paresis in vitamin D treated and control previous history and no previous history cows.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;.5% Ca, &gt;.25% P</td>
<td>&lt;.5% Ca, &gt;.25% P</td>
<td>NRC Recommendations</td>
</tr>
<tr>
<td>Treated previous history</td>
<td>14/37 = 37.8%</td>
<td>7/22 = 31.8%</td>
<td>0/18 = 0.0%</td>
</tr>
<tr>
<td>Control previous history</td>
<td>9/15 = 60.0%</td>
<td>2/4 = 50.0%</td>
<td>1/10 = 10.0%</td>
</tr>
<tr>
<td>Treated no previous history</td>
<td>17/60 = 28.0%</td>
<td>6/25 = 24.0%</td>
<td>1/20 = 5.0%</td>
</tr>
<tr>
<td>Control no previous history</td>
<td>29/93 = 31.0%</td>
<td>4/28 = 14.2%</td>
<td>3/32 = 9.3%</td>
</tr>
</tbody>
</table>
Incidence of parturient paresis was found to be significantly affected by dietary Ca and P. Animals consuming the high Ca, high P diets had an overall incidence of 33.6% while incidence in the low Ca, high P fed cows was 24.0%. Those animals fed according to NRC recommendations, however, had an incidence of only 6.2%, a significant reduction from the other groups (Table 5). When examined in relation to previous and non-previous history, incidence varied from 60% in group 1 cows, and 50% in the group 2 animals to 10% for those animals consuming the recommended .5% of their dry matter as Ca and .25% as P (Table 4). In non-previous history cows, a significant reduction in incidence was noted between the high Ca, high P cows (31%) and those on the recommended levels (9.3%). A difference in incidence between the low Ca, high P cows (14.2%) and the recommended level group was noted but was not significant (Table 4).

A stepwise multiple regression resulted in four significant variables (Table 6). These were dietary Ca, dietary P, blood P and number of animals per herd 4 years of age or older. When placed in the equation: $+21.5 + 1.48 (A) + 1.93 (B) - 1.5 (C) + .2 (D) + 1.2$: where $A =$ percent Ca in the diet, $B =$ percent P in the diet, $C =$ number of animals 4 years of age or older, and $D =$ the percent P in the blood, and 21.5 equals the mean incidence of the sampled populations and 1.2 equals the standard error of the estimate, these values indicated the following.

1) For every increase of .1% of dietary Ca above .59% incidence of parturient paresis increased by 14%.
TABLE 5. Effect of dietary calcium and phosphorus on total incidence of parturient paresis in previous and no previous history cows.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;.5% Ca, &gt;.25% P</td>
<td>&lt;.5% Ca, &gt;.25% P</td>
<td>NRC Recommendations</td>
</tr>
<tr>
<td>Reported cases</td>
<td>69</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>Total observations</td>
<td>205</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>Incidence, %</td>
<td>33.6</td>
<td>24.05</td>
<td>6.20</td>
</tr>
</tbody>
</table>
TABLE 6. Significant variables observed in the vitamin D₃ field trial as shown by stepwise multiple regression analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>Standard deviation coefficient</th>
<th>t value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary calcium</td>
<td>0.14382433</td>
<td>0.6038781</td>
<td>2.381</td>
</tr>
<tr>
<td>Dietary phosphorus</td>
<td>0.19278515</td>
<td>0.15796041</td>
<td>1.22</td>
</tr>
<tr>
<td>Blood phosphorus</td>
<td>0.20814834</td>
<td>0.14892376</td>
<td>1.397</td>
</tr>
<tr>
<td>Number of animals 4 yr or older</td>
<td>-0.15053165</td>
<td>0.10250819</td>
<td>-1.468</td>
</tr>
</tbody>
</table>

Multiple correlation coefficients = 0.358
Goodness of fit (4, 95) = 3.505
Standard error of estimate = 0.11937497
2) For every increase in dietary P of .1% above .37% incidence of parturient paresis increased by 19%.
3) Increases in dietary P were reflected in total blood P.
4) For every increase in blood P of 1 mg % above 5.80 mg % incidence of parturient paresis increased by 20%.

2.12 Discussion

The observation of Payne (122) as to the prophylactic value of a 10 million unit intramuscular injection of vitamin D₃ in a period 24 to 192 hr prepartum was confirmed in this study. Efficacy, however, was apparently dependent upon both the physiological state of the animal and the prepartal dietary intake of calcium and phosphorus.

The significant reduction in parturient paresis in treated previous history cows and the apparent lack of response in treated nonprevious history animals (Table 1) was noted in an earlier study conducted by Hibbs et al. (66). This variation in animal response can be explained in terms of the premise that physiological differences exist between previous milk fever and nonprevious milk fever history cows.

Data collected in the 1966 study and during the current field trial showed no significant differences between previous and nonprevious history animals in respect to reproductive performance, susceptibility to disease, or pounds of milk produced in the previous lactation. A difference did exist, however, in the means of the ages of the two groups. The previous history animals were older, averaging 7.4 yr in the Hibbs experiment and 6.89 yr in the field trial. The mean age of nonprevious history cows was less; 5.4 yr and 3.95 yr
respectively for the two studies. Previous history animals averaged 2.43 yr older than the nonprevious history group for both studies though all had had 3 or more calvings.

The period from 5 to 10 yr of age is recognized as the time of greatest susceptibility to parturient paresis. It is also the period in which an animal should realize her production potential. This fact has resulted in the generalization that parturient paresis is to be strongly associated with total production, heavier milkers being more likely to contract milk fever than animals producing at lower levels. In this study, the production levels of previous and non-previous history animals compared favorably, with no significant differences observed (Table 7). These data support the earlier observations of Hibbs (64) who found no differences between normal and paretic, parturient animals in respect to colostrum and total milk yield. The fact that negative Ca balances have been reported in preparturient and mastectomized animals (105, 134, 157) tends to support the idea that the effect of age upon incidence and therefore efficacy of vitamin D₃ is not mediated through increased milk synthesis associated with age.

Ca and P homeostasis is a function of endogenous and exogenous ionic inflow and excretory outflow. The relative importance of endogenous and exogenous Ca and P to regulation has been shown to vary with physiologic condition (130), which is a function of age. Endogenous Ca and P is primarily supplied by mobilization of labile and cancellous bone (131, 4). Availability of bone mineral, however, tends to decrease with increasing age (52). At 3 yr of age the
TABLE 7. Average milk production by breeds in previous and no previous history animals.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Previous history</th>
<th>No previous history</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guernsey</td>
<td>5028.4</td>
<td>4886.3</td>
</tr>
<tr>
<td>Holstein</td>
<td>8090.9</td>
<td>8522.7</td>
</tr>
<tr>
<td>Jersey</td>
<td>4908.4</td>
<td>4461.3</td>
</tr>
<tr>
<td>Ayrshire</td>
<td>4595.0</td>
<td>5300.0</td>
</tr>
<tr>
<td>Average for all breeds</td>
<td>5655.7</td>
<td>5792.6</td>
</tr>
</tbody>
</table>
percent of exchangeable bone Ca is believed to have a minimum value of 6.0% and a maximum of 20%. By the time the cow reaches 13 yr of age this has decreased to a minimum of 2% and a maximum of 5% (52). Other workers have expressed these readily available endogenous Ca bone pools to be even less than this figure (77). It is estimated that the mature dairy cow has only 6.39 g of Ca available to her from endogenous stores (122). This has been shown to be mobilized at the rate of only .5 g per hr (122). Thus the relative importance of endogenous mobilization of Ca in the older animal tends to be negligible in maintaining homeostasis.

The Ca requirement of the parturient cow may reach 19 g or better within a 24 hr period of time (122). The older animal, being unable to mobilize sufficient endogenous Ca, is forced to rely more heavily upon intestinal absorption. This is supported by the fact that depressions in dry matter intake in mature cows have been shown to cause a corresponding depression in serum Ca and P (101, 51, 135). The older animal, however, is again challenged in that intestinal absorption efficiency decreases with age. A typical excretion rate of dietary Ca has been shown to increase by threefold when animals 6 mo of age are compared to animals 35-70 mo (52). Compounding this problem is the fact that parturition has been shown to be associated with inappetance in cows, the degree of which is also a function of age (75). Inappetance is believed to be a consequence of elevated blood estrogen (101, 40, 103), the net affect of which is reduced net absorption of Ca (4), and a corresponding drop in plasma Ca (103).
In summary, a characterization of the previous history cow would show that she is an aged animal, who due to the physiological changes associated with cessation of skeletal growth, has reduced available endogenous Ca reserves and a reduced ability to absorb and retain exogenous Ca. The fact that not all mature cows have milk fever indicates that other physiological differences exist which separate these animals from the normal population. The individual animal response at parturition could be important. Several hormones associated with parturition; namely estrogen, the glucocorticoids, ACTH and prostaglandin have been shown to inhibit the normal mechanisms of Ca homeostasis (102, 153). Animals that tend to maintain higher or prolonged circulatory levels of certain hormones may be made more susceptible to attack due to interference with the blood Ca regulatory system already made inefficient after skeletal maturity.

In contrast there is the non-previous history animal. This animal is younger, and thus perhaps somewhat more efficient at resorbing bone Ca and absorbing dietary Ca for blood Ca maintenance. The individual parturient hormonal responses which might be as variable as in the older cow, would tend to be negated somewhat by this fact.

The proposed physiological differences between previous and non-previous history animals and the effect they have upon efficacy is born out somewhat by the physiological function of vitamin D and the overall efficacy data. Vitamin D₃ has been shown to be essential for the absorption of dietary Ca (116) and synergizes with parathormone
to induce resorption of previously formed bone (54). The efficiency of these two processes in the older previous history cows would be expected to be low. Individual endocrine response would offer further interference. The non-previous history cow subject to similar endocrine response, is more efficient at maintaining homeostasis, perhaps due to the fact that she is younger. Supplementation with massive doses of vitamin D₃ would be expected to elicit the greatest response in the system which is the most inefficient, i.e. the previous history group. This is indicated by the reduction in incidence in this population from 41% to 27% (Table 1). The non-previous history cows would not be expected to benefit as much or at all from vitamin D supplementation as they are already at a higher physiological homeostatic efficiency. This is born out by the non-significant differences in incidence between treated and control no previous history animals. This is also supported by the fact that the incidence in the treated previous history group was reduced only to that of the population mean (23%) of the non-previous history cows. By equalizing the physiological differences between the treated previous history cows and the non-previous history population, vitamin D₃ supplementation reduced the incidence of the former to the normal incidence level for the sampled population or 23%, which is the mean incidence of the non-previous history cows. This level of efficacy, is an expression of the interaction of vitamin D₃ with the inherent physiological homeostatic mechanisms of the animal. It would be impossible to reduce the incidence in previous history cows further based upon changes in the vitamin D₃-physiological interaction alone.
This is indicated by the fact that no reduction in incidence was noted between the non-previous history treated animals and non-previous history controls, the control animal incidence representing the normal population incidence. Further reduction perhaps would be possible by changes in other parameters which affect this interaction, however. The significant variation in efficacy of vitamin D₃ treatment as effected by total daily consumption of Ca and P in both previous and non-previous history cows is indicative of the fact that the prepartal nutrition is such a parameter.

Efficacy values indicated by analysis of the data on the basis of dietary Ca and P input show that the overall incidence of the three populations as related to diet, varied considerably with changes in these parameters (Table 2). Groups 1 and 2, representing the extremes in mineral intake noted in the study, showed no significant differences between them with respect to reaction to treatment. They were both, however, significantly less effected by treatment than group 3. Also evident is the fact that vitamin D₃ was unable to reduce the incidence of group 1 to levels comparable to the population norm of 23%, had no apparent significant effect on incidence in group 2, but provided 98% efficacy in group 3. This is strong evidence supporting the contention that diet is important to the effective use of vitamin D as a prophylactic agent.

That diet does in fact effect the efficacy of vitamin D₃ has a biological basis for support. Recent advances in vitamin D metabolism show that this vitamin undergoes two metabolic changes before it produces a measurable physiological response. The first of these, an
hydroxylation at the 25 position by an hepatic enzyme (57, 9) results in the metabolite, 25-OH-D$_3$ which is 40% more biologically active than vitamin D$_3$ (10). The second metabolic change involves a renal enzyme (109) which causes an additional hydroxylation at the 1$\alpha$ position. This metabolite, 1,25-OH-D$_3$ (109, 72) has been shown to be 80 to 100 times more rapid in biological activity than the 25 form (110, 118, 129). Evidence suggests that this is the active form of the vitamin and the one needed for maximal biological effect (150, 42).

The hepatic and renal enzymatic systems that catalyze the necessary hydroxylation are subject to regulation of which the primary mediators are the plasma ionic levels of Ca and P. These pathways are outlined in Figure 1. In brief, normal or high levels of plasma ionic Ca and P inhibit the hydroxylation reaction leading to 1,25-OH-D$_3$, and favor an alternate pathway which produces the metabolite 24,25-OH-D$_3$ which possesses limited biological activity (118).

Plasma ionic Ca and P concentrations are primarily a function of the relative dietary concentrations and availability of these ions. High Ca diets have been shown to decrease the percent absorption but increase the total amount of Ca absorbed (4). A strong correlation between available digestible Ca and Ca balance has been shown to exist (121). In essence, high dietary levels of Ca tend to promote positive Ca balance, the physiological response to which, is the reduced release of parathormone and increased release of TCT. Conversely, low Ca diets would have the opposite effect, and stimulate parathormone but inhibit TCT release.
Dietary P similarly effects its own physiological balance. P is believed to be absorbed passively (96). Dietary availability is generally reflected in plasma inorganic P values as shown through multiple regression analysis of the inorganic P contents of the diets sampled in this experiment and the plasma P values obtained from the sampled population (Table 6). Thus high dietary P concentrations cause elevated plasma inorganic P values and low availability depresses plasma values.

The interaction of this diet mediated physiological response in Ca and P, with vitamin D3 metabolism, vitamin D3 efficacy, and group incidence can be categorized as either negative as in groups 1 and 2 or positive as in group 3. This is reflected in efficacy data for the treated population (Table 2) and overall incidence (Table 4) of each group.

In the negative interactions of groups 1 and 2, there exists a difference, indicated by the failure of treated animals of group 1 to reach the lower incidence level of the normal population. This is not the case in group 2, where incidence of treated animals is insignificantly different from this value. In essence, the interaction in group 1 is of greater intensity than in group 2. Group 1 animals, through consumption of high levels of both Ca and P have inhibited exogenous inflow of Ca and endogenous mobilization of Ca by inactivating the homeostatic systems responsible for both actions. Exogenous inflow is blocked in that elevated levels of plasma ionic Ca and P, reflections of the dietary intake, inhibit renal hydroxylation of 25-OH-D3 and favor the alternate synthesis of the inactive 24,25-OH-D3.
Endogenous mobilization is blocked because elevated ionic Ca levels 1) inhibit PTH release and 2) inhibit 1,25-OH-D₃ which synergizes with PTH to release bone stores of Ca to the blood. As these animals possess limited prophylactic protection from vitamin D₃ treatment and are also without natural homeostatic control, the severity of incidence and therefore the intensity of interaction between diet and incidence is greater. This is supported by the significantly higher treatment incidence of group 1 as compared to group 2 (Table 4) as well as the significantly higher overall incidence of group 1 over group 2 and group 3 (Table 5). However, this leads to a false conclusion with regard to the interaction of diet and efficacy. This is made clear after examining the incidence and efficacy of group 2. These animals were not significantly different in their reaction to treatment than group 1. Treatment incidence values of group 2, however, were not significantly different from those of the population norm. Group 2 animals by consuming low Ca, high P diets inhibit exogenous inflow but apparently only partially inhibit endogenous mobilization. Exogenous inflow is inhibited by the elevated renal cortex concentrations of inorganic P (151), a function of plasma concentration, which block renal hydroxylation of 25-OH-D₃ to 1,25-OH-D₃, as in group 1. Endogenous mobilization of bone Ca, however, would only be partially blocked in that low plasma levels of Ca stimulate PTH release which mobilizes these stores. This happens most efficiently only in the presence of 1,25-OH-D₃, which in these animals is absent or limited. Group 2 animals then have limited prophylactic action of vitamin D₃ as indicated by the insignificant difference between treatment
efficacy values of groups 1 and 2 but are able to call upon natural homeostatic mechanisms, something group 1 animals are unable to do. Thus, treatment incidence would not be significantly different from the population norm as in this case. The negative interaction of diet with incidence of group 2 is less severe – but the negative interaction of the diet with efficacy is the same as that of group 1. The differences in treated incidence of groups 1 and 2 are not a direct consequence of differences in efficacy which is a function of diet, but rather of differences in physiological reaction, not mediated through D₃, to diet.

In contrast to the inhibiting or negative interaction of groups 1 and 2 is the positive or stimulating interaction of group 3. Both efficacy (98%) values and treatment incidence (2%) were significantly greater than in groups 1 and 2 (Table 2). Of special interest is the fact that overall incidence (Table 5) was also considerably lower than that of groups 1 and 2 and the population mean (23%). When examined in respect to previous and non-previous history (Table 4), group 3 incidence was still lower than in the other groups or populations as a whole.

These data strongly indicate that the feeding of .5% of the total DM as Ca and .25% of the total DM as P insures efficacy at 95% or better. This is also the strongest example of dietary interaction with vitamin D. However, the overall significantly lower incidence of group 3 population would suggest that it is the diet and not the treatment which caused this. The 10% incidence of the previous history group (control animals) supports this contention. These animals as
previous history cows are physiologically the most susceptible to attack, which is verified by the overall incidence of the previous history control population (Table 1). By feeding the NRC recommended amounts of Ca and P, their incidence value is below that of treated previous and non-previous history animals of groups 1 and 2. Furthermore, their incidence is not significantly different from that of the non-previous group 3 history controls, the animals which would establish the norm from the population of group 3 animals. In effect, they have been brought to a level of efficiency equal to that of the younger non-previous history cows. This can be explained on the basis of Ca and P balance. Animals must be in positive Ca and P balance for proper absorption and utilization of both. Hibbs et al. (66) and Young (166) have shown that exchangeable bone pools of Ca as well as rate of dietary absorption decreases when animals are in negative P balance. However, high ionic plasma levels of P also decrease absorption and resorption by inhibiting vitamin D₃ metabolism (151). Ca absorption in the older animal increases with increasing dietary concentration (4). This relationship is linear only until the upper limit of normal plasma concentration is reached. Then homeostatic mechanisms favoring absorption and resorption are inhibited and net loss of ionic Ca results. Thus .50% Ca and .25% P apparently represents the dietary levels needed to allow natural homeostatic systems to continue to function properly and thus gives the animal protection against negative balances of Ca and P.

These data indicate that proper dietary Ca and P intake can be as effective as vitamin D₃ in the prevention of parturient paresis.
Diet apparently has a profound effect upon the efficacy of vitamin D. Thus, there exists the possibility that diet and vitamin D can be used synergistically for effective control. This is supported by the work of Hibbs et al. (66) who found significantly different responses in the Ca metabolism of vitamin D fed animals depending upon dietary intake of P. This interaction appears to be additive.

Support for this reasoning comes from the incidence and efficacy data of groups 1, 2 and 3 (Table 4). The incidence of the control animals of each group represents the population norm of each group. In group 1 this is 35.1%; group 2, 15.6%; group 3, 9.5%. This is the incidence set by the dietary (Ca and P) inputs. When compared to the overall incidence of the entire sampled population (23%), it can be concluded that diet has either increased as in group 1 or decreased incidence (groups 2 and 3). The incidence level of the control previous history and control nonprevious history animals (Table 4) represents the incidence level set by dietary and physiological interaction. The efficacy of vitamin D₃ treatment is indicated in the incidence of treated previous and non-previous history cows.

The strongest evidence in support of a synergistic action is in group 3. Here, the overall population incidence of 23% has been reduced to 9.5%, the incidence level set by the diet alone. This value is not significantly different from the 10% established by physiological and dietary interaction in previous history animals. Vitamin D treatment reduced the value to 0% incidence in the treated previous history group. The dietary change plus vitamin D treatment reduced potential population incidence from 23% to 0%.
This theory can also be indirectly supported by group 1 data (Table 4). Here, dietary incidence (35.1%) exceeded significantly the population norm value of 23%. Thus diet is not, as in group 3 prophylactic, but rather it is causative, interacting with physiological factors as reflected in the high incidence (60%) of the control previous history cows. Vitamin D treatment reduced this to 37.8%, a value not significantly greater than that set by diet alone. The inability of vitamin D to bring further reduction can be explained in that vitamin D is the sole prophylactic agent working without the additive effect of diet as seen in group 3.

In group 2 incidence set by diet was 15.6% which was significantly less than the 23% of the population norm. However, vitamin D$_3$ treatment only reduced incidence in previous history animals to 31.8% which was significantly greater than both the population and the group means. This apparent inability of vitamin D to compensate for physiological-dietary interaction was not seen in group 1 and is not expected based upon the lack of significant differences between incidence and efficacy values between groups 1 and 2. A possible explanation as to the discrepancy, can be found in the relatively low value for the group mean which approaches the mean of group 3. This was determined by averaging the total observations of the control animals for each group and arriving at percent incidence. The relatively small sampling population of the control previous history animals, allows for the control non-previous history animals to make up 87.5% of the sampled population. As the incidence in these animals is low, but was found to be significantly different from the
treated non-previous history incidence, a sampling error in this population is possible, thus giving a false value to the group mean.

The general lack of significant differences between treated and control non-previous history cows of all groups agrees with the earlier conclusion that these animals are already near the peak of homeostatic efficiency. Incidence values are dictated by dietary input alone. This is evident by the fact that both treated and control non-previous history incidence values are not significantly different from group mean incidences, nor are they, in the case of group 1 and 2 significantly different from the population norm. Group 3 non-previous history incidence is significantly lower than the population mean and incidence value of 1 and 2, which is further evidence of the importance of dietary input in prophylactic action. These data generally support the additive theory in that no effect is seen because no added response is necessary nor could be expressed because of the physiological makeup of the non-previous history population.

2.13 Conclusions

Intramuscular injection of 10 million units of vitamin D₃ 24 to 192 hrs prepartum is an effective prophylactic agent for the prevention of parturient paresis. Response, however, is greatly dependent upon the physiological condition of the animal injected, and the prepartal intake of Ca and P.

Efficacy tends to increase with age, this relationship being an expression of an increasing inefficiency in the normal homeostatic mechanisms to provide protection. Incidence also tends to increase
with age which supports this contention. Thus, vitamin D₃ is most efficiently used as a prophylactic agent in the older cow with previous milk fever history.

Properly balanced Ca and P nutrition of the preparturient and parturient cow is of vital importance in the etiology of the disease and in the natural prophylactic protection against its expression. Ca and P intakes that vary significantly from the NRC recommended total ration dry matter levels of .5% Ca and .25% P cause a significant increase in incidence and reduce the protection afforded by the prophylactic use of vitamin D₃. Conversely, by feeding .5% Ca and .25% P prepartum, paretic incidence can be reduced drastically. A properly balanced Ca and P intake when fed in conjunction with vitamin D injection resulted in even further reduction of the incidence of parturient paresis.
LIST OF REFERENCES


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Within the last 35 years reports of a myoparalytic disease of the parturient dairy cow, sometimes referred to as atypical parturient paresis (milk fever), have appeared from time to time in the literature. Incidence of this disorder has risen at an alarming rate in recent years (8).

These cases have been commonly diagnosed as cases of parturient paresis and treated as such. Prognosis, however, is poor in that these animals fail to respond to infusions of calcium salts used for treatment of hypocalcemia. Furthermore, stricken animals appear to be exceptionally susceptible to invasion by pathogenic bacteria and consequently, mortality rates are exceedingly high.

This disease, designated as the "Fat", "Downer" or "Creeper" Cow syndrome remains generally undefined as to cause and prevention. The experiments were designed to study the etiology of this disease, its prevention and possible methods of therapy.

3.1 Etiology of the Downer Cow Syndrome

Based upon current knowledge, this disease has been classified as an aberrant form of parturient paresis. Examination of the animal, however, reveals certain recognizable visual differences that would
separate the two syndromes. Parturient paresis normally includes recumbency accompanied by a comatose condition. Downer cows although recumbent are not comatose. Hallgren (27) described these animals as alert, and clinically normal in every respect except for the inability to rise. Johnson (39) likened these animals to those under high epidural anaesthesia; alert and active in the forequarter, but unable to coordinate muscular activity in the rear. Bjorsell et al. (8) defines downers as cows with normal psyches, maintaining normal appetites but simply unable to rise.

The clinical variations between the downer cow and the true milk fever cow can be explained as the basis of difference in the hemochemical, pathological and histochemical histories of the two groups. Hemochemically, downer cows show a considerable variation in parturient ionic blood response when compared to confirmed cases of parturient paresis. In a summary of the blood Ca, P and Mg concentrations of 77 relapsed cases of parturient paresis, 65 of which were diagnosed as downer cows, mean serum blood concentrations were: Ca, 7.99 mg%, P, 3.83 mg%, and Mg, 2.43 mg% (27) in the downer cow as compared to 5.10 mg%, 2.55 mg% and 3.51 mg% for Ca, P and Mg respectively in the confirmed cases of parturient paresis. The blood values of the downer animals show no significant variation from that reported as normal for parturient animals (47), while blood values of the parturient paresis cow reflected what is normal for parturient paresis (47). The insignificant change in blood Ca, P and Mg in downer cows as compared to normal postparturient cows has also been
reported by Jacobson et al. (38), Jonsson et al. (41), and others (62, 8).

Patho-anatomical examinations of downer cows show several other important differences from animals affected by parturient paresis. Pathologists generally agree that normal cases of parturient paresis produce no discernible lesions that are useful in diagnosis. Necropsies performed on downer cows reveal that a distinct pathology is common. The organs most frequently involved are the heart, liver, abomasum and skeletal muscle.

1) Heart. Hallgren (27) reported that 20% of the downer cows examined had myocardosis. Acute focal myocardosis of varying severity was histologically demonstrable in 49% of the downer cows in another study (41). It was concluded that these degenerative changes occurred after calving. Fifty-nine percent of the myocardiac cases had heart rates exceeding 100 beats per minute and arrhythmia was observed in 41% of the affected animals.

2) Liver. Puerpheral fatty degeneration of the liver was observed in 14% of the affected animals in Hallgren's (27) study. Jonsson et al. (41) reported that acute hepatosis characterized by swollen liver cells, severe fatty infiltration, cytoplasmic eosinophilia, and degenerative nuclear changes were observed in 50% of the downer cows necropsied in a Swedish study. Garm (24) observed similar degenerative changes as did Noorsdsy et al. (62).

3) Abomasum. Acute abomasal changes were observed in 74% of the downer cows in one study (41). Petechial hemorrhage were seen in 6% and erosions and/or ulcers in 68%. Most of the erosions or ulcers
were in the pyloric region, varying in size and configuration from one to 20. Observations of peptic ulcers were reported by other workers as well (27).

4) **Skeletal Muscle.** Damage of skeletal muscle generally restricted to hindleg adductors was seen in 62% of the necropsied animals in the study of Jonsson et al. (41). The main pathological lesions consisted of hemorrhages and general cellular degeneration, varying considerably in severity from case to case. Bjorsell et al. (8) concluded that the major pathological change in the downer cow was traumatic damage to the adductor muscle mass.

Histochemically, downer cows exhibit changes in serum enzymes which correlate with the aforementioned patho-anatomical findings. Elevated levels of serum glutamine-oxaloacetic transaminase (41) indicative of the hepatic and muscular necrosis, glutamate dehydrogenase, a hepatic diagnostic enzyme and alanine transaminase, an enzyme somewhat specific for muscular necrosis have been reported (9).

Perhaps one of the most useful clinical evaluations to confirm the diagnosis of a recumbent animal is her response to treatment. Downer cows do not appear to be severely hypocalemic, and therapy initiated for cases of parturient paresis elicit little therapeutic response. Indeed, in a recent Swedish study (8) downers were defined as cows which had not responded 24 hr after the first treatment for parturient paresis. This lack of response to calcium therapy initially brought the disease to the attention of veterinarians and was a differentiating factor used by many early workers (24, 27).
In summary, the downer syndrome is a disease of the parturient cow. Clinically, it expresses itself as alert recumbency caused by an apparent myoparalysis of the hindleg adductor muscles. It often involves degenerative changes in hepatic, cardiac, abomasal and skeletal tissue. Therapeutically it is unresponsive to the regime of treatment indicated for normal parturient paresis and consequently, mortality in affected animals is high.

3.2 Causative Theories

The downer cow syndrome has been the object of limited scientific interest primarily due to failure to recognize that such a problem exists. The increasing number of case histories of these recumbent animals with poor prognoses has generated several theories as to reasons for their response or lack thereof to the usual methods of therapy.

These can be summarized as follows:

1) Atypical Parturient Paresis. This theory is the oldest and currently the one with widest acceptance. Hallgren (27) concluded that the increasing number of cases of downer cows was indicative of a structural change in the clinical aspect of parturient paresis and consequently the disease had changed its character. The contention is supported by several authors (41, 8, 66, 62, 23).

Theories as to the reasons for prolonged recumbency after calcium treatment include: A) muscle injury, localized primarily in the thigh musculature, induced by the struggling of the cow to rise (41, 8); B) limb disfunction caused by prolonged unnatural recumbency due to insufficient calcium (39); and C) alterations in the plasma calcium
to phosphorus ratio (62). The assumption made by proponents of this theory is that in all cases of recumbency, animals become classically hypocalcemic (41). In three studies conducted to establish the etiology of this apparent aberration of normal parturient paresis, blood calcium concentrations averaged 8.3 mg%. This value is well within the range of the normal postparturient cow and is significantly different from the average figure of $4.5 \pm 1.4$ mg% value most commonly associated with parturient paresis (47). Thus the basic premise upon which this theory is based is open to question.

2) Hypokalemia. Garm (24) believed that prolonged recumbency was caused by a potassium deficiency of the skeletal muscles. He theorized that increases in the circulating levels of adrenocortical hormones caused imbalances in electrolytic regulation. Physiological circumstances could cause a varying response in the adrenal cortex which in turn led to a different degree of disturbance in the intermediary metabolism of the animal. In the parturient cow, potassium loss due to parturition and milk synthesis could be significantly great enough to place the animal in negative potassium balance, which could not necessarily be monitored in serum concentrations.

Muscle potassium depletion has been reported in several cases of prolonged recumbency in the bovine (41, 45). Physiological depression in extracellular and intracellular potassium causes a decreased excitability of nerve and muscle cells with weakness and flacid paralysis often seen as clinical consequences (10).

Several authors (25, 39) have shown a favorable response to potassium infusion in some cases of downer cows.
3) **Hypophosphotemia.** Hallgren (27) reported that hypophosphotemia was common in downer cows, and an infusion of calcium hypophosphate could reverse the condition. A persistent hypophosphotemia in downer cows was also reported by Jonsson et al. (41) although the etiology of the condition was not known. Hypophosphotemia, however, appears not to be common in all cases. Kronfeld (45) reported that a hyper rather than reduced plasma phosphorus concentration was important to downer etiology. Furthermore, values given as hypophosphotemic by several authors do not vary significantly from those observed in normal postparturient animals (47), and thus whether hypophosphotemia exists in the cases cited in these studies appears to be a matter of self interpretation.

4) **Mechanical Factors.** A) Obesity: One factor which is commonly reported in most studies of the downer cow syndrome is that animals which are usually affected tend to be overconditioned, frequently to the point of obesity. Obesity has been associated with contributing to the complications in recumbent animals. Such animals, due to physical size would appear to be more susceptible to traumatic muscle and nerve injury brought on by attempting to rise or pressure injury caused by the recumbency itself (41). B) Nerve injury: Parturient nerve damage in obese animals inter alia is a consequence of struggling. C) Muscle injury: As in nerve damage this is a condition believed brought on by attempts to rise. However, several authors (8, 41) concluded that traumatic muscle damage is primarily responsible for prolonged recumbency.
5) **Nutritional Factors.** Garm (24) suggested that endocrine disturbances involving the hypophysis and adrenal cortex in conjunction with unsuitable feed were involved in the etiology of this disease. Garm suggested that high protein intakes and abundant sodium supplies in the diet in some way induced this syndrome. Osinga (66) reached similar conclusions. Noordsy et al. (62) proposed that long predisposing factors involving limited calcium intake was the common denominator in this syndrome. Jonsson et al. (41), however, concluded that there was no reason to suppose that variations in respect to quality or quantity of roughage, concentrate of water intake during the prepartum period was of significance in the downer syndrome. Nutrition, however, could be involved inasmuch as overfeeding tended to make for overconditioned animals.

These theories summarize the present knowledge of the downer cow syndrome. None of these, however, have provided an adequate scientific basis for a prophylactic or a therapeutic means to effectively deal with the condition. The corresponding rise in incidence of myocardiosis, hepatosis, and gastric disorders all significantly correlated to prolonged recumbency, also cannot adequately be explained by current theories. Hallgren (27) perhaps sums this problem up best in the statement "the fact is that we are now faced with a number of metabolic disorders that occur in high-producing cows after calving and that clinical manifestations of this disorder can be puzzlingly like parturient paresis and occasionally in ambulatory practice, impossible to distinguish from this disease". The present knowledge
indicates that this puzzling disease involves something other than hypocalcemia.

3.3 Development of a New Hypothesis

Within recent years, the number of complaints of metabolic and/or infectious conditions associated with high milk production have become increasingly common (62). The most frequently reported of these include myocardosis, hepatosis, reproductive problems, unresponsive mastitis problems, retained placenta, intestinal disorders, and high death losses following parturition (62, 27). Frequently, several of these disorders are found in the downer cow (41, 27). That these disorders can be directly linked to hypocalcemia, as is proposed by several authors (41, 38) is open to question.

Hallgren (27) observed that the unexplainable increase in this metabolic disturbance began subsequent to the end of the Second World War, and from all indications has steadily increased from this period, to the present. The increasingly heavier production potential of the modern dairy animal is commonly cited as the basis for this occurrence. Noorsdsy et al. (62) observed that normally, complaints of metabolic disease came from longtime dairymen whose cows exceeded average production. Statistical analysis, however, has shown no correlation between the total pounds of milk produced and the evidence of metabolic disturbance (61).

A key to the reconciliation of the differences in the above statements can perhaps be found in the generalization that better managed cows tend to produce more milk than poorly managed ones, and that dairy management is a highly variable situation related to
economics, geography, etc. The importance of management practices to metabolic disease incidence is shown in the study of Noorsdsy et al. (62) who observed that certain trends in management and feeding did exist in high incidence herds. The conclusion that management, particularly nutritional management, is a factor in metabolic disease incidence has also been reached by several other authors (61, 6, 15, 71).

The agricultural enterprise, as any business, must respond to the prevailing economic situations in order to remain viable. Dairy management, being one of the most intensified forms of animal agriculture, is a prime example of this reaction. Labor shortages and rising costs of raw materials have forced dairy management practices to change significantly within the last two decades. Management systems have been adopted that minimize the number of man hours spent per head per day. Although designed to maximize profits, these systems have brought with them their own special problems. These changes and their respective problems can be categorized as follows.

1) Drylot Confinement. The use of a free stall barn design reduces time spent in animal care. It also limits the direct personal supervision of the cow as indicated by increased calving intervals and a subsequent new interest in natural service. Nutritionally, distinctions are frequently no longer made between an animal's productivity and nutrient consumption; thus dry cows often consume rations balanced for the animal producing 50 or more pounds of milk, simply because they are maintained and fed with the milking herd.
The mechanical feeding systems needed to efficiently use confinement housing also make it difficult to restrict animal intake based upon need.

2) **Feeding High Yielding Corn Silage and/or Haylage as Roughage.**
The general trend towards increasing use of ensilage, both corn and hay crop, is associated somewhat with the demands of confinement housing feeding systems, these materials being readily adapted to mechanical handling and the fact that they provide a more economically efficient use of crop land and labor than conventional roughages. Several authors, however, have shown significantly higher incidences of metabolic diseases in herds where ensilage, particularly corn silage, is fed (61, 71, 6).

3) **Use of Complete Feeds.** Mechanical feeding and confinement housing have justified the use of mixing ensilage and concentrate and simultaneously feeding them together. This increases the likelihood of meeting nutritional requirements of the high-producing cow which can be difficult when grain is fed independently from roughage. Unfortunately, this feeding program compounds the problems outlined above in that it provides a further source of luxury consumption of nutrients to less productive and nonproductive animals.

An illustration as to the involvement of these parameters in metabolic disturbance is provided by a study of 3 yr duration conducted at the North Central Branch of the Ohio Agricultural Research and Development Center. Starting in October of 1970, the dairy herd was divided into two groups, one being maintained on an all corn silage-concentrate ration, the other on an alfalfa-hay concentrate ration.
All animals were maintained in loose housing with milking and dry cows of each group running together. Roughage was fed ad libitum and top-dressed with 14 to 16 lb. of either 16% (hay group) or 24% (corn silage group) crude protein concentrate mix, so as to make the diets isocaloric and isonitrogenous. Milking cows received in addition 16% crude protein concentrate based upon milk production. During the first year of the experiment, 66% of the animals in the corn silage group were affected with a clinically expressed metabolic disturbance. The hay fed animals had an incidence of only 5.2% in comparison. As a result, in June 1971, the dry cows of both groups were separated from the milking cows and intake of corn silage and grain in the corn silage fed dry cows restricted to 2 lb. of 24% crude protein concentrate and corn silage ad libitum. Consequently the incidence of metabolic disturbance was reduced to 0%. The dry cow ration was again modified in October 1971 when dry cows of both groups were restricted to 8 lb. of 16% crude protein concentrate, 20 lb. of corn silage and hay ad libitum with no change in incidence of metabolic disturbance. These data are outlined in Table 1. This study typifies what is most commonly seen, in herds where metabolic disturbance poses a serious problem.

Parturient metabolic disease in the bovine appears to be significant only in the dairy cow. There are no reports, at present, of these diseases in the beef brood cow. The reasons for this would not appear to be physiological in that no differences metabolically have thus far been established between the pregnant dry beef cow and the pregnant dry dairy animal. Nutritionally, however, there is
TABLE 1. A summary of metabolic disorders observed at the North Central Branch of the Ohio Agricultural Research and Development Center during the corn silage feeding experiment from 1970 to 1973a.

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows in groups</td>
<td>18</td>
<td>18</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>LDA cases</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Died from LDAb</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Died, other causes</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Milk fever cases</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Metritis cases</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Ketosis cases</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

aCourtesy of H. W. Newland (unpublished data).

bLeft displaced abomasum.
considerable variation in feedstuffs used, and nutritional management in general, between the two groups. It is estimated that 92% of the dry matter intake of the beef brood cow comes from homegrown forages in the form of pasture and/or hay (27). Research has shown that in most major beef producing areas of the country, forages can be used to provide 100% of the nutrient requirements of the brood cow (80). Forages utilized are generally mixed native grasses, species used being dependent upon geographic location. In Ohio, tall fescue, bluegrass and orchardgrass are the most common. Such a ration fed to a 600 kg cow provides approximately 8.4% crude protein and 22 MCal of metabolizable energy and adequately meets the NRC requirements of 5.81% crude protein and 15.5 MCal of metabolizable energy established for this animal (63).

The dry dairy cow in comparison generally receives only 50% to 70% of her dry matter intake as roughage and frequently two-thirds or more of this input is in the form of either grass or corn silage. The roughages have been implicated by several authors as involved in the etiology of metabolic disturbances (61, 6, 7). The remainder of the diet is often fed as a concentrate at the rate of 1 lb per 100 lb of body weight. Animals so fed often consume 65% of their dry matter intake as concentrate. Dry dairy cow rations also tend to contain significantly more protein and energy than consumed by the beef brood cow. They also eat more than is actually required for maintenance and pregnancy. This is particularly true in herds where milking and dry cows are housed and fed as a single unit. A
comparison of a typical beef brood cow ration, the NRC requirements of a mature dry dairy cow for maintenance and pregnancy (64) and the ration fed to the dry cows in the above experiment is outlined in Table 2. The incidence of metabolic disturbance in animals consuming this ration was particularly high. It should be noted that the protein intake of these animals was 2 times as great as that of the brood cows and that established as a dietary requirement. Energy intake was also higher but the megacalories consumed by the dairy cow varies with individual animal intake, while protein consumption per unit of dry matter consumed remains relatively constant. Thus, protein consumption is significantly different between these two classes of animals.

The dietary difference observed between beef and dairy cows also exists within the animal classes themselves. Protein and energy variability can be seen from farm to farm and from season to season even within the same management system. Variations are caused by differences in feeding systems, by availability of feedstuffs, by changes in quality of feedstuffs and by the herdsman's individual beliefs and prejudices. The brood cow is generally faced with a single variable, changes in forage quality. Dairy animals due to the intensity of management, are frequently the object of all these inputs. An example of the extremes that are seen is presented in Table 3. The herds represented are both registered Holsteins and average body weight per animal is about 600 kg. Herd B, referred to earlier in the discussion had a 66% incidence of metabolic disturbance. Herd A had a 0% incidence. Actual nutrient intake is relatively similar
TABLE 2. A comparison of the nutrient intake of A) a typical beef brood cow ration; B) the NRC recommended nutrient intake for the dry preparturient dairy cow; and C) the nutrient intake of a dairy herd with a high incidence of metabolic disease.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>9.7</td>
<td>8.84</td>
<td>16.0</td>
</tr>
<tr>
<td>Total digestible nutrients</td>
<td>57.0</td>
<td>55.7</td>
<td>60.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>.45</td>
<td>.50</td>
<td>.50</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>.35</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Magnesium</td>
<td>.19</td>
<td>.19</td>
<td>.19</td>
</tr>
</tbody>
</table>

TABLE 3. Comparisons in nutrient intake of Holstein herds with A) no incidence of metabolic diseases; and B) a high incidence of metabolic diseases.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>9.7</td>
<td>16.0</td>
</tr>
<tr>
<td>Total digestible nutrients</td>
<td>46.3</td>
<td>60.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>.54</td>
<td>.54</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>.28</td>
<td>.25</td>
</tr>
<tr>
<td>Magnesium</td>
<td>.11</td>
<td>.19</td>
</tr>
</tbody>
</table>
between the two herds except for the amount of protein fed. Herd B consumed nearly two times the amount of protein that was fed to cows of herd A. Another significant difference was the nutrient source itself. Herd A cows consumed 50% of their dry matter as concentrate with the remainder being supplied as corn silage. Herd B animals consumed an all roughage diet 75% of which was orchardgrass hay and the remaining 25% was fed as corn silage. These data are typical of the variability of dry dairy cow nutritional management.

The dry cow ration of herd B and the beef brood cow ration are strikingly similar in nutrient source and content. Their incidence of metabolic disturbance is also similar. However, the significantly different nutrient sources and intake of herd A, and the severe incidence of metabolic disturbances that accompany them suggest that there are factors here not seen in the rations of herd B or the beef cow. The silage-concentrate ration is one variable. As has been mentioned silage-concentrate rations have been implicated by several authors (79, 6, 61) as important to the etiology of metabolic diseases. Holter et al. (34), however, reported no detrimental effects associated with maintaining dry dairy cows on such rations when intake was regulated to prevent overconditioning. A comparison of Holter et al.'s (34) study with those of Belyea et al. (6), Trimberger et al. (79) and Newland et al. (61) which implicate these rations as detrimental, show that although nutrient sources were similar in all studies, the relative amounts consumed and the composition in regard to percent crude protein were different. Holter's cows were placed on restricted intakes and consumed a ration that had a crude protein concentrate of
11.89%. The other studies allowed animals essentially ad libitum intakes at protein levels ranging from 13.70% to 17.0%. Thus the incidence of metabolic disturbance rather than being a simple consequence of an ensilage-concentrate dry cow ration, would appear to be more significantly affected by the relative amounts of ensilage and concentrate consumed, compounded by the percentage of crude protein that the consumed materials contain.

The significant variations observed in protein intake and nutrient sources between beef brood cow, the dairy herd in which there is limited problems of metabolic origin and the herd in which metabolic disease is a chronic condition is the basis for the working hypothesis of this experiment. It is our thesis that dietary protein when consumed in excess of actual physiological requirement in some way increases the susceptibility of the dry parturient dairy cow to the downer syndrome and other metabolic disturbances. The increased susceptibility is compounded by the use of the ensilage-concentrate rations when fed in excess of dietary needs; however, it is the actual consumption of protein in the form of ensilage and concentrate, rather than the ensilage and concentrate themselves that is physiologically detrimental in the case of the downer cow.

3.4 Experimental Procedures

This series of experiments initiated on August 27, 1974 and terminated on May 18, 1975, were conducted at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Fifty-three dry cows from the Research Center dairy herd were involved in a 2 x 2 factorial experimental design.
The primary objectives of these experiments were:

1) To define the "downer" cow syndrome.

2) To determine if the amount of dietary protein influences the incidence of this disease.

3) To determine possible relationship between mineral metabolism and the "downer" cow syndrome.

Experiment I. The Effect of Dietary Protein and Phosphorus on Incidence of the "Downer" Cow Syndrome

Fifty-three dry cows at the Ohio Agricultural Research and Development Center dairy herd were placed in one of four pens for the entire dry period. All received a corn silage-concentrate ration. Pens 1 and 2, however, were maintained on an 8% crude protein intake while pens 3 and 4 received a 15% crude protein ration. Pens 1 and 3 also received a .65% of their dry matter intake as Ca and .29% as P. Pen 2 received .70% as Ca and .70% as P while pen 4 received .66% as Ca and .65% as P (Table 4).

Only animals 3 yr of age or older were used in the study. The age, lactation number, lifetime production average, past health history, body weight at the onset of the previous lactation and body weight prior to pen assignment were recorded. The cows included both Jerseys and Holsteins.

Cows were randomly assigned to treatment subsequent to the final milking. All cows were bled from the jugular or tail vein prior to entering their respective treatment group and at regular intervals thereafter. Bleedings were also made within 6 hr post freshening or prior to the initiation of any therapy. Approximately 20 ml of blood
### TABLE 4. Nutrient content of rations fed pens 1, 2, 3, and 4.

<table>
<thead>
<tr>
<th>Pen no.</th>
<th>Ration constituents</th>
<th>Crude protein</th>
<th>Crude fiber</th>
<th>Total digestible nutrients</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60% Corn silage 40% D-401&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.5</td>
<td>22.0</td>
<td>72.6</td>
<td>.65</td>
<td>.29</td>
<td>.20</td>
</tr>
<tr>
<td>2</td>
<td>60% Corn silage 40% D-402&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.5</td>
<td>21.0</td>
<td>70.1</td>
<td>.70</td>
<td>.70</td>
<td>.23</td>
</tr>
<tr>
<td>3</td>
<td>61% Corn silage 24% Alfalfa pellets 15% D-403&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.0</td>
<td>23.0</td>
<td>68.7</td>
<td>.66</td>
<td>.30</td>
<td>.26</td>
</tr>
<tr>
<td>4</td>
<td>70% Corn silage 30% D-404&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.2</td>
<td>31.0</td>
<td>70.1</td>
<td>.63</td>
<td>.65</td>
<td>.19</td>
</tr>
</tbody>
</table>

<sup>a</sup>See Appendix Table 4.
was collected in heparinized tubes at each bleeding for plasma analysis of inorganic Ca, P, Mg, sodium, potassium, plasma glucose, total blood protein, and plasma urea nitrogen. Blood samples were also collected several times during the feeding period for analysis of serum glutamic oxaloacetic transaminase and isocitrate dehydrogenase. All animals were weighed prior to treatment assignment and at least once during the feeding period. Weights were also recorded within 3 days postpartum.

Cows of all four treatments were fed a corn silage-concentrate ration. In pens 1 and 2, corn silage comprised 60% of the dry matter intake and concentrate made up the remaining 40%. In pen 3, 61% of the dry matter was corn silage, 24% alfalfa pellets and 15% was concentrate. In pen 4, corn silage was 70% of the total dry matter and concentrate 30% (Table 4). The diets fed varied only in protein and mineral intake. Otherwise TDN and crude fiber values were not significantly different (Table 4). The relative amounts of corn silage and concentrate fed varied with the average body weight and number of animals that were in the treatment groups at any particular time. This was based on an estimated dry matter consumption rate of 2% of the body weight per head per day. Silage and concentrate were fed to allow for a 10% refusal over this estimate. Animals were fed only once daily. Water and iodized salt were offered ad libitum.

Corn silage (34.9%, DM) used in this experiment was stored in an upright silo, with all groups being fed from this single source. Variations in protein and mineral intake were mediated through the
concentrate rations fed each group. The nutrient content of the concentrate and roughage fed are outlined in Table 5.

Samples of corn silage and concentrate were taken at monthly intervals and submitted to the Ohio Ration Evaluation Laboratory for analysis of protein, energy and mineral content.

**Experiment II. The Effect of High and Low Protein Corn Silage-Concentrate Rations on Insulin Response**

This experiment was designed to test the theory that the downer syndrome might be caused by a dietary induced insulin insufficiency, which is aggravated by parturient response. This was tested by glucose tolerance response according to the method of Reid (69).

Four cows, representing treatment groups 1, 2, 3 and 4 were removed from the group pens and isolated in individual box stalls approximately 96 h prior to parturition. Pre-calving body weights were recorded prior to the animals being placed in their respective box stalls. Animals continued to receive the treatment ration designated for their group (Experiment I).

After a 24 h adjustment period, one jugular vein of each cow was catheterized with Silastic Medical-Grade Tubing (.040 in. ID, .085 in. OD). Catheter length was approximately 1 meter, 15.24 cm of which were in the jugular vein itself. Catheters were then infused with heparinized physiological saline to prevent clotting.

The actual experiment was initiated approximately 24 h prepartum which was determined by visual inspection and projected calving date. At approximately 8 h postfeeding, a basal blood sample was taken via the jugular catheter. A glucose solution (50% w/v) was then injected
TABLE 5. Nutrient content of concentrate and roughage fed, pens 1, 2, 3 and 4.

<table>
<thead>
<tr>
<th></th>
<th>Dry matter</th>
<th>Total digestible nutrients</th>
<th>Crude protein</th>
<th>Crude fiber</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-401&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.9</td>
<td>82.0 (E)</td>
<td>7.8</td>
<td>15.43</td>
<td>1.28</td>
<td>.31</td>
<td>.25</td>
</tr>
<tr>
<td>D-402&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.2</td>
<td>82.0 (E)</td>
<td>8.5</td>
<td>13.52</td>
<td>1.55</td>
<td>1.52</td>
<td>.32</td>
</tr>
<tr>
<td>D-403&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.2</td>
<td>82.0 (E)</td>
<td>38.4</td>
<td>7.35</td>
<td>.93</td>
<td>1.69</td>
<td>.27</td>
</tr>
<tr>
<td>D-404&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.8</td>
<td>80.0 (E)</td>
<td>39.7</td>
<td>6.66</td>
<td>1.49</td>
<td>1.76</td>
<td>.24</td>
</tr>
<tr>
<td>Alfalfa pellets</td>
<td>93.2</td>
<td>63.5 (E)</td>
<td>17.1</td>
<td>24.32</td>
<td>1.81</td>
<td>.27</td>
<td>.31</td>
</tr>
<tr>
<td>Corn silage</td>
<td>34.9</td>
<td>67.8 (E)</td>
<td>8.9</td>
<td>27.73</td>
<td>.24</td>
<td>.28</td>
<td>.18</td>
</tr>
</tbody>
</table>

<sup>a</sup>See Appendix Table 4.
via the jugular catheter; the dose rate was .4 g of glucose/kg of body weight given over 5 min. The catheter was then flooded with 30 cc of physiological saline. A blood sample was then drawn via the tail vein and designated as T-0. Subsequently, samples were drawn in this manner every 10 min out to T-60 minutes. Samples were then drawn every 30 min to T-150 and every 60 min out to T-270.

At each bleeding 20 ml of whole blood was collected in heparinized tubes and immediately centrifuged at 2000 rpm for 15 min. Plasma was then extracted and frozen for analysis of inorganic Ca, P, Mg and glucose concentration.

3.5 General Methods

Preparation of glucose infusion

Dextrose was added to deionized distilled water to make a 50% w/v solution. The total grams of dextrose used to make this solution was determined on the basis of a dose rate of .4 g of glucose being infused per kg of body weight (69).

Analytical procedure

Plasma Ca and Mg determinations were made by atomic absorption spectroscopy using the procedures of Willis (83) with certain modifications (58). Plasma sodium and potassium were also done by atomic absorption spectroscopy using the method of Preston (67). Plasma inorganic P analysis was done by colorimetry using a modified AOAC procedure (67). Plasma glucose was measured by the glucostate reaction (76). Total serum protein was analyzed by the colorimetric procedure of Henery (31). Blood urea nitrogen was measured by the biuret method as modified by Preston (67). Serum glutamic
oxaloacetic transaminase was measured by the colorimetric method of Karmen (43). Isocitrate dehydrogenase assay was done by the colorimetric procedure of Taylor et al. (77) as modified in the Sigma Technical Bulletin No. 175 (75).

Mineral content of feed samples was determined by spectrographic analysis using an emission spectrograph.

Nitrogen determinations were done by kjeldahl procedures. Net energy values were estimated by methods outlined in OARDC Bulletin No. 554 (68).

3.6 Results

Experiment I

A highly significant difference in incidence of the "downer" cow syndrome was found to exist between animals consuming 8% crude protein (pens 1 and 2) and those consuming 15% crude protein (pens 3 and 4). In pens 1 and 2 no downer cows were observed. In pen 3 three out of twelve animals (25%) were diagnosed as downer cows, all of which subsequently died. In pen 4, five out of 14 animals (35%) calving in this group developed the downer syndrome. Of these, two responded to therapy (Table 6).

Incidence of downer cows was not significantly affected by mineral intake or abnormalities in mineral metabolism. This was indicated by: 1) lack of a significant difference between incidence in pens 3 and 4 despite the variation in Ca and P fed the two groups; 2) the 0% incidence in pen 1 as compared to the 25% incidence of pen 3 where both groups were on similar Ca and P intake. This was also true where group 2 is compared to group 4 (Table 6); 3) the normal
TABLE 6. Incidence of downer cows and all metabolic disturbance at parturition in pens 1 and 2 (low protein) and pens 3 and 4 (high protein).

<table>
<thead>
<tr>
<th></th>
<th>Pen 1</th>
<th>Pen 2</th>
<th>Pen 3</th>
<th>Pen 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Downer cows</td>
<td>-</td>
<td>-</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>All other metabolic disturbances</td>
<td>1</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Deaths</td>
<td>-</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total metabolic disturbance incidence, %</td>
<td>1/14 = 7</td>
<td>1/13 = 7.6</td>
<td>8/12 = 66.6</td>
<td>10/14 = 71</td>
</tr>
<tr>
<td>Total metabolic disturbance incidence by protein level, %</td>
<td>7.14</td>
<td>69.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Observed in same animal
parturient response in blood minerals of 5 of the 6 downer cows examined (Table 7); 4) the lack of plasma mineral changes in "downer" cows that would be indicative of impaired mineral balance, i.e. hypocalcemia, hypophosphotemia, hyperphosphotemia, hyper- or hypomagnesimia, hypo- or hyperkalemia (Table 7).

A highly significant difference in incidence of all metabolic disturbances was also found to exist between protein intake groups. Total incidence of metabolic disturbances was 7.14% in the 8% crude protein pens 1 and 2 as compared to 69.2% in animals consuming the 15% level (Table 6).

As in the downer syndrome comparisons, no significant effect was observed in total incidence of all metabolic disturbances when based upon Ca and P intake. This was indicated by incidence levels when comparisons were made between animals consuming identical dietary levels of Ca and P (pens 1 and 3 and pens 2 and 4) and by comparison between pens 3 and 4 that varied in Ca and P intake, but demonstrate insignificant differences in total incidence, 66.6% and 71%, respectively (Table 6).

No significant differences were found to exist in serum Ca, Mg, sodium and potassium among the four treatment groups (Table 8). Serum phosphorus values, however, tended to be lower in the high protein fed cows of pens 3 and 4 than those of pens 1 and 2 (Table 8). All blood minerals measured were within the range given as normal for the preparturient dry dairy cow (47).

No significant differences were seen in plasma glucose or total protein values among the four treatments (Table 9). Plasma urea
TABLE 7. Specific serum values for blood minerals of downer cows of pens 3 and 4 (high protein).

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Pen 3</th>
<th>Pen 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2072</td>
<td>1956</td>
</tr>
<tr>
<td></td>
<td>12/12</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>11/17</td>
<td>12/26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bleeding dates</th>
<th>Cow No.</th>
<th>Pen 3</th>
<th>Pen 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2072</td>
<td>1956</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12/12</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11/17</td>
<td>12/26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>Serum Ca</th>
<th>Serum P</th>
<th>Serum Mg</th>
<th>Serum Na</th>
<th>Serum K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>9.7</td>
<td>5.8</td>
<td>1.8</td>
<td>34.1</td>
<td>28.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.5</td>
<td>6.2</td>
<td>2.2</td>
<td>32.6</td>
<td>34.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.6</td>
<td>5.2</td>
<td>2.3</td>
<td>32.6</td>
<td>16.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.4</td>
<td>4.7</td>
<td>1.8</td>
<td>35.3</td>
<td>22.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.7</td>
<td>--</td>
<td>2.4</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.7</td>
<td>--</td>
<td>1.9</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td>6.5</td>
<td>2.5</td>
<td>34.5</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.6</td>
<td>3.3</td>
<td>3.3</td>
<td>36.0</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.6</td>
<td>5.5</td>
<td>2.5</td>
<td>35.6</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.2</td>
<td>4.3</td>
<td>2.6</td>
<td>33.6</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.6</td>
<td>5.9</td>
<td>1.4</td>
<td>34.0</td>
<td>26.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.7</td>
<td>4.0</td>
<td>1.4</td>
<td>32.6</td>
<td>22.4</td>
</tr>
</tbody>
</table>

* Taken approximately 30 days prepartum

* Taken previous to treatment.
### TABLE 8. Average values for blood minerals of cows on low (pens 1 and 2) and high (pens 3 and 4) protein.

<table>
<thead>
<tr>
<th></th>
<th>Pen 1</th>
<th>Pen 2</th>
<th>Pen 3</th>
<th>Pen 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ca</td>
<td>10.4</td>
<td>10.8</td>
<td>10.9</td>
<td>10.1</td>
</tr>
<tr>
<td>Serum P</td>
<td>6.2</td>
<td>6.6</td>
<td>5.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Serum Mg</td>
<td>2.6</td>
<td>2.4</td>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Serum Na</td>
<td>35.4</td>
<td>34.8</td>
<td>34.8</td>
<td>36.0</td>
</tr>
<tr>
<td>Serum K</td>
<td>24.7</td>
<td>27.3</td>
<td>26.5</td>
<td>24.1</td>
</tr>
</tbody>
</table>

### TABLE 9. Average values of plasma glucose and other blood constituents for pens 1, 2, 3, and 4.

<table>
<thead>
<tr>
<th></th>
<th>Pen 1</th>
<th>Pen 2</th>
<th>Pen 3</th>
<th>Pen 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma glucose, mg %</td>
<td>75.0</td>
<td>68.8</td>
<td>73.5</td>
<td>72.7</td>
</tr>
<tr>
<td>Total blood protein, mg %</td>
<td>7.2</td>
<td>7.2</td>
<td>7.3</td>
<td>7.1</td>
</tr>
<tr>
<td>PUN, mg %</td>
<td>5.6</td>
<td>4.1</td>
<td>13.1</td>
<td>14.8</td>
</tr>
<tr>
<td>SGOT, Sigma units</td>
<td>41.0</td>
<td>57.0</td>
<td>57.5</td>
<td>51.3</td>
</tr>
<tr>
<td>ICD, Sigma units</td>
<td>340.0</td>
<td>377.5</td>
<td>448.8</td>
<td>425.0</td>
</tr>
</tbody>
</table>
nitrogen values were not significantly different in pens 1 and 2 but were significantly less than seen in pens 3 and 4 (Table 9).

Serum glutamic oxaloacetic transaminase values for all groups were within normal range for the dry preparturient dairy cow. Average isocitrate dehydrogenase values were higher in pens 3 and 4 than those in pens 1 and 2, but whether these differences can be taken as indicative of group response to treatment has not yet been determined (Table 9).

No differences were found to exist in the average period on feed between groups or between "downer" cows and normal parturient cows (Tables 10 and 11). Weight gains were not significantly different between groups, averaging 75.77 kg per animal (Table 10).

Experiment II

No significant differences in glucose tolerance was noted in any of the four cows. Peak concentrations of plasma glucose were reached in 10 min after termination of infusion in cows from pens 1, 2 and 4 and at 20 min from T-0 in the animal from pen 3. Tolerance curves for each of the animals are shown in Figure 1.

3.7 Discussion

The highly significant correlation between dietary protein intake and the development of the "downer" cow syndrome in parturient dairy cows, confirms the hypothesis presented at the onset. This effect is apparently directly attributable to protein intake alone although the form in which the animal consumes its protein appears to be important. Thus the "downer" cow syndrome would appear not to be associated with a mineral imbalance. These data also suggest that this adverse effect
TABLE 10. Average number of lactations, feeding period length, and weight gains of pens 1, 2, 3, and 4.

<table>
<thead>
<tr>
<th></th>
<th>Pen 1</th>
<th>Pen 2</th>
<th>Pen 3</th>
<th>Pen 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calvings</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Average No. of lactations</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>Average length (mo) of feeding period</td>
<td>3</td>
<td>2.2</td>
<td>3</td>
<td>2.2</td>
</tr>
<tr>
<td>Average gain(^a), kg</td>
<td>81.8</td>
<td>72.7</td>
<td>77.7</td>
<td>70.9</td>
</tr>
</tbody>
</table>

\(^a\) Gain from previous parturition

TABLE 11. Specific data for downer cows of pens 3 and 4 (high protein).

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Pen 3</th>
<th></th>
<th>Pen 4</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2072</td>
<td>1956</td>
<td>2141</td>
<td>1912</td>
</tr>
<tr>
<td>Age, yr</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Period on feed (mo)</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>
FIG. 1. Glucose tolerance curves for cows of pens 1 and 2 (low protein) and pens 3 and 4 (high protein).
of high dietary protein may carry over into the etiology of several other metabolic disturbances of the parturient dairy cow (Table 6). Finally, these data offer a possible explanation as to the cause of the alleged detrimental effects of silage-concentrate rations on ruminant health.

That high levels of dietary protein are directly involved in the etiology of the "downer" cow syndrome is supported by several direct lines of evidence. The most significant of these is the high clinical expression of the disease itself in the high protein groups. In pen 3, three out of twelve animals (25%) and in pen 4, five out of fourteen animals (35%) were diagnosed as downer cows. This is to be compared to the 0% incidence of pens 1 and 2 (Table 6).

The diagnosis of the downer syndrome was based upon clinical, biochemical and pathology criteria reported in the literature. Clinically affected animals were in a state of alert recumbency, maintaining appetite, having normal psyche, but apparently affected by a myoparalysis of the hindleg adductor muscles. These animals also failed to respond to normal therapeutic methods used to alleviate hypocalcemia, and except in the case of two animals, failed to respond to any supportive therapy, remaining recumbent until death. These clinical observations reflect those reported by several authors in the literature (39, 8, 38, 41).

Pathologically affected animals were consistent in their physiological response to disease. Hepatic and renal fatty degeneration, Figures 2 and 3, ulceration of the pyloric region of the abomasum, Figure 4, and a general necrosis of muscle and uterine tissue was
FIG. 2. Hepatic fatty degeneration typical of the downer cows.
FIG. 3. Renal degeneration typical of the downer cow.
FIG. 4. Abomasal tissue, showing pyloric ulcerations as seen in the downer cow.
found in five out of six animals examined. Myocardosis was found in four of the six animals. These pathological observations are the same as that reported by Jonsson et al. (41), Hallgren (27), Osinga (66) and others (8, 24, 62).

Biochemically, animal response to the disease as measured by changes in blood minerals, plasma glucose total serum protein and plasma urea nitrogen were also identical, although concentration of the materials varied from animal to animal as would be expected due to animal individuality (Tables 7 and 12). These values were also similar to those established as characteristic for the "downer" cow syndrome (38, 41, 27).

These clinical, pathological and biochemical changes from normal in the "downer" cow syndrome have been implicated by several authors to be a consequence of hypocalcemia or hypokalemia. Indeed, the downer syndrome is generally believed to be an atypical form of parturient paresis or an expression of potassium deficiency. The observations made during these studies do not support these contentions. The rations of pens 1, 2, 3 and 4 were balanced to meet the NRC established requirements for all macro elements, except phosphorus, which was an experimental variable, and all micro elements. Thus, any clinical disorders that could be attributable to mineral nutrition and metabolism would have to be related to the intake and utilization of phosphorus. No significant difference in incidence of the "downer" cow syndrome was found between pens 3 and 4 in spite of the variation in phosphorus intake between the two groups. The animals of pen 4 received .66% of the dry matter as calcium and .65% as phosphorus.
TABLE 12. Specific plasma glucose values and other blood constituents of downer cows of pens 3 and 4 (high protein).

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Pen 3</th>
<th>Pen 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2072</td>
<td>1956</td>
</tr>
<tr>
<td>Bleeding dates</td>
<td>12/12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1/3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total protein</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>PUN</td>
<td>14.2</td>
<td>65.6</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>71</td>
<td>82</td>
</tr>
</tbody>
</table>

<sup>a</sup>Taken approximately 30 days prepartum.

<sup>b</sup>Taken previous to treatment.
Ca and P intakes similar to these have been found to greatly increase the incidence of parturient paresis (42). In contrast, the animals of pen 3 receiving .66% of their dry matter as calcium and .30% as phosphorus consumed amounts of calcium and phosphorus that approach the recommended levels of intake for the pregnant non-lactating dairy cow (NRC). The lack of a significant difference in incidence between animals on the balanced ration (pen 3) and animals consuming the unbalanced ration (pen 4) suggest that differences in mineral intake had little effect as to whether an animal clinically develops the "downer" cow syndrome. Furthermore, the significant differences in downer incidence between pen 1 (0%) and pen 3 (25%), where animals consumed identically balanced calcium and phosphorus rations, and pen 2 (0%) and pen 4 (35%), where animals also had comparable calcium and phosphorus intakes, support this contention. The plasma mineral changes observed in the downer cows (Table 7) when prepartum plasma electrolytes are compared to postpartum (6 h) or pretreatment values, indicate that in all but one case (cow 1912) there is no reason to suspect a mineral imbalance in any of these animals. The levels observed in five of the six were not significantly different from those observed in the normal parturient cow (47). Therefore, the statement that the "downer" cow syndrome and its concurrent pathology is a clinical form of an aggravated mineral imbalance or deficiency is unfounded. These findings agree with those of Jacobsson (38), who reported normal serum calcium and phosphorus values in recumbent animals which were clinically and pathologically similar to those examined in this study. Finally the lack of response in recumbent
animals to intravenous infusion of calcium in the form of calcium borogluconate is further indication that calcium imbalance atypical or otherwise is not involved in this problem. Several animals were infused with as much as 750 cc of calcium borogluconate solution without any improvement in condition. This type of therapy was discontinued after serum calcium values were found not to be in the range that would cause impairment of neuromuscular function (10).

A common observation made in downer cows is that they are often overconditioned. This has given rise to the use of the synonymous term of "fat cow syndrome" for downer cows. Obesity has been blamed by veterinary practitioners as the cause for prolonged recumbency, through increasing the chances for neurological and muscular injury by the struggling of the obese animals to rise. The evidence obtained in this study indicates that obesity itself is not a primary factor in the etiology of the downer cow, since weight gain in all groups averaged 75.77 kg per animal (Table 10). Visually, animals in all treatments resembled those in Figure 5. Despite the fact that obesity was equally prevalent in all groups, pens 1 and 2 had zero "downer" cow incidence. Furthermore, muscle injury in the cases observed in pens 3 and 4 was extensive enough to cause recumbency in only one animal and this was believed to be due to mishandling following treatment (cow 2141). Pathologically, the muscle damage observed in the other animals was believed to be pressure induced and insufficient to be the reason for the animal's inability to rise. That neurological damage was involved is also doubtful. In none of the affected animals was skin sensibility of the hindlegs lost. This is
FIG. 5. A typical dry cow.
commonly used as a diagnostic test for nerve injury (41). Peroneal paralysis is usually recognized as a consequence of traumatic birth injury when the fetus passes the pelvic cavity (41). Considering that the animals of all four groups were in a similar physical condition, it is unlikely that the animals of pens 3 and 4 had a higher incidence of traumatic birth injuries than those of pens 1 and 2. That neurological damage is a secondary factor in downer etiology is supported by the work of Jonsson et al. (41) who could find no degenerative lesions in the hindleg nerve fibers of the downer cows examined in his study. Perineal hemorrhages were common, however, but these are generally associated with pressure induced injury, and are not usually sufficient to inhibit neural impulse.

The high mortality of cows fed corn silage-concentrate rations reported by Trimberger et al. (79) and the overall high incidence of metabolic disturbances in other experiments where animals consumed such rations (6, 61, 71) was verified in this experiment but with one significant qualification. The relative proportion of silage and concentrate fed to all treatment groups in this study were similar to those fed in the experiments referred to above; however, total protein level was varied. The animals of pens 1 and 2 received significantly less protein (8%) than those of pens 3 or 4 (16%) where nutrient consumption data closely resembled the aforementioned experiments. Incidence levels and mortality rates of pens 3 and 4 are not significantly different from those reported in the literature as common for such diets. Pens 1 and 2, however, had a significantly lower incidence rate as compared to pens 3 and 4 or to the previous
experiments where such diets were shown to be detrimental. This difference is attributable to the variation in protein intake. Silage in this experiment was harvested and stored in a similar manner for all groups fed. Consumption data based upon relative amounts fed and refused were identical. Weight gains were also similar. The lack of a significant difference in the blood parameters measured, except for PUN values, a function of dietary protein level, between pens 1, 2, 3 and 4 (Tables 8 and 9) and the fact that these values all were within normal ranges for the preparturient dry dairy cow indicate that the pathological response cannot be attributed to a diet of corn silage or concentrate per se. The lack of a significant incidence of metabolic disease in general or downer syndrome in particular in pens 1 and 2 further supports the premise. The high incidence levels and mortality rates of pens 3 and 4 as well as those reported by Trimberger and others where a minimum of 13.75% crude protein was fed indicates that protein level in the diet was the significant factor. It is concluded that level of protein in the concentrate is the critical factor in incidence of the "downer" cow syndrome in cows fed corn silage-concentrate diets.

The metabolic problems encountered in this experiment when protein consumption far exceeded needs of dry cows would implicate protein as the origin of parturient metabolic disease. It can thus be assumed that "downer" cow syndrome and related sicknesses of overfed dairy cows are somehow related to the processes of protein degradation and metabolism. There is nonspecific information in the literature that would support the contention of a protein induced clinical condition
In the well-fed ruminant. Nevertheless, an abundance of information may be brought to bear on the likelihood of this clinical circumstance.

Dietary nitrogen either in the form of protein or non-protein nitrogen is subject to extensive changes in the rumen by ruminal microbial activity. This normally results in the transformation of a diversity of nitrogen sources into a more uniform mass; this being microbial protein and ammonia. Subsequently, microbial protein passes on to the abomasum and after enzymatic degradation to amino acids there and in the small intestine, is absorbed. Ammonia is either directly used as a nitrogen source for further microbial synthesis or is absorbed through the rumen wall as ammonia and recycled via salivary flow as urea. These processes have been extensively examined in reviews by Hungate (35) and Mugerwa (57).

There are several factors which affect the rate and efficiency at which the fermentative processes of ruminant protein degradation occur. Hungate (36) has shown that yield of microbial protein depends considerably on the presence of readily fermentable carbohydrate. In diets high in protein but low in fermentable carbohydrate, the amount of protein degraded in the rumen far exceeds the amount synthesized (44). Such conditions have been demonstrated to seriously limit microbial protein synthesis and thus curb ruminant protein synthesis and growth (13). Protein solubility has also been shown to have a significant effect upon fermentation. The less soluble proteins are generally less fully fermented and thus are less available for rumen microbial synthesis (44). The differential fractional clearance of fluid from the rumen has also been shown to reduce microbial
fermentation by flushing dietary protein from the rumen before it can be acted upon (78).

The nitrogenous fractions that leave the rumen are primarily bacterial, protozoal and undegraded dietary proteins. The relative contribution of each of these materials to abomasal contents is dependent upon the aforementioned factors. The abomasal digestive process appears similar to that observed in other mammals. Enzymatic degradation yields amino acids (64-68% of the total nitrogen), ammonia (6-10% of the total nitrogen) and unidentified fractions (44).

Intestinal digestion of protein is an enzymatic process regulated by pancreatic secretion. These reactions are significantly affected by intestinal pH, which in turn is affected by abomasal acid secretion. Abomasal acid secretion control is thought to be an integrated function of inflow of ruminal digesta, acid secretion of the fundic glands, and outflow of digesta from the abomasum (3). It has been shown that inflow stimulates acid secretion but it is the rate of passage that determines the pattern of secretion, this being a linear relationship (3). Blockage of abomasal outflow inhibits acid secretion as does abomasal pH values below 2.0 in the sheep. Under normal conditions, abomasal secretion of acid is believed to be continuous, causing intestinal contents to remain acid throughout the upper intestine. Harrison et al. (29) found that in sheep, duodenal pH rises only gradually from a value of 2.7 at the pylorus to a value of 4.0 at the common bile and pancreatic ducts. The low pH values of the duodenum influence both abomasal (3) and pancreatic secretions as well (49). However, the low pH while extending abomasal pepsin
activity (44) apparently inhibits pancreatic peptidase (49) and will continue to do so until pH values approach the alkaline side of neutrality.

Physiologically, the net effect of these factors on ruminant protein digestion is a negative one, in that the completion of protein degradation, an intestinal process, is apparently somewhat inhibited as a consequence.

In summary the digestion of protein in the ruminant involves microbial fermentation in the rumen and enzymatic degradation in the abomasum and small intestine. As a physiological process, hydrolysis and absorption of potentially digestible protein is often slow and inefficient, leaving a significant amount of the dietary protein unabsorbed.

The efficiency of nutrient degradation, besides being a function related to the physiological processes of digestion, is also related to the physical and biochemical properties of the nutrient source. These factors have a profound influence upon fermentation, on rate of passage, on efficiency of enzymatic degradation, and on net absorption. As such it would appear that nutrient source and physical characteristics must be considered in the etiology of any metabolic disturbance.

Corn silage can be characterized as a highly digestible (<70%) (15) readily fermentable roughage. It is an excellent energy source, having a M.E. of 2.57 Mcal/kg for the mature dry cow (NRC). Most of the available energy is in the form of the insoluble carbohydrate, cellulose; the soluble forms including particulate starch, representing less than 23% of the total carbohydrate content (40). It is
relatively low in protein, averaging around 8.4% crude protein. A considerable portion of the total nitrogen is in a soluble form (<50%) (26). As with all forages, digestion of corn silage is primarily a rumen process due to its physical form, specific gravity and the nature of its nutrient content (74). The rate at which it is fermented is relatively rapid. In data taken from the work of Johnson et al. (40), cellulose turnover rate of corn stalks alone was found to be 5% of the total consumed per hour. As this portion represents the least digestible fraction of the ensiled plant, turnover time can be expected to be even more rapid. Microbial fermentation is relatively efficient, leaving little digestible material for enzymatic degradation in the lower gut. The efficiency with which this process occurs is due primarily to the nature of the nutritional constituents of silage itself. As was previously mentioned, 50% of the total dietary nitrogen in silage is in a readily exessible soluble form. In addition, the primary energy source of silage, digestible cellulose, is 90% digested before leaving the rumen (56). The ingesta from a meal of corn silage upon leaving the rumen is thus little changed before it is voided in the feces. McClure (53) has found that fecal material from corn silage fed animals has a TDN value of only 19% which supports these contentions.

Concentrates are important sources of energy and protein in the ruminant ration. Until recently, they were considered to be almost completely digested by rumen microbial action. There are several lines of evidence which suggest that this is not the case, particularly in cattle. Retention time within the rumen and thus efficiency of
fermentation is considerably less for concentrates than for forages. Reduced retention is a result of the physical form and specific gravity of this material which cause a considerable proportion of the ingested amount to pass from the rumen without having undergone fermentation. The rate of loss or washout tends to be increased with the dietary level of concentrate fed. This results in large quantities of starch, the primary energy source of concentrate accumulating in the lower gut and feces (46, 48). In addition, the relatively low solubility of many commonly used protein supplements of concentrate rations (84) compounded by their rapid rate of passage results in large amounts of previously undigested dietary nitrogen entering the lower digestive tract also. As a consequence, a considerable quantity of both carbohydrate and protein must be enzymatically digested directly by the animal to realize full feeding value.

A corn silage-concentrate ration is a high grain ration in that as much as 65% or more of the total dry matter consumed may be derived from grain. Consequently it is a ration that is generally high in starch and insoluble dietary nitrogen. Its physical properties favor a considerable bypass of rumen fermentation, although this may be best described as biphasic as the physical properties of the corn stalk and leaves causes this material to remain in the rumen while the grain portion largely passes on. In respect to digestive physiology these diets result in the following conditions.

1) Rapid rates of passage generate a constant inflow and outflow of ingesta in the abomasum. This stimulates continuous acid
secretion from the fundic glands of the abomasum, helping to maintain low pH levels in the upper intestine (3).

2) Ingesta entering the upper duodenum would contain considerable quantities of unfermented material, in the form of starch and dietary protein.

3) The starch has been shown to be inefficiently used by the ruminant (46) when consumed in large quantities due to the inherent nature of bovine amylases (14).

4) Dietary protein while subject to abomasal pepsin degradation is often not entirely digested because of the limited secretion of pancreatic peptides as well as the inhibition of these peptidases by the acid environment of the upper intestine (49).

5) The animal is then left with a significant amount of potentially digestible material that remain undigested and unabsorbed. The relatively high TDN values (40%) of fecal material collected for concentrate fed animals support this contention (53).

The rations fed in this experiment would be expected to elicit similar physiological responses to those outlined above. The fact that the pen 3 and 4 cows had an overall metabolic disease incidence of 69.2% as compared to the 7.14% of pens 1 and 2 indicates that the dietary-physiological interaction was different between the two groups and in the case of pens 3 and 4 possibly of a pathological nature (Table 6). Considering that protein was the major independent variable imposed between the two sets of cows this effect must somehow be a consequence of protein metabolism as effected through the sequence of conditions mentioned above.
In pens 1 and 2, fed at the 8% crude protein level, an 1500 lb. animal consumed approximately 2.82 lb. of dietary protein per day, of which .92 lb. was in a soluble form as determined by the nitrogen solubility data of Wohlt (84). Thus, approximately 1.68 lb. of dietary protein bypassed rumen fermentation. In pens 3 and 4 protein consumption averaged 4.86 lb. for a 1500 lb. cow per day. The amount of soluble protein in these diets was estimated as being 1.92 lb., thus leaving 2.94 lb. to be degraded in the lower gut. This amounts to a 75% difference between the low and high protein pens in the amount of bypass protein in the lower gut.

In the initial phases of this study into downer syndrome etiology, it was observed that animals consuming the experimental rations were hyperglycemic, a condition that was noted in all pens (Table 10). Several of the animals of pens 3 and 4, however, were significantly more so; blood glucose values being greater than 100 mg %. As the hyperglycemic condition was common in all pens it was obvious that it was a consequence in all dietary conditions.

A considerable proportion of ruminant blood glucose comes after the synthesis from propionate and other end products of rumen fermentation. Due to the high concentration of starch that reach the intestine when concentrate rations are fed, absorption of monosaccharide after enzymatic degradation is a second source. Thirdly, dietary protein is itself glucogenic in the ruminant, and in many diets, significantly so (7). In the normal diet of the ruminant only two of these pathways, synthesis from protein and propionate are important. Carbohydrate digestion is normally insignificant in the ruminant, as is
monosaccharide absorption. This is indicated by the limited physiological dependence of the ruminant directly upon carbohydrate metabolism. Instead, animals have adapted to allow efficient use of the fermentation byproducts of the functioning rumen as energy sources and as generators of glucose. As cattle are not inherently capable of coping with a substantial increase in carbohydrate input in metabolism, carbohydrate in excessive amounts may be considered a stress. Hinsworth (32) noted that in human subjects who consumed diets that were normally rich in fat but restricted in carbohydrates, the exposure to high carbohydrate diets resulted in a blood glucose response to ingested glucose that assumed diabetic characteristics. The incidence of diabetes mellitus in human populations which after generations of dietary consumption of high fat diets (i.e. Eskimo diets), were forced to carbohydrate energy sources was significantly higher than the population norm. This can be likened to the bovine that normally consumes ensilage-concentrate rations. Genetically, the animal is adapted to the use of volatile fatty acids as an energy source. High carbohydrate diets are not well tolerated as indicated by the hyperglycemic state. That bovine hyperglycemia is indicative of a diabetic condition must also be considered as hyperglycemia and a diabetic-like syndrome has become increasingly more common in cattle (82).

In these experiments all groups received the glucogenic diets. The animals of pens 3 and 4 on the elevated protein intakes were subjected to an additional pathway for glucose synthesis, thus their problem would appear to be compounded. This was reflected in the high
blood glucose values of the first animals sampled in pens 3 and 4 when compared to those of pens 1 and 2. The likelihood of a diabetic condition developing as a result of dietary stress was greater in these animals that were subjected to the greater stress. This hypothesis was strengthened by the fact that several confirmed cases of bovine diabetes mellitus (82) resembled the clinical condition of the downer animals of pens 3 and 4. A report of a positive response to insulin therapy in several cases of the downer syndrome (45) further supported this premise. It was thus postulated that the downer syndrome was clinically a diabetic condition, caused by the high protein glucogenic ration. This diet fed throughout the dry period placed a continual demand upon pancreatic secretion of insulin, as stimulated by the elevated circulatory levels of propionate, butyrate, glucogenic amino acids, their metabolites and glucose, all of which were elevated in pens 3 and 4 as a result of the high protein, high energy intake. This condition though present in pens 1 and 2 as indicated by the hyperglycemia, was not as severe due to the limited influence of protein on gluconeogenic and possibly pancreatic insulin response. In other species prolonged hyperglycemia has been postulated to strain pancreatic islet function to a point at which insulin supply becomes inadequate (22). In the parturient cow, diet induced hyperglycemia is aggravated by the hormonal changes at parturition, which are simultaneously glucogenic and inhibitory to insulin mediated cellular response (82). This inability to provide glucose for cellular metabolism could result in neurological injury and possibly be an associated cause of downer cows at parturition. Diabetes has also been
shown in humans to elicit several degenerative changes characterized by fatty infiltration of the liver, and renal degeneration and failure (22). Both of these lesions are common in the downer cow.

This hypothesis was tested and rejected on the basis of a number of lines of evidence. The glucose tolerance tests performed on individuals from all pens showed no significant differences in response to glucose infusion (Figure 1). The tolerance curves themselves were normal for all animals, indicating that the observed hyperglycemia could not be taken as indicative of a pathological condition. This was true in spite of the 24 to 48 h prepartum sampling time, a period during which the hormonal changes of parturition could be expected to produce some effect. Furthermore, the lack of glycosuria or ketonuria in any of the animals is indicative of normal pancreatic islet cell function. Finally, a failure of downer cows to respond to insulin therapy, even though the hyperglycemia itself was reduced after injection, indicates that the problem was not one related to glucose metabolism.

There are a number of reports in the literature of metabolic disorders associated with concentrate rations. One of the more important of these is grain engorgement toxemia. Clinically, the symptoms exhibited are similar to those observed in many cases of acute indigestion. The pathogenesis of this disorder remains somewhat undetermined. It was generally believed that etiology of engorgement toxemia was due to lactacidemia, specifically the accumulation of the D-form of lactate, a poorly utilized metabolite in the rumen and the cow as a result of soluble carbohydrate fermentation. Hungate (36)
found that the intraruminal feeding of grain or glucose could easily reproduce the clinical manifestation, but that intraruminal infusion of acetate or lactate although reducing rumen motility, produced no adverse effect upon animal health. Scaribrick (73) and others (11), through infusion of dilute solutions of hydrochloric acid, sulfuric acid or lactate also failed to reproduce the disease.

Hungate (36) proposed that as this was a disease related to the abrupt alteration of the nature of the nutrients supplied for fermentation, it was probable that changes in the diet would cause significant changes in microbiological activities, which in time might affect the host. However, no changes were observed by Hungate (36) in total numbers of bacteria before or after grain feeding, although the population of gram positive organisms increased significantly subsequent to grain ingestion. Thus was also true when glucose was infused. However, no demonstrable toxins could be identified nor could the changes in fermentation byproducts in either case be shown to produce a clinical disorder. Dougherty et al. (19), however, using animals with clinical and experimentally induced grain engorgement, reported the presence of a substance(s) in the ruminal ingesta and blood plasma which was an active blood pressure depressant. Mullenax et al. (59) extracted a substance having some of the properties of classic endotoxin of gram negative bacteria from ruminal bacteria and ruminal fluid. Endotoxin was also detected in three sheep and one steer experimentally "overfed" with a concentrate ration. Dain et al. (17) isolated considerable amounts of histamine and tyramine in ingesta of experimentally overfed sheep as did Irwin (37) and Ahrens (1).
The pharmacological action of both amines, specifically histamine and bacterial endotoxins have been extensively demonstrated in a number of species. The presence of these substances and their relationship to incidence of metabolic diseases associated with high grain intakes, have been suggested by a number of authors (17, 37, 20, 1, 59). It is the belief of this author that these substances are also important to the etiology of the downer syndrome.

The proliferation and accumulation of both amines and gram negative bacterial endotoxins are correlated with the modification of physiological conditions associated with grain overload in ruminants. These include reduced ruminal pH, decreased gastrointestinal motility and increased fermentable substrate availability. It has been shown for both the sheep and the cow that high levels of carbohydrate ingestion in animals not preconditioned to such diets will cause decreased ruminal contraction when rumen pH approaches 5 and atony at lower values (36, 21). Dougherty (21) has shown a similar reaction to reduced pH in the caecum, small intestine and colon.

Gastrointestinal pH of the ruminant is significantly affected by diet. The low pH values frequently observed on high concentrate rations or in cases of engorgement toxemia are related to microbial changes in population and fermentation (36, 50). High concentrate rations favor the rapid growth of acidophilic bacteria (both streptococci and lactobacilli). These organisms generate considerable quantities of lactate as a fermentative byproduct. Allison (2) reported that 24 h after overfeeding grain to cattle and sheep, similar microbial population changes were noted in the lower gut and
caecum as well, with pH reductions corresponding to those seen in the rumen. The reduction in pH besides inhibiting gastric motility is apparently an etiological agent in amine production and gram negative microbe proliferation. Irwin (37) showed a significant linear relationship between decreasing ruminal pH and increasing intraruminal formation of histamine, tyramine and tryptamine in glucose induced lactacidosis. Similar conclusions were reached by Dain (17). Rodwell (70) isolated eight species of Lactobacilli from the rumen ingesta of overfed sheep which have the ability to decarboxylate histidine to form histamine. Amine production in the lower gut has not been quantitated. That it does occur is likely however. Allison (2) reported that the lactic acid bacteria were the predominant forms in the lower gut and ceacum of overfed sheep and cattle. These acid conditions have also been shown to favor intestinal growth of coliform organisms, among them, E. coli and Clostridium perfringens, both active producers of endotoxin.

The pathogenicity of both histamine and endotoxin in grain over-load has only been shown indirectly. Dain (17) was able to correlate severity of illness of overfed sheep with concentrations of histamine in the ingesta. Histamine was found by Neumark (60) to significantly reduce the amount of feed consumed when present in the feed itself, although no pathogenicity was shown. Ahrens (1) correlated the severity of rumenitis in cattle with histamine concentration. Endotoxin produced by clostridial organisms has long been associated with enterotoxemia in sheep. Mullenax (59) and Dougherty et al. (19) were both able to reproduce a condition similar in clinical expression
to engorgement toxicity through the intravenous injection of endotoxic material obtained from ruminal bacteria. Dougherty (20) successfully isolated endotoxin from the blood of sheep and cattle which were experimentally overfed and expressed clinical engorgement toxemia symptoms.

Histamine and endotoxin production, although a function of microbial activity, is indirectly attributable to the physical and nutrient characteristics of the diet. In physical form and nutrient consistency, the concentrate rations implicated in the etiology of engorgement toxemia are not significantly different from the silage-concentrate ratio fed in this experiment; thus it is extremely likely that they are physiologically handled in a similar manner. Due to the physical characteristics of a concentrate diet, a certain percentage of it can be expected to bypass rumen fermentation. This was shown to happen by Dougherty (21) in all concentrate diets fed to sheep and steers. A considerable portion of the corn silage-concentrate ration due to its high grain content also may be expected to bypass the rumen. This contention is supported by a considerable amount of indirect evidence as has been previously presented. The ingested material of the silage-concentrate ration that has bypassed the rumen is not efficiently utilized by the animal due to inherent characteristics of the feed as well as inefficiency of the lower gut digestive process in the ruminant. In effect a significant amount of potentially digestible material remain. This material is a source of substrate for intestinal microbial fermentation. This substrate is largely soluble carbohydrate and nonsoluble protein, and would support a rapid fermentation initially favoring acidophilic bacteria as described by
Allison (2) for overfed sheep and cattle. In essence it also favors the production of amines, i.e., histamine and the growth of gram negative bacteria, i.e. endotoxin.

In engorgement toxemia, the production of histamine or endotoxin in quantities sufficient to be pathogenic is only possible after gastrointestinal motility is reduced or completely inhibited. This disease then is caused primarily by smooth muscle reaction to low pH values generated by the soluble carbohydrate fermentation. This only appears to occur in animals unaccustomed to using soluble carbohydrate as an energy source (36). The animals in the downer experiment differ in that they have been maintained successfully on this ration for an extended period as indicated by the average weight gain of 71 kg per head. Thus they are apparently well adjusted to the diet. However at parturition, hormonal changes, specifically increases in plasma estrogen have been shown to inhibit feed intake and gastric motility in some cows (58). This estrogen induced gastrointestinal stasis could negate this adaptation and allow for accumulation of both histamine and endotoxin.

Intestinal fermentation of silage-concentrate material alone is not sufficient to explain the downer syndrome. This is indicated by the significantly lower mortality in cases of engorgement toxemia as compared to the downer cows of pens 3 and 4 as well as the lack of downer cows in pens 1 and 2. These differences between the three groups of animals can be attributable to dietary protein. The luxury consumption of protein by pens 3 and 4 especially the insoluble proteins of these rations can be expected to allow for a considerable
amount of protein bypass and accumulation of protein in the lower gut. Nitrogen, when there is an adequate supply of energy is the limiting factor in microbial synthesis and growth. It is safe to assume that the experimental rations consumed in pens 3 and 4 have provided an ample intestinal microbial energy supply. This bypassed protein can thus be expected to be efficiently converted to microbial mass in the small intestine. It is also likely that this theoretically abundant nitrogen supply would support microbial growth and therefore fermentation for a much longer period of time than would be the case in pens 1 and 2. This would result in a considerable increase in the intestinal population of lactobacilli organisms, important in decarboxylation reactions and therefore amine production, and coliform organisms, generators of endotoxin. These reactions are enhanced in the high protein fed cows not only by the availability of protein but the concentration of histamine itself. Histamine is a potent inhibitor of gastrointestinal motility (60, 51). This is detrimental to the high protein fed cow in that gastric motility is inhibited beyond the point of time when hormonal influence is no longer significant. Thus the reactions can theoretically continue until substrate supply is exhausted due to the lack of washout of substrate. This stasis also favors the increasing accumulation of fermentation byproducts, i.e., amines and endotoxin, which like substrate, remain and are absorbed, simply because they are not moved out.

The importance of these premises to downer etiology is supported by several lines of evidence. The lesions and clinical symptoms which are common in cases of downer cows closely resemble those caused by
both endotoxin and histamine. Endotoxin has numerous biological effects. These include dyspnea, diarrhea, a flaccid paralysis of the posterior extremities, a progressive general weakness and death. Histopathologically, endotoxin exerts its effect upon the blood vessels, damage to which results in degenerative changes to the tissue supplied (12). They are generally pyrogenic and appear to adversely alter nonspecific immunity to bacterial infection (18). Histamine reactivity varies with species in clinical expression and intensity. Generally, histamine causes hypotension and hypothermia, inflammation of the abdominal viscera, vasodilation, increased capillary permeability (18), and stimulates gastric secretion (65). Histamine injection in dogs have been shown to produce a hypotensive state characterized by a fall in blood pressure, a decrease in myocardial contractibility, decreased cardiac output and a paralysis of the hindlegs (85). These effects when applied to downer cow symptomology and pathology offer a scientific basis for explanation. The general degenerative changes of the viscera, the liver and kidney in particular, can be attributed to the vascular changes and inhibition of blood supply mediated by both. Pyloric ulcerations, also common, are believed to be a consequence of impairment of normal mucosal blood flow, gastric hyperacidity and stress (81). As vasodilators, inhibitors of cardiac function, and general destructive agents in the vascular system, the first of these etiological factors can be attributed to both histamine and endotoxic action. Histamines as stimulants to gastric secretion, promote gastric hyperacidity, a condition which is further enhanced by parturient stress and hormonal
Influences in the early postpartum period (81). Both endotoxins and histamine have been shown to cause paralysis of the posterior extremities and a hypotensive state culminating in progressive weakness and death. These are classical symptoms of downer syndrome.

Further support comes from the reaction of downer cows to therapy as initiated to counteract these agents. Prior to the development of the current hypothesis, any form of therapy in downer cows was futile. When this hypothesis was tested, treatment consisting of clearing the intestinal tract with a cathartic, supplemented by use of a broad spectrum antibiotic, resulted in complete recovery in the animals. The treatment has since proven consistently effective in alleviating the clinical symptoms of the downer syndrome.

In summary, the biological effects of histamine and endotoxins are capable of producing the clinical symptoms and histopathological lesions of the downer cow. The proliferation of these toxic substances is apparently dependent upon microbiological activity, the nature of which is a consequence of fermentation of soluble carbohydrates but more significantly protein in the lower gut. The physiological condition of the parturient cow serves as a triggering mechanism, providing an ideal environment in which these reactions can occur.

A conclusion to this discussion and the theories presented therein is provided in an observation made by Hansen (28) of a toxic liver injury in parturient ruminants. Hansen reported a disease of unknown origin that he designated as toxic hepatitis. The affected animals were parturient dairy cows and ewes which had been liberally
fed a high concentrate, high protein ration in which herring meal, a
nonsoluble bypass form of protein supplement (84) was used as the
protein source. Symptoms were distinct; alert recumbency caused by
an apparent ataxia of the hind limbs. Appetite remained normal but
animals became progressively weaker. Bradycardia and tachycardia
were common. Death occurred within a week of the development of
symptoms. Necropsy revealed a fatty degeneration of hepatic and
renal tissue. Cause of the disease was undetermined. It is of some
significance that the clinical and histopathological description of
affected animals is strikingly similar to the downer cow. More
importantly, the expression of Hensen's unknown disease was subsequent
to the ingestion of a high protein concentrate ration, the protein of
which largely bypasses rumen fermentation.

3.8 Notes on Therapy

A successful therapeutic method for alleviating the clinical
symptoms of the downer syndrome was devised during this study.
Treatment was based upon the assumption that the disease was caused
by an intestinal toxemia, the agents of which are histamine and
bacterial endotoxin.

It was decided that to effect recovery, removal of these toxic
materials and the elimination of the conditions that further their
proliferation was necessary. Secondly, the apparent lack of immune
response and the resulting increased pathogenicity of secondary
infections in affected animals had to be compensated for. Thus the
use of an oral cathartic, a stomachic and broadspectrum antibiotic
was indicated.
Therapy was initiated soon after the initial clinical symptoms of recumbency developed. A cathartic was prepared by dissolving .9 kg of magnesium sulfate in 3.3 l of water. This solution was administered orally as a drench. The stomachic, *Nux Vomica*, containing 35 mg of strychnine was also given orally at this time. Effectiveness of treatment was indicated by a purgative action of intestinal contents and the resumption of gastric motility. If no response was observed within a 24 h period following dosing, treatment was repeated.

As supportive therapy a broad spectrum antibiotic containing procaine penicillin (300,000 units/cc) and dihydrostreptomycin (500 mg/cc) was given as a 20 cc intramuscular injection beginning at the initial treatment and every 12 h thereafter. Injections were continued until recovery was complete.

During the treatment period animals were offered only roughage in the form of coarse, stemmy hay and water *ad libitum*.

Animals so treated were found to respond within 3 days. Recovery was considered complete based upon physical condition and feed consumption data. This method has been shown to be 100% effective in reversing the downer syndrome under controlled and varied field conditions.

### 3.8 Conclusions

The downer syndrome may be defined as a metabolic disturbance of the parturient dry cow usually occurring within a 3 wk period post-partum. Clinically, affected animals are recumbent but alert, maintaining normal psyches and appetites but unable to rise, apparently due to a flaccid paralysis of the posterior extremities. Animals
appear to have an inhibited immune response as indicated by an increased susceptibility to all pathogenicity. Animals become progressively weaker and eventually enter a comatose condition which precedes death. The course of this disease is normally no longer than 7 days. Necropsy shows a general visceral degeneration which is most severe in hepatic, renal and myocardial tissue. Ulceration of the pyloric region of the abomasum is also common. Cause of death is generally attributed to an acute septicemia resulting in hepatic and renal failure.

It is commonly believed that this disease is an aberrant form of parturient paresis or possibly a clinical manifestation of some other abnormality of mineral metabolism. The inability to correlate downer incidence with mineral intake and the failure to establish significant differences in blood mineral concentrations between downers and normal postparturient cows in this study causes one to question this theory.

A highly significant correlation between downer incidence and the level of dietary protein intake during the dry period was found to exist. It is therefore concluded that the heavy consumption of protein during the dry period is significant in the etiology of this disease. Earlier studies made of this syndrome associated it with the consumption of corn silage-concentrate ration. The ability to successfully maintain dry preparturient dairy cows on similar rations provided that dietary crude protein content was limited to less than 10% indicates that the apparent pathogenicity of corn silage-concentrate diets is related to the protein content of the concentrate
and that the pathogenicity of protein itself is related to its physical and biological properties, i.e. solubility and physical form.

The manner in which protein is involved in downer etiology remains undetermined. However, from the parameters measured in this study, it is not likely that the downer syndrome is a clinical manifestation of a protein induced derangement of metabolism.

A new theory as to the etiology of the downer syndrome and protein involvement in this disease was developed during this study. It is thought that the clinical symptoms and pathology of downer cows are indicative of a toxic condition originating in the lower gut. The presence of excessive partially digested material in the form of soluble carbohydrate and more significantly protein in the intestine of the parturient cow provides substrate and ideal environmental conditions for microbial fermentation. Similar fermentations have been demonstrated in animals suffering from grain engorgement toxemia. Among the byproducts of this fermentation is histamine and endotoxin, both of which are capable of producing the symptomology and pathology of the downer cow. It is believed that this intestinal fermentation and its resulting proliferation of histamine and endotoxin are critical in downer etiology. Work is currently in progress to measure intestinal amines and to test this theory.
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CHAPTER 4

THE ROLE OF SELENIUM AND VITAMIN E IN THE INCIDENCE OF RETAINED PLACENTA IN THE PARTURIENT DAIRY COW

Retained placenta is the failure of the fetal placenta to separate from the maternal placenta. It has been estimated that the normal incidence of placental retention in parturient dairy cows averages 10.3% (25), although the incidence in individual herds can be two to three times greater (8). Placental retention has been reported to result in increases in incidence of uterine infection of 54% in affected animals as compared to 10% for cows which expelled their fetal placenta normally (71). Resistance to uterine infection is enhanced by rapid uterine involution, closing of the cervix and a return of the vagina and vulva to the nongravid state (8). In animals with retained placenta, the rate at which these processes occur is reduced (119, 90). Considering that nearly 25% of the professionally treated diseases of dairy cattle are associated with genital infections (90), the economic significance of retained placenta cannot be overestimated.

Trinder et al. (113) observed that the incidence of retained placenta was high in those herds with correspondingly high incidences of selenium/vitamin E responsive diseases, and that plasma selenium
values were lower than those observed in herds where retained placenta was not a significant problem. As the state of Ohio is an area uniformly deficient in selenium, the possibility that a selenium deficiency exists in dairy herds in this state is likely. These experiments were therefore initiated to determine if incidence of retained placenta could be reduced through supplementation in the preparturient cow with selenium and vitamin E. The possibility that retained placenta may be an expression of a selenium deficiency in the mature dairy cow was also examined.

4.1 Etiology of Fetal Membrane Retention

Retention of the fetal membranes is basically due to the failure of the villi of the fetal cotyledons to detach from the maternal crypts in the caruncles (90). This process normally occurs within 2 to 8 hours postpartum. If the placenta is retained for longer than 12 hours, the condition is considered pathological (90, 119).

The symptoms of retained placenta are usually obvious; a portion of the fetal membrane hangs from the vulva 12 hours or more following abortion, parturition or dystocia (90). Occasionally the fetal membranes are not entirely expelled but remain in the vagina or uterus (90). About 75 to 80% of affected animals show no marked illness. Fifty to 60% of affected animals may exhibit light to moderate anorexia and a reduction in milk flow. About 20 to 25% may exhibit moderate to severe symptoms of metritis or septic metritis as shown in some cases by anorexia, depression, elevation of body temperature, increase in pulse rate, decreased milk flow and loss of weight (90). In severely affected animals, retained placenta may be associated with, or
complicated by other diseases; metritis, septic metritis with peri-
metritis, peritonitis, severe straining, vaginitis, parturient paresis,
acetonemia (90) and displaced abomasum (48). It was found that
infertility is also a problem in 10% of all affected animals (25).

In a normal parturition the expulsion of the fetus produces
changes in both the maternal and fetal placentae. With the rupture of
the umbilicus there is no longer a blood supply to the fetal villi and
subsequently, these structures begin to shrink (90). In the dam,
umbilical rupture also marks a reduction in the uterine blood supply
which is associated with a reduction in the size of the maternal
carunucles (90) and a vasoconstriction of the carunuclar stalk (54).
Degeneration of the epithelial cells of the placenta begins at this
time (119). Histological examination of fetal cotyledons and maternal
carunucles show degeneration and necrosis of both the fetal villi and
maternal placental epithelium and crypts (90). This degeneration has
been proposed by McDonald (66) to be enzymatically induced but this has
not yet been proven. Placental degeneration is believed to be essential
for normal detachment (90).

Throughout the postpartum period the uterus tends to remain
active. In the first few hours following calving, the uterus contracts
strongly at a rate of 14 times per hour, each contraction lasting from
1 to 2.5 minutes. These gradually decrease to 1 per hour at 42 hours
(116). Detachment of the fetal cotyledons from the maternal carunucle
has been shown to occur most easily during the times of contraction
(90).
In summary, fetal membrane expulsion is a complex process, involving a reduction in blood supply followed by shrinking of both maternal and fetal placental structures, degenerative changes, and strong uterine contraction. It is logical to assume that any factor which would interfere with any of these processes could be a causative agent in fetal placenta retention.

4.2 Factors Related to Retained Placenta

Fetal placenta retention has not been shown to be a consequence of a specific syndrome, but rather it is related to a number of factors. Researchers have documented physiological, environmental and nutritional causes for retention.

Physiological factors in retained placenta

Those factors relating directly to the physiological state of the animal as affecting parturition itself may be categorized as physiological. Included here are age, gestation length, twinning, abortion (not caused by infection), uterine inertia, and hormonal imbalance.

1) Age. In relating age to incidence, Erb (25) found that placenta retention rate was only 5.4% of all first calf heifer calvings. This incidence increased to 25% of all calving for animals in their ninth pregnancy. Wetherhill (119) reported that incidence of retention was actually reduced at the 2nd, 3rd, or 4th calvings compared to either older or younger animals. Although age has been shown to have some effect upon incidence, the reason is not known.

2) Gestation Length. Boyd (6) reported that gestation length affected retention rate. Gestations terminating in less than 274
days or extending beyond 291 days tended to result in a higher incidence of retained placenta than those that did not. Muller (71) found that gestation length was significantly shorter for cows with retained placenta.

3) **Multiple Birth and Abortion.** Multiple birth and abortion although accounting for only 13.2% of all calvings accounted for 37.7% of all reported retentions in one study (25). In another study, the incidence of retained placenta in cows twinning or aborting was as high as 80% of all reported cases (90).

4) **Dystocia.** DeSutter (21) has shown that animals affected by dystocia at parturition had a 70% retention rate, regardless of the cause of the problem.

5) **Endocrine Influence.** Placental expulsion is also believed to be influenced by the hormonal changes occurring at parturition, although evidence is inconclusive. MacDonald (61) showed that by removing the corpus luteum prior to parturition, the tendency to retain the fetal membranes was increased. He concluded that this was due to lowered progesterone levels which are enhanced by the corpus luteum. Roberts (90) reported that progesterone deficiency may directly cause retention or it may do so indirectly by predisposing an animal to early parturition. In contrast, Erb (25) proposed that an estrogen, progesterone balance favoring progesterone may lead to increased susceptibility to infection, thus interfering with normal uterine function. It has been shown that the luteal phase uterus of the cow is much more susceptible to infection than the follicular phase uterus (2). High progesterone, then, may lower the resistance of the
pregnant bovine to uterine infection and thus increase the likelihood of retention due to uterine disease.

6) Uterine Activity. Venable (116) reported that a correlation existed between uterine contractability and retention rate. Jordan (53) found that in cases of retained placenta, the uterus was in a static state immediately postpartum and remained in this condition for up to 72 hours, when noticeable contraction resumed. This is not the case in normal parturition where uterine contractability is evident throughout the period (116). Roberts (90) concluded that any condition which causes uterine inactivity whether it be infective, hereditary, nutritional, circulatory or hormonal, could predispose an animal to retention of the placenta.

Environmental factors in retained placenta

Placental retention rates vary with the season. A significantly lower rate of retention was observed during the period of August through November than compared to the winter months (December through March) (71, 37). Similar findings were reported by Wetherhill (119) who related this to change in plane of nutrition. Similar conclusions were reached by Guierro (37). Erb, however, reported a higher incidence during the months of July through October than at other times of the year, although this could be related to the relatively larger calving population during this period (25).

Disease and retained placenta

There is much evidence suggesting that infection of the uterus during gestation is a cause of retained placenta. Boyd (6) concluded that the rate of membrane retention was three times higher in a herd
that was infected with *Brucella abortus* compared to one that was brucellosis free. Fincher reported that 20 to 25% of 282 cases were associated with *Brucella* infection. Roberts (90) reported that according to Bernard et al., 30% of all retained placentas in cattle in Germany were the result of brucellosis. Hallman (38) reported that in *Brucella* infections, an inflammatory reaction of the cotyledons and caruncles is commonly reported as part of the pathology of the disease. Such reactions could cause endometritis, placentitis and fetal membrane retention (90). McDonald et al. (66) reported an increased incidence in dairy herds affected with *Vibrio fetus*. Mold infections due mostly to aspergillus and mucor molds have been noted by Roberts (90) as being a cause of marked cotyledonitis and severe retention of the fetal membranes. Any disease or organism that could cause abortion or premature birth, or lead to an inflammatory reaction of the maternal endometrium could increase susceptibility to fetal placenta retention (90).

**Nutritional factors in retained placenta**

Several workers have concluded that the apparent seasonal variation in retention rate is a consequence of cattle coming off the green feeds of spring and summer and being maintained on stored feed (37, 119). The reduced vitamin availability associated with stored feed, compounded by mismanagement of vitamin and mineral supplementation during this period is believed to be directly involved in retention pathology (71, 85, 5).

Iodine deficiency has been postulated to predispose animals to retained placenta and other herd reproductive problems (66, 68). In
studying the possibility of an all corn silage-forage program, Hemken (43) found that in diets that were unsupplemented with iodine, several cows produced calves with small goiters; these cows all retained their fetal placentae. Allcroft (1) demonstrated subnormal PBI levels in herds having a high incidence of abortions and retained placenta. Similar results were reported by Moberg (68).

Reproductive failure is often the outstanding manifestation of iodine deficiency and is believed to be a consequence of the impairment of thyroid function (115). It is lowered thyroid hormone that is most likely responsible for observable effects of iodine deficiency. Thyroidectomy has been reported to cause abortion in ewes (29). The feeding of goitrogenic substances which block iodine uptake by the thyroid and thus inhibit thyroxin synthesis, have also been known to cause abortion and fetal death (115). The increase in placenta retention rate associated with iodine deficiency may be secondary to a deficiency in thyroid hormone and the changes that this deficiency would induce.

Pelessier (85) reported that a highly significant relationship was found to exist between retained placenta and milk fever. Noorsdy et al. (76) reported that the incidence of retained placenta was higher in herds in which managerial practices had altered calcium metabolism. They suggest that the precipitous drop in blood calcium observed at parturition may adversely affect smooth muscle function. A resulting atony of the smooth muscle of the uterus could interfere with normal uterine involution and thus increase the likelihood of placental retention.
Boslov (5) investigated the causes of retention incidence in a 250 head herd of Friesian cattle. He reported that supplementation of the fat soluble vitamins A, D₃ and E, reduced the incidence of retention to 6% in treated animals as compared to 25% for controls. Several workers have reported similar findings, thus suggesting a possible role of the fat soluble vitamins in normal uterine health.

Ronning (91) in examining the role of vitamin A in reproduction reported a significantly higher incidence of retained placenta in Guernsey cows consuming 66 to 110 mg/kg of body weight of carotene, the precursor of vitamin A, when compared to animals on higher intakes. Pope (86) reported that in beef cows, depleted of vitamin A stores through a deficiency diet, a marked incidence of abortion, fetal death and abnormal necrosis of the fetal membranes were observed. Feeding vitamin A at a level of 20,000 IU per day to pregnant beef cows had little effect upon reducing the incidence of retention but when this was increased to 50,000 IU per day the retention rate was significantly reduced, as compared to unsupplemented animals (74).

An important function of vitamin A in mammalian systems is the protection and maintenance of epithelial tissue. Vitamin A deficiency leads to varying degrees of keratinization of epithelial cells. This process reduces the ability of epithelia to resist bacterial invasion and infection (40). In cases of hyperkeratosis, the incidence of retained placenta, metritis and fetal death is high (90). Warren (118) noted that the histological changes within placental tissue, associated with an infection of *Brucella abortus*
were similar to those seen in avitaminosis A. He concluded that the high incidence of fetal death associated with vitamin A deficiency is secondary to a marked placental injury. Thus the essentiality of vitamin A for the maintenance of the health and resistance to disease of uterine tissue is likely (90).

Observing an abnormally high incidence of retained placenta in an area with a history of white muscle disease, Trinder et al. (114) suggested that retention may be related to a deficiency of vitamin E, but implicated the element selenium as well, white muscle disease being responsive to both. In a series of experiments, these workers found that injection of vitamin E and/or selenium significantly reduced retention rate and that injection of E and selenium together was more effective than either substance injected alone in its prevention. In a later study, dry cows fed a ration containing .025-.047 mg per g of dry matter of selenium still had a high incidence of retention, although in another group of dry cows a combined injection of vitamin E and 15 mg of selenium one month before the projected calving date was found effective in reducing this incidence. Fifteen mg of selenium injected as potassium selenate was found slightly less effective. Blood levels of selenium in herds with high retention rates were found to be significantly lower than in herds with no history of retained placenta (113). These data would indicate that there is a possible relationship between uterine health and selenium and/or vitamin E deficiency. This hypothesis is strengthened somewhat in that the herds studied were on summer pasture which more than adequately met their vitamin A requirements, and the ration was balanced for all the
nutrients except selenium and vitamin E. However, Horvath (49) in a study of three years duration was unable to show a significant prophylactic effect when 15 mg of selenium and vitamin E was injected 20 days prepartum in dairy cows. Both source and concentration of selenium and vitamin E were identical to those used in Trinder's study. The reason for the apparent discrepancy in the results in the two studies is not known.

Both selenium and vitamin E have recently been shown to inhibit cellular damage associated with autoxidation of lipoproteins of the cell and microsomal membranes. The importance of this antioxidative property of selenium and vitamin E to the prevention of retained placenta is not known. Oksanen (78) suggests that vitamin E and selenium functioning as biological antioxidants, may be involved in maintaining vitamin A, a substance known to influence retention rate, in a proper oxidative state for physiological activity. Trinder et al. (113) failed to draw conclusions as to the apparent prophylactic action of vitamin E and selenium in his study.

4.3 Therapeutic Value of Selenium and Vitamin E

During the 1920's, Evans et al. (26), first demonstrated a physiological need for the supplementation of alpha tocopherol in the diet of rats. Subsequently, a dietary vitamin E requirement was found to exist for a number of species (47). Selenium by comparison has only recently been shown to be essential for normal physiological function and then only in a limited number of species (98, 84).

Due to the apparent biological similarities in animal response to vitamin E and selenium, many investigators questioned the need for
selenium in vivo, provided that there was an adequate availability of vitamin E. Demonstration of selenium deficiency symptoms in chicks (111), and rats (65), in the presence of vitamin E supplementation, and lack of response to selenium in several avitaminosis E related diseases (51), established a physiological need for each of these nutrients. Schwarz (96) thus concluded that both selenium and vitamin E are required nutrients which apparently interrelate in their metabolic function. Recent work delineating the pathology of selenium/vitamin E responsive diseases and the discovery of an apparent mode of action for selenium (93) which is not duplicated by alpha tocopherol, gives biochemical credence to this theory.

It is interesting to note that the biological response, whether mediated through vitamin E or selenium, is apparently enhanced by the presence of the other nutrient. Thompson et al. (111) found that protection against exudative diathesis, a vitamin E deficiency disease of chicks, was also selenium responsive but that this response was affected by vitamin E. Diets containing 100 ppm vitamin E were found to afford 100% protection when selenium was fed at less than .01 ppm whereas with 10 ppm of vitamin E, a comparable level of protection was realized only when selenium supplementation was greater than .02 ppm. In chicks fed no vitamin E, selenium was required at levels greater than .05 ppm.

Enzootic nutritional white muscle disease, the most clearly defined selenium/vitamin E responsive disease of ruminants, was found by California workers to be unresponsive to vitamin E alone, but could be successfully dealt with when vitamin E was used conjunctively with
selenite (122). Muth (72) and Hartley et al. (42) observed a variable response of white muscle disease to vitamin E supplementation under some conditions, but found it more effective when selenium also was fed. In lambs fed a Torula yeast, milk replacer diet, the onset of white muscle disease could be prevented by vitamin E alone. A growth response, however, was observed when selenium was added to the diet, and it was concluded that these nutrients were acting independently, with selenium slowing the development of white muscle disease and thus allowing weight gain. Evans (26) observed that in treating dystrophic lambs, no improvement was noted when selenium or vitamin E was used alone, but when used together at levels comparable to those used when each was the sole therapeutic agent, rapid improvement was noted. Similar results for other species are in the literature (32, 89, 62, 100, 9, 83). Buchanan-Smith (7) reported that reproductive performance of ewes and rams fed diets deficient in both selenium and vitamin E could only be improved through supplementation of both. Myocardial necrosis was noted in all animals except those treated with vitamin E and selenium, and only lambs from ewes treated with the combination survived beyond weaning.

4.4 Physiological Function of Vitamin E

The fact that alpha tocopherol possesses antioxidative properties was known prior to the complete delineation of its chemical nature (80). Davies et al. (17) observed that vitamin E reduced the rate of depletion of vitamin A from rat liver, thus suggesting a function as a chemical stabilizer under physiological conditions. Tappel (107), based on observations made both in his, and other laboratories, proposed that
vitamin E functioned as a biological antioxidant, protecting the structural integrity of various functional cell components, i.e. cell membranes, and subcellular particles. This theory has been the object of considerable controversy in recent years (33, 35, 24), but its basic concepts are defendable from several lines of evidence.

In examining the pathology of vitamin E deficiency one generally finds a nonspecific degenerative, necrotic type of myopathy (58), however, fetal death and resorption, renal degeneration and failure, vascular degeneration, hemorrhage and erythrocyte hemolysis have also been reported (47). Blaxter (3) in reviewing seven distinct myopathies associated with avitaminosis E in cattle, sheep and swine noted all exhibited degeneration of the muscle cell, which was not secondary to neural involvement. Lesions of the cardiac and skeletal muscles were histologically similar in all species. Supportive evidence for these conclusions have been published by Hibbs (45) in cattle, Trapp (112), and others (26, 34) in swine, and Stowe (105) in mink. In fowl, the conditions associated with avitaminosis E are similar in expression to those observed in mammals. Exudative diathesis and encephalomacia in chicks, as well as gizzard myopathy and perosis observed in poults, have all been shown to involve degenerative lesions. Lesions vary from a degeneration of Purkinje cells and cerebral necrosis in the case of encephalomacia (63) to a general necrosis of both smooth and skeletal muscle as observed in gizzard myopathy (55) and perosis (99) respectively.

These data show that a common interspecies reaction to a deficiency of alpha tocopherol is necrosis and degeneration of cellular structures.
Early workers observed that most of the diseases associated with avitaminosis E could be prevented or treated with synthetic antioxidants, thus concluding that autooxidation of cellular components was in some way responsible for this myopathic damage (51). Tappel (107) later showed a correlation between the protective properties of synthetic antioxidants and the apparent biological function of vitamin E. He proposed that the observed cellular damage was a result of lipid peroxidation of cell and microsomal membranes. The fact that lipid peroxidation does occur in vivo, a concept questioned by critics of this theory (35), was recently proven by Demopoulos (18). Of equal importance was the confirmation of measurable accumulations of lipid peroxides in damaged tissues in cases of vitamin E deficiency in vivo (10, 102, 33). Thus, the hypothesis stating that vitamin E functions as a biological antioxidant appears to be a valid one, although the manner in which it accomplishes this function is still not clear.

4.5 Physiological Function of Selenium

Selenium was not recognized as mediating a physiological response until the discovery of Schwarz et al. (98), demonstrating its effectiveness in preventing liver necrosis in rats fed a Torula yeast supplemented diet, deficient in vitamin E. Prior to this work, selenium received little attention in objective dietary requirement studies, other than those related to its toxic properties. However, with the establishment of an actual dietary requirement, there soon followed reports of a number of selenium responsive diseases which
have prompted further investigation into possible biological mechanisms to explain physiological function.

Most of the known pathologies of selenium deficiency can be summarized as noninflammatory degenerative or necrotic lesions of a variety of tissues, the most common being muscle tissue. Clinically and histologically, these lesions are identical to those seen in avitaminosis E. With the exception of Ill Thrift of calves and lambs (42), all of the selenium responsive diseases can be prophylactically and therapeutically treated with vitamin E. Several have been shown to respond as well to the addition of the sulphur amino acids, methionine and cystine. Conversely, most of the diseases associated with a deficiency of vitamin E, with the exception of encephalomacia of the chick, nutritional muscular dystrophy of the rabbit, pig and chick and fetal resorption in the rat are selenium responsive (51).

The apparent ability of selenium to prevent most diseases of avitaminosis E prompted the conclusion that selenium also was a biological antioxidant or an antioxidant precursor. Tappel (108) stated that the oxidation-reduction reaction of seleno compounds which appear related to its biological activity can be classified as: 1) lipid antioxidants inhibiting peroxidation; or 2) peroxide decomposition. Olcott et al. (79) showed that selenomethionine is a stronger antioxidant than methionine and was also capable of decomposing lipid peroxides. Tappel (108) verified this and reported on a molar basis, the selenium antioxidants have 50 to 500 times the antioxidant activity of vitamin E; 3) free radical scavenging; and
4) repair of molecular damage sites. Evidence in support of reactions 3 and 4 is indirect, coming principally from systems of amino acids and enzymes subjected to ionizing radiation (106), but indicative of these contentions.

The relegation of simple antioxidative properties to selenium or to its organic derivatives has not gained wide acceptance. Glavind (33) in reviewing the antioxidant theory stated "It seems awkward to interpret the protective properties of trace amounts of selenium against manifestation of vitamin E deficiency by attributing exceptional antioxidant properties to selenium compounds". Several laboratories suggested a more complex role for selenium. Schwarz (96) proposed that selenium and vitamin E functioned separately in a cofactor-type, probably enzymatic role at sites in metabolism that were closely related. Recently selenium has been found to be an essential component of glutathione peroxidase, an enzyme which apparently serves as a protective mechanism against peroxidative damage. This discovery is important in that it allows for the encompassment of the antioxidant theory of Tappel and the cofactor theory of Schwarz and thus reconciles this dilemma.

4.6 Glutathione-Peroxidase Theory

An enzyme, isolated in the bovine erythrocyte by Mills (67) and identified as glutathione peroxidase, was found to be active in the prevention of cellular damage caused by hydrogen peroxide. No association was made at this time between this enzyme and selenium or alpha tocopherol. Rotruck et al. (92) in attempting to clarify the role of alpha tocopherol, selenium and glucose in the prevention of
cellular oxidative damage to rat erythrocytes discovered that vitamin E alone protected the cell membrane, but could not prevent oxidative damage to hemoglobin. Selenium, as dietary selenite, decreased autohemolysis and also prevented ascorbic acid induced oxidation of hemoglobin, but only in the presence of glucose. Glucose in the incubation medium, protected both membrane and hemoglobin but only in the presence of selenium. It had been presumed that the protective action of glucose was a result of the synthesis of reduced glutathione by generating NADPH via glucose-6-phosphate dehydrogenase, with NADPH maintaining GSH via glutathione reductase (12). Selenium's role was initially assumed to be catalytic in the generation of glutathione, but it was found that the GSH concentrations were not influenced by the absence of selenium. It was then proposed that selenium might be required for the utilization of glutathione in maintaining cell integrity. It was further suggested that selenium prevented oxidative damage to the membrane and hemoglobin in a distinct manner, other than that mediated through vitamin E, and that this action was related to the presence of the enzyme GSH peroxidase. Purification of GSH peroxidase from rats injected earlier with $^{75}$Se, in sodium selenite, revealed that 60% of the injected $^{75}$Se cochromatographed with GSH peroxidase activity, indicating that selenium was a component of the enzyme system (93). Analytical purification of ovine erythrocyte glutathionine peroxidase revealed four atoms of selenium per mole of protein of 88,000 molecular weight or an average of one selenium per protein subunit of 22,000 molecular weight (77). Scott et al. (102) found that glutathione peroxidase level of chick plasma was directly
related to selenium in the diet and to the effectiveness of selenium in prevention of exudative diathesis. Similar responses have been reported by Omaye (82) for the rat and Smith (104) for the chick.

4.7 Mode of Action of GSH Peroxidase

In studying the oxidation of hemoglobin of rat erythrocytes, Mills (67) indicated hemoglobin may be protected by reduced glutathione which serves as a hydrogen donor in the presence of GSH peroxidase. Hoekstra (47) proposed that GSH peroxidase is responsible for the degradation of both H₂O₂ and organic hydroperoxides into less damaging alcohol, although the fate of these alcohols is still not clear.

4.8 The Pathology of Selenium-Vitamin E Responsive Disease

There is ample evidence in the literature that would suggest that the lesions of the selenium/vitamin E responsive diseases are a consequence of cellular injury. Tappel (107) proposed that this was caused by lipid peroxidation of cellular constituents. This theory has been shown to be true in a number of selenium/vitamin E responsive diseases studied in several species (10, 102, 33).

Lipid peroxidation in vivo, has been identified as a deteriorative reaction of cellular components and is a normal part of cell physiological function (19). It has also been associated with cellular mechanisms involved in the aging process (106) and several diverse pathological conditions (110, 22, 50, 19).

Biochemically, peroxidation of lipids is defined as the oxidative deterioration of polyunsaturated lipids, and involves the direct reaction of oxygen and lipid to yield free radical intermediates and semi-stable peroxides (109). The reaction is autocatalytic, the
velocity of which is linearly proportional to the extent of oxidation (60). Early workers observed certain similarities to inorganic antioxidative systems, and concluded that peroxidation involved a chain reaction that resulted in the formation of hydroperoxides. These structures serve as a catalyst for further reaction, as decomposition of peroxides in unsaturated systems initiates polymerization reaction chains (60). Schematically, these reactions are:

Initiation

\[
\begin{align*}
\text{RH} + O_2 & \rightarrow \text{ROOH} \\
\text{ROOH} & \rightarrow \text{ROOH}_2
\end{align*}
\]

Propagation

\[
\begin{align*}
\text{R}_1 + \text{R}_1 & \rightarrow \text{R} + \text{ROOH} \\
\text{RO}_2 + \text{RH} & \rightarrow \text{R} + \text{ROOH}
\end{align*}
\]

Termination

\[
\begin{align*}
\text{R}_1 + \text{R}_1 & \rightarrow \text{RO}_2 + \text{RO}_2 \\
\text{R}_1 + \text{RO}_2 & \rightarrow \text{stable (non radical) end products.}
\end{align*}
\]

That these reactions occur in vivo has been the object of considerable controversy (35). Initially, supporters of this theory drew correlations between radiation poisoning, which is caused by tissue peroxidation (120) as well as the aging process (120) and concluded that the physiological and cellular impairments observed in both could be caused by an analogous chemical reaction. This was believed to be lipid peroxidation. Hendley (44) in characterizing the lipofuscin pigments associated with senescent tissues, reported that these were a complex lipid-protein structure, whose composition indicates that
they are derived from lipid peroxidation. Tappel (109) concluded that accumulations of lipofuscin pigments in tissue is indicative of cellular damage as caused by peroxidation. The discovery that peroxidative pathology can be prevented by antioxidants, both synthetic and biological and the isolation of measurable accumulations of these lipid peroxides in tissue damaged by peroxidation (10, 18) is further evidence as to the importance of these reactions in vivo. The readiness with which unsaturated lipids enter into free radical reactions (94) led workers concerned with modeling autooxidation systems in vivo to suspect those tissues composed of unsaturated lipids to be most susceptible to peroxidative reaction. Biomembranes have been shown to have unsaturated lipid as a major structural component, and have also been shown to be the primary site of peroxidative damage (109). Biomembranes include the plasma membrane and the membrane of subcellular organelles such as in the mitochondria and lysosomes (18).

Although differing in composition (59, 30), depending upon structural site, biomembranes are primarily protein and phospholipid, the phospholipid component being somewhat unsaturated. Subcellular membranes contain a greater proportion of phospholipid and protein per unit of size than the plasma membrane. Consequently, they contain more unsaturated structures than the plasma membrane (30), and are more susceptible to peroxidative damage (18). Structurally, the plasma membrane is a bimolecular leaflet of phospholipid and other amphipatic substances such as cholesterol (19). These integrate with protein and form hydrophobic associations at the midzone of the membrane (59).
The organelle membrane lacks the leaflet structure and is composed of intertwined units of protein and phospholipid.

The disassociation of biomembranes by peroxidative reactions is initiated in the polyunsaturated fatty acids of the phospholipid (18, 109). These reactions and their consequences are described in detail by Demapaulos and others in a recent symposium on free radical pathology (18). Once initiated they have been shown not only to affect membrane structure and function, but to produce a drastic effect upon subcellular organelles, i.e. ribosomes and mitochondria, as well as affecting surface glycoproteins and various complex enzymatic receptor sites and several enzymatic systems themselves (19, 109). The net effect of defects in the systems is cell injury and death.

4.9 Peroxidation, Selenium and Vitamin E

Recent evidence suggests that peroxidative reactions are in some fashion inhibited by the presence of vitamin E and selenium. Hoekstra (47) in a recent review of literature of the function of selenium and vitamin E, proposed that these nutrients have a dual role in protecting the cell against autoxidation. The vitamin E-selenium interaction is seen as a role of the vitamin preventing the formation of lipid hydroperoxides and the consequent autocatalytic lipid peroxidation and selenium, as a component of glutathione peroxidase, in converting hydroperoxides into less damaging alcohols. It has been suggested that vitamin E be thought of as a lipid antioxidant, acting possibly by restricting lipid peroxidation through favoring alternate routes of oxygen metabolism. Selenium has the dual role of degrading both
H₂O₂ and organic hydroperoxides, a function that has not been demonstrated for vitamin E. This hypothesis suggests that the therapeutic and prophylactic value of these nutrients in the prevention disease syndromes, would be greater when they are used together than when either is used alone.

4.10 Experimental Procedures

This series of experiments was initiated on August 27, 1974 and terminated on February 1, 1976. Two experimental methods were employed: the controlled experiment and the field trial.

Experiment 1

The controlled experiment was conducted at the Ohio Agricultural Research and Development Center in Wooster from August 27, 1974 to May 18, 1975. Fifty-three dry cows for the Research Center dairy herd were involved in a 2 x 2 x 2 factorial design.

The primary objectives of this experiment were:

1) To determine if placental retention in dairy cows is one of the selenium-vitamin E responsive diseases.

2) To determine the possible role of dietary protein intake in placental retention etiology.

3) To determine the possible involvement of dietary calcium and phosphorus in retained placental retention etiology.

Fifty-three dry cows at the Ohio Agricultural Research and Development Center dairy herd were placed in one of four pens for the entire dry period. All cows received a corn silage-concentrate ration. Pens 1 and 2, however, were maintained on an 8% crude protein intake while pens 3 and 4 received a 15% crude protein ratio. Pens 1
and 3 also received .68% of the dry matter intake as calcium and .29% as phosphorus. Pen 2 received .70% as calcium and .70% as phosphorus while pen 4 received .63% as calcium and .65% as phosphorus (Table 1).

Only animals 3 yr of age or older were used in this study. The age, lactation number, lifetime production average and past health history was considered in selecting animals for this experiment prior to pen assignment. Animals were either of the Jersey or Holstein breed.

Cows were randomly assigned to treatment subsequent to the final milking. All cows were bled from the jugular or tail vein prior to entering their respective treatment group and at regular intervals thereafter. Approximately 20 ml of blood was collected in heparinized tubes at each bleeding for plasma analysis of inorganic calcium, phosphorus, magnesium and selenium.

Cows of all four treatments were fed a corn silage-concentrate ration. In pens 1 and 2 corn silage comprised 60% of the dry matter consumed and concentrate made up the remaining 40%. In pen 3, 61% of the dry matter was corn silage, 24% alfalfa pellets and 15% was concentrate. In pen 4, corn silage was 70% of the total dry matter and concentrate 30% (Table 1). The diets varied only in protein and mineral intake. Otherwise, TDN and crude fiber values were not significantly different (Table 2). The average amounts of corn silage and concentrate fed varied with average body weight and number of animals that were in a treatment group at any particular time. This was based upon an estimated dry matter consumption of 2% of the body weight per head per day. Silage and concentrate was fed to allow for
TABLE 1. Nutrient content of rations fed in pens 1, 2, 3, and 4.

<table>
<thead>
<tr>
<th>Pen no.</th>
<th>Ration constituents</th>
<th>Crude protein</th>
<th>Crude fiber</th>
<th>TDN</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60% Corn silage</td>
<td>8.5</td>
<td>22.0</td>
<td>72.6</td>
<td>.65</td>
<td>.29</td>
<td>.20</td>
<td>.02</td>
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<td></td>
<td>40% D-401&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>2</td>
<td>60% Corn silage</td>
<td>8.5</td>
<td>21.0</td>
<td>70.1</td>
<td>.70</td>
<td>.70</td>
<td>.23</td>
<td>.02</td>
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<tr>
<td></td>
<td>40% D-402&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>3</td>
<td>61% Corn silage</td>
<td>15.0</td>
<td>23.0</td>
<td>68.7</td>
<td>.66</td>
<td>.30</td>
<td>.26</td>
<td>.07</td>
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<tr>
<td></td>
<td>24% Alfalfa pellets</td>
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<td></td>
<td>15% D-403&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>4</td>
<td>70% Corn silage</td>
<td>15.2</td>
<td>31.0</td>
<td>70.1</td>
<td>.63</td>
<td>.65</td>
<td>.19</td>
<td>.05</td>
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<tr>
<td></td>
<td>30% D-404&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>See Appendix Table 4.
TABLE 2. Nutrient content of concentrate and roughage fed in pens 1, 2, 3 and 4.

<table>
<thead>
<tr>
<th></th>
<th>Dry matter</th>
<th>Total digestible nutrients</th>
<th>Crude protein</th>
<th>Crude fiber</th>
<th>Ca</th>
<th>P</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-401&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.9</td>
<td>82.0 (E)</td>
<td>7.8</td>
<td>15.43</td>
<td>1.28</td>
<td>.31</td>
<td>.25</td>
</tr>
<tr>
<td>D-402&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.2</td>
<td>82.0 (E)</td>
<td>8.5</td>
<td>13.52</td>
<td>1.55</td>
<td>1.52</td>
<td>.32</td>
</tr>
<tr>
<td>D-403&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.2</td>
<td>82.0 (E)</td>
<td>38.4</td>
<td>7.35</td>
<td>.93</td>
<td>1.69</td>
<td>.27</td>
</tr>
<tr>
<td>D-404&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.8</td>
<td>80.0 (E)</td>
<td>39.7</td>
<td>6.66</td>
<td>1.49</td>
<td>1.76</td>
<td>.24</td>
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<tr>
<td>Alfalfa pellets</td>
<td>93.2</td>
<td>63.5 (E)</td>
<td>17.1</td>
<td>24.32</td>
<td>1.81</td>
<td>.27</td>
<td>.31</td>
</tr>
<tr>
<td>Corn silage</td>
<td>34.9</td>
<td>67.8 (E)</td>
<td>8.9</td>
<td>27.73</td>
<td>.24</td>
<td>.28</td>
<td>.18</td>
</tr>
</tbody>
</table>

<sup>a</sup>See Appendix Table 4.
a 10% refusal over this estimate. Animals were fed once daily. Water and iodized salt were offered ad libitum.

Corn silage (34.9% dry matter) used in the experiment was stored in an upright silo with all groups being fed from this single source. Variations in protein and mineral intake were mediated through the concentrate rations fed. The nutrient of the concentrate and roughage fed are outlined in Table 2.

Samples of corn silage and concentrate were taken at monthly intervals and submitted to the Ohio Ration Evaluation Laboratory for analysis of protein, energy and minerals. Selenium content of rations fed was determined in the laboratory of the Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster.

Selenium was administered to half the animals in each of the four pens. Initially selenium was given orally in bolus form; 6 mg of selenium in the form of sodium selenite being given at the time cows were assigned to treatment. This was revised on October 24, 1974 so that 12 mg of selenium as sodium selenite was given orally as a bolus on a daily basis for a period of 5 days after treatment assignment and were weekly thereafter. This treatment was again revised on December 1, 1974 so that designated animals received a 10 cc intramuscular injection of the selenium/vitamin E preparation MuSe, supplied by Burns Biotec Lab., 50 mg of selenium as sodium selenite and 680 IU of vitamin E as alpha tocopherol acetate, approximately 21 days prior to freshening.

Animals were considered as having retained their fetal placentas if the membranes were still visible or palpable after a 12 h postpartum period.
Experiment 2

The field trial involved four dairy operations located in northeastern, central and southwestern Ohio. Two dairy breeds, Holstein and Guernsey, were represented in the sampling. The total number of observations made was 193, 113 of which were in the treatment group.

The primary objectives of this experiment were:

1) To verify and expand upon the results obtained in Experiment 1, under a number of field conditions.

2) To establish a method whereby an intramuscular injection of selenium and vitamin E can be most effectively used as a prophylactic agent in the prevention of retained placenta.

Four dairy farms located in the counties of Wayne and Tuscarawas in northeastern Ohio, Union in central Ohio, and Greene in southwestern Ohio cooperated in the study (Appendix Table 4). The herds involved had been referred to the author as a result of their exceptionally high chronic incidence of placenta membrane retention (> 45%). Data were collected on the nutritional, reproductive and health management of each herd.

The age and past health history was considered in selection of the animals for the experiment. Animals were then randomly assigned to either one of two treatments or designated as controls.

Twenty-five percent of the animals involved in each herd were bled prior to treatment from the jugular or tail vein upon group assignment. Approximately 20 ml of whole blood was collected in heparinized tubes for plasma analysis of calcium, phosphorus, magnesium and selenium content.
There were two treatment groups in the experiment. Treatment A involved a series of two intramuscular injections of the selenium/vitamin E preparation, MuSe, supplied by Burns Biotec Laboratories, with 10 cc of the preparation given at 40 days and again at 20 days prepartum. Each injection contained 50 mg of selenium in the form of sodium selenite and 680 IU of vitamin E as alpha tocopherol acetate. Treatment B was a single intramuscular 10 cc injection of MuSe at 20 days prepartum only. Injections were administered in the flank of each animal by the herdsman. Efficacy was based upon the criteria outlined in Experiment 1.

To determine the dietary intake of protein, calcium and phosphorus and the components of the rations fed in each herd were collected and relative amounts consumed, recorded and submitted to the Ohio Ration Evaluation Laboratory for analyses. Selenium content of feeds was determined by similar sampling methods, in the laboratories of the Department of Dairy Science, Ohio Agricultural Research and Development Center, Wooster.

4.11 General Methods

Analytical procedures

Calcium and magnesium determinations were accomplished by atomic absorption spectroscopy using the procedure of Willis (121) with certain modifications (70). Plasma phosphorus determination was done by colorimetric analysis using a modified AOAC procedure (87).

Mineral content of feed samples other than selenium were determined by spectrographic analysis with an emission spectrograph.
Nitrogen determinations were done by AOAC procedures. Net energy values were estimated by methods outlined in OARDC Bull. No. 554 (88).

Selenium content of plasma and feeds were done by the method of Olson (81) with certain modifications (69).

4.12 Results

Experiment 1

Incidence of retained placenta was found to be significantly affected by protein intake, selenium, and selenium plus vitamin E. No significant effect was noted when retention rates were correlated to phosphorus intake, however.

Protein level in the diet was found to significantly affect retained placenta. Overall incidence of cows consuming the 8% crude protein (pens 1 and 2) ratio was found to be 33% as compared to 11% for the cows fed the 15% crude protein ratio (pens 3 and 4) (Table 3). Significant differences were also noted in the retention incidence of the control animals consuming the low protein diet (50%) when compared to the high protein controls (20%) (Table 4). No significant differences were observed between protein intake levels in treatment groups, however (Table 4).

Selenium supplementation significantly reduced overall incidence of retained placenta from 38% to 0% in this experiment (Table 5). Incidence in treated animals was similar to all groups (0%). Reduction in incidence in the low protein groups was significant, dropping from 50% for controls to 0% in treated animals (Table 4). A reduction in incidence in the high protein groups from 20% to 0% was observed but this effect was not significant, possibly due to the limited sample
### TABLE 3. Overall incidence of retained placenta in low and high protein fed parturient cows (experiment 1).

<table>
<thead>
<tr>
<th>Protein level</th>
<th>8%</th>
<th>15%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total incidence</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Total observations</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>Incidence, %</td>
<td>33</td>
<td>11</td>
</tr>
</tbody>
</table>

### TABLE 4. Incidence of retained placenta in treated and untreated parturient cows on low and high protein rations (experiment 1).

<table>
<thead>
<tr>
<th>Protein level</th>
<th>8%</th>
<th>15%</th>
</tr>
</thead>
<tbody>
<tr>
<td>- - - - - - (%) - - - - -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated incidence</td>
<td>0/8 = 0</td>
<td>0/7 = 0</td>
</tr>
<tr>
<td>Control incidence</td>
<td>8/16 = 50</td>
<td>2/10 = 20</td>
</tr>
</tbody>
</table>

### TABLE 5. Incidence of retained placenta in treated and untreated dry cows for experiment 1.

<table>
<thead>
<tr>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated incidence</td>
</tr>
<tr>
<td>Control incidence</td>
</tr>
</tbody>
</table>
population in pens 3 and 4 (Table 4). The prophylactic action of selenium was similar regardless of the route of supplementation and did not appear to be dependent upon the presence of vitamin E (Table 6).

No significant effect was noticed in incidence levels when compared on the basis of calcium and phosphorus intake (Table 6). Incidence of both control and treatment animals was identical when calcium and phosphorus intakes were compared within protein levels. A significant difference was noted in control cows on identical calcium and phosphorus diets when comparison of the intakes were made between protein levels, however (Table 6).

**Experiment 2**

The intramuscular injection of 50 mg of selenium and 680 units of vitamin E approximately 20 days prepartum significantly reduced overall incidence in the cooperating herds from 51.2% to 8.84% (Table 7). No significant difference in efficacy was noted between animals who received two injections, 40 and 20 days prepartum, as compared to a single 20 day prepartum injection, however (Table 8).

Significant reduction in the incidence was noted in all herds (Table 7).

**4.13 Discussion**

The highly significant reduction in incidence of retained placenta in treated animals of both the field trial (Table 7) and controlled experiment (Table 5) indicates that retained placenta is a selenium/vitamin E responsive disease. The results obtained further suggest that the observed prophylactic effect is possibly more significantly related to supplementation of selenium than of vitamin E,
TABLE 6. Incidence of retained placenta in pens 1, 2, 3, and 4 in treated and untreated parturient cows.

<table>
<thead>
<tr>
<th>Pen 1</th>
<th>Pen 2</th>
<th>Pen 3</th>
<th>Pen 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>8</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Calcium in diet</td>
<td>.65</td>
<td>.70</td>
<td>.66</td>
</tr>
<tr>
<td>Phosphorus in diet</td>
<td>.29</td>
<td>.70</td>
<td>.30</td>
</tr>
<tr>
<td>Treated incidence</td>
<td>0/4 = 0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/4 = 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/3 = 0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control incidence</td>
<td>4/8 = 50</td>
<td>4/8 = 50</td>
<td>1/5 = 20</td>
</tr>
</tbody>
</table>

<sup>a</sup>Two animals were supplemented intramuscularly with 50 mg of selenium and 680 IU of vitamin E, the remaining animals were orally supplemented with 100 mg of selenium.

<sup>b</sup>One animal was supplemented intramuscularly as outlined in (a), the remaining animals were orally supplemented as in (a).

TABLE 7. Incidence of retained placenta in treated and control parturient cows by farm.

<table>
<thead>
<tr>
<th>Incidence by Farm</th>
<th>Incidence</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>Franchester Farms</td>
<td>6/53 = 11.3</td>
<td>16/39 = 41.0</td>
</tr>
<tr>
<td>Joseph Wedding</td>
<td>4/37 = 10.8</td>
<td>12/23 = 52.0</td>
</tr>
<tr>
<td>Monroe Pyles</td>
<td>0/14 = 0</td>
<td>7/9 = 77.7</td>
</tr>
<tr>
<td>John Young</td>
<td>0/9 = 0</td>
<td>6/9 = 66.6</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10/113 = 8.8</td>
<td>41/80 = 51.2</td>
</tr>
</tbody>
</table>
TABLE 8. Efficacy of A) two injections of 50 mg of selenium and 680 IU of vitamin E 40 and 20 days prepartum and B) a single injection of 50 mg of selenium and 680 IU of vitamin E 20 days prepartum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td>90.0</td>
</tr>
<tr>
<td>Treatment B</td>
<td>91.9</td>
</tr>
</tbody>
</table>

TABLE 9. Selenium content of the dry cow ration of cooperating farms.

<table>
<thead>
<tr>
<th>Name</th>
<th>Selenium (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Franchise Farms</td>
<td>.04</td>
</tr>
<tr>
<td>Joseph Wedding</td>
<td>.02</td>
</tr>
<tr>
<td>Monroe Pyles</td>
<td>.035</td>
</tr>
<tr>
<td>John Young</td>
<td>--</td>
</tr>
</tbody>
</table>
and as such, the incidence of retained placenta may be a clinical expression of a selenium deficiency in the mature dairy cow.

The prophylactic value of selenium and vitamin E in placental membrane retention is significant. Strong evidence in support of this conclusion is indicated by the field trial results. The 20 day prepurtnum injection of 50 mg of selenium and 680 IU of alpha tocopherol successfully controlled placental retention in herds in which the disease had been a serious and chronic problem. The incidence of the control groups in each herd is typical of the past history of retention for that particular herd (Table 7). Aside from the injection, there were no changes in nutritional or herd health management that would account for the sudden, significant reduction in retention incidence. The field trial results verify the conclusions reached during the controlled experiment, that retained placenta is responsive to selenium and vitamin E.

The inability to demonstrate a selenium deficiency in the mature dairy cow has cast some doubt as to the dietary requirement of this microelement in dairy cattle nutrition. The results of these experiments show, however, that retained placenta is selenium and vitamin E responsive. Furthermore, there is evidence that the favorable prophylactic response observed in these experiments is mediated through selenium supplementation. In the controlled experiment, only 5 of the 15 animals treated with selenium received additional vitamin E supplementation, yet no difference in efficacy was noted between the vitamin E supplemented and unsupplemented treatment cows (Table 1). The amount of biologically active selenium which was administered in
the vitamin E unsupplemented group was calculated, based upon the data of Waite et al. (117) to be 20.36 mg. This is not significantly different than the 26.3 mg of selenium estimated to be the amount that was biologically available to the cows receiving the vitamin E supplementation. Thus the 680 IU of vitamin E which was given in addition to the selenium in the 5 treated animals apparently was unnecessary to afford protection with these basal rations. This contention is further supported by the significant difference in retention incidence between the control groups of the high and low protein fed groups as the cows of both groups consumed an average of 60% of their dry matter as corn silage and 40% as concentrate. In such a ration the primary source of tocopherol would be in the silage. Thus these animals can be expected to consume approximately equal amounts of alpha tocopherol. However, incidence in the low as compared to the high protein fed controls was significantly different (50% as compared to 20%). The low incidence observed in the high protein group can possible be explained on the basis of the differences in selenium consumption as reflected by protein intake. The high protein fed cows consumed approximately 6.1 mg of selenium per head per day in the ration, 40% of which was contained in the protein in the concentrate (soybean meal, 44%). The low protein group consumed approximately 3.5 mg of selenium per head per day, which is half the amount consumed by the high protein group. Based upon Waite et al.'s (117) data, the total amount of biologically active selenium contributed by the ration would be 1.2 mg per day or more in the high protein group and .7 mg per day or less in the low protein group, this two-fold difference favoring the high
protein fed cows, although unable to entirely alleviate an apparent deficiency condition as indicated by the low blood selenium levels of the control (average .03 ppm) high protein cows, apparently had some sparing effect on incidence of retained placenta. This amount, however, is still inadequate to provide complete protection as indicated by the 20% incidence which is still significantly high when compared to the normal incidence of retained placenta in the dairy cow (10%) or when compared to the selenium treated cows who received complete protection through supplementation.

In the field trial, dietary management of the cooperating herds was very similar to that used in the controlled experiment, thus alpha tocopherol intake was relatively similar. The low selenium values of the feeds sampled on each of the farms (Table 9) are reflected in the blood values of sampled cows (.035 ppm). These values are not significantly different to the basal blood values of the cows in the controlled experiment prior to treatment. The significant response to treatment in all herds is further evidence that selenium supplementation in animals where this nutrient is limiting was beneficial.

There has been a belief among nutritionists that retained placenta was in some way related to calcium and phosphorus metabolism (76). The lack of a significant difference in incidence between mineral intake levels within protein groups (pen 1 compared to pen 2, pen 3 compared to pen 4; Table 6) in both control and treated animals would indicate that this is unfounded. The significant reduction in incidence in all cases is significantly correlated with selenium supplementation, and would appear incidental to calcium and phosphorus intake.
4.14 Conclusion

Retained placenta in the parturient dairy cow can be effectively controlled by either an intramuscular injection of 50 mg of selenium and 680 IU of vitamin E or 100 mg of orally supplemented selenium alone, when given approximately 21 days prepartum. This indicates that retained placenta is a selenium/vitamin E responsive disease, and that selenium alone is sufficient if given in sufficient quantity.

The correlation between supplementation of selenium and reduction in incidence, plasma selenium values in supplemented and unsupplemented cows in relation to incidence, and the inverse relationship between selenium content in the diet and retention incidence, all indicate that retained placenta is an expression of a selenium deficiency in the mature dairy cow. A failure to correlate retained placenta incidence with any other variable, i.e. dietary calcium and phosphorus, aside from protein intake which increased selenium intake, further supports this conclusion.
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the intake of selenium and vitamin E on the incidence of retained

of vitamin E and selenium on the incidence of retained placentae


uterine motility - normal and after experimentally produced re-

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ponents. I. Lipid peroxide formation in the endoplasmic reten-

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serum by atomic absorption spectroscopy. I. Calcium. Spectro-
chemi Acta 16:259.

11:355.
APPENDIX TABLE 1. Index to cooperators in the vitamin D₃ field trial conducted September 17, 1974 to June 1, 1975.

<table>
<thead>
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<th>Name</th>
<th>Location</th>
<th>County</th>
<th>Breed</th>
<th>ID No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Westbrook Farms</td>
<td>Seville</td>
<td>Medina</td>
<td>Holstein</td>
<td>1</td>
</tr>
<tr>
<td>Zimmerman's Jerseys</td>
<td>Salem</td>
<td>Columbiana</td>
<td>Jersey</td>
<td>2</td>
</tr>
<tr>
<td>Welcome View Farms</td>
<td>Salem</td>
<td>Columbiana</td>
<td>Jersey</td>
<td>3</td>
</tr>
<tr>
<td>G. R. Santee</td>
<td>Beloit</td>
<td>Mahoning</td>
<td>Jersey</td>
<td>4</td>
</tr>
<tr>
<td>J. A. Rhodes</td>
<td>Salem</td>
<td>Columbiana</td>
<td>Jersey</td>
<td>5</td>
</tr>
<tr>
<td>C. D. Irey</td>
<td>Lisbonville</td>
<td>Columbiana</td>
<td>Jersey</td>
<td>6</td>
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<tr>
<td>Franchester Farms</td>
<td>West Salem</td>
<td>Wayne</td>
<td>Guernsey</td>
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<tr>
<td>Edindell Farms</td>
<td>Streetsboro</td>
<td>Portage</td>
<td>Holstein</td>
<td>8</td>
</tr>
<tr>
<td>Dan Harper</td>
<td>Mantua</td>
<td>Portage</td>
<td>Holstein</td>
<td>9</td>
</tr>
<tr>
<td>Wendrol Farms</td>
<td>Streetsboro</td>
<td>Portage</td>
<td>Guernsey</td>
<td>10</td>
</tr>
<tr>
<td>Oliverdale Farms</td>
<td>Randolph</td>
<td>Portage</td>
<td>Holstein</td>
<td>11</td>
</tr>
<tr>
<td>Wendell Sediler</td>
<td>Palmyra</td>
<td>Portage</td>
<td>Holstein</td>
<td>12</td>
</tr>
<tr>
<td>Don McCarthy</td>
<td>Nelson</td>
<td>Portage</td>
<td>Holstein</td>
<td>13</td>
</tr>
<tr>
<td>Ruparts Jerseys</td>
<td>New Waterford</td>
<td>Columbiana</td>
<td>Jersey</td>
<td>14</td>
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<tr>
<td>Eastview Dairy</td>
<td>Canfield</td>
<td>Columbiana</td>
<td>Holstein</td>
<td>15</td>
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<tr>
<td>David Weaver</td>
<td>Columbiana</td>
<td>Columbiana</td>
<td>Holstein</td>
<td>16</td>
</tr>
<tr>
<td>Ohio State University</td>
<td>Columbus</td>
<td>Franklin</td>
<td>Ayrshire</td>
<td>17</td>
</tr>
</tbody>
</table>
APPENDIX TABLE 2. Index to cooperating veterinarians in the vitamin D₃ field trial conducted September 17, 1974 to June 1, 1975.

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. D. L. Noah</td>
<td>Smithville, Ohio</td>
</tr>
<tr>
<td>Dr. J. D. Liggett</td>
<td>Salem, Ohio</td>
</tr>
<tr>
<td>Dr. J. Richardson</td>
<td>Ravenna, Ohio</td>
</tr>
<tr>
<td>Dr. A. Dimick</td>
<td>Ravenna, Ohio</td>
</tr>
<tr>
<td>Dr. R. Urmsen</td>
<td>Columbiana, Ohio</td>
</tr>
<tr>
<td>Dr. T. Hensen</td>
<td>The Ohio State University</td>
</tr>
<tr>
<td></td>
<td>College of Veterinary Medicine</td>
</tr>
</tbody>
</table>
APPENDIX TABLE 3. Incidence by farm of parturient paresis in vitamin D$_3$ treated and non-treated cows as influenced by calcium and phosphorus intake of previous history.

<table>
<thead>
<tr>
<th>Farm No.</th>
<th>Breed</th>
<th>Ca Intake (g)</th>
<th>P Intake (g)</th>
<th>No previous history Treated</th>
<th>Control</th>
<th>Previous history Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Holstein</td>
<td>113.0</td>
<td>43.6</td>
<td>3/6$^a$</td>
<td>2/5$^a$</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Jersey</td>
<td>26.9</td>
<td>32.7</td>
<td>2/2</td>
<td>2/4</td>
<td>1/2$^a$</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Jersey</td>
<td>43.3</td>
<td>28.7</td>
<td>3/5</td>
<td>4/9</td>
<td>2/6</td>
<td>1/3</td>
</tr>
<tr>
<td>4</td>
<td>Jersey</td>
<td>45.8</td>
<td>31.0</td>
<td>0/1</td>
<td>1/4</td>
<td>1/1</td>
<td>3/3</td>
</tr>
<tr>
<td>5</td>
<td>Jersey</td>
<td>59.7</td>
<td>40.9</td>
<td>1/2</td>
<td>0/3</td>
<td>2/3</td>
<td>1/1</td>
</tr>
<tr>
<td>6</td>
<td>Jersey</td>
<td>40.9</td>
<td>16.3</td>
<td>0/2</td>
<td>0/3</td>
<td>0/3</td>
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<tr>
<td>7</td>
<td>Guernsey</td>
<td>85.0</td>
<td>58.9</td>
<td>0/1</td>
<td>0/4</td>
<td>2/2</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>Holstein</td>
<td>84.9</td>
<td>40.1</td>
<td>1/3</td>
<td>0/3</td>
<td>0/1</td>
<td>--</td>
</tr>
<tr>
<td>9</td>
<td>Holstein</td>
<td>66.0</td>
<td>40.1</td>
<td>0/3</td>
<td>2/6</td>
<td>0/4</td>
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</tr>
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<td>10</td>
<td>Guernsey</td>
<td>109.0</td>
<td>33.8</td>
<td>0/7</td>
<td>6/18</td>
<td>0/4</td>
<td>2/3</td>
</tr>
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<td>11</td>
<td>Holstein</td>
<td>64.9</td>
<td>44.8</td>
<td>0/6</td>
<td>2/7</td>
<td>1/1</td>
<td>--</td>
</tr>
<tr>
<td>12</td>
<td>Holstein</td>
<td>41.3</td>
<td>37.7</td>
<td>1/8</td>
<td>0/5</td>
<td>0/2</td>
<td>--</td>
</tr>
<tr>
<td>13</td>
<td>Holstein</td>
<td>80.2</td>
<td>48.3</td>
<td>1/5</td>
<td>1/4</td>
<td>0/2</td>
<td>--</td>
</tr>
<tr>
<td>14</td>
<td>Jersey</td>
<td>81.8</td>
<td>33.5</td>
<td>4/12</td>
<td>4/14</td>
<td>2/7</td>
<td>--</td>
</tr>
<tr>
<td>15</td>
<td>Holstein</td>
<td>66.0</td>
<td>35.4</td>
<td>0/5</td>
<td>0/4</td>
<td>0/2</td>
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</tr>
<tr>
<td>16</td>
<td>Holstein</td>
<td>36.5</td>
<td>59.0</td>
<td>0/3</td>
<td>1/6</td>
<td>1/3</td>
<td>1/1</td>
</tr>
<tr>
<td>17</td>
<td>Ayrshire</td>
<td>52.1</td>
<td>27.2</td>
<td>0/11</td>
<td>0/6</td>
<td>0/3</td>
<td>0/4</td>
</tr>
</tbody>
</table>

$^a$Incidence over total observations.
APPENDIX TABLE 4. Ingredients list for grain mixes D-401, D-402, D-403, and D-404.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>D-401 (%)</th>
<th>D-402 (%)</th>
<th>D-403 (%)</th>
<th>D-404 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, rolled</td>
<td>67.128</td>
<td>64.578</td>
<td>15.798</td>
<td>16.228</td>
</tr>
<tr>
<td>Corn cobs, ground</td>
<td>26.29</td>
<td>25.33</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>--</td>
<td>--</td>
<td>69.82</td>
<td>70.45</td>
</tr>
<tr>
<td>Molasses, Sweetone</td>
<td>1.23</td>
<td>1.37</td>
<td>7.9</td>
<td>2.12</td>
</tr>
<tr>
<td>Dynamate</td>
<td>1.23</td>
<td>1.37</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Biofos</td>
<td>--</td>
<td>5.55</td>
<td>5.98</td>
<td>10.7</td>
</tr>
<tr>
<td>Salt, trace mineralized</td>
<td>.5</td>
<td>.5</td>
<td>.5</td>
<td>.5</td>
</tr>
<tr>
<td>Vitamin A-30</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
<td>.001</td>
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<tr>
<td>Limestone</td>
<td>3.62</td>
<td>1.30</td>
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APPENDIX TABLE 5. Index to cooperators in the selenium/vitamin E field trial conducted June 1, 1975 to February 1, 1976.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Location</th>
<th>County</th>
<th>Breed</th>
<th>I.D. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Franchester Farms</td>
<td>West Salem</td>
<td>Wayne</td>
<td>Guernsey</td>
<td>1</td>
</tr>
<tr>
<td>Joseph Wedding</td>
<td>Raymond</td>
<td>Union</td>
<td>Holstein</td>
<td>2</td>
</tr>
<tr>
<td>Monroe Pyles</td>
<td>Cedarville</td>
<td>Greene</td>
<td>Holstein</td>
<td>3</td>
</tr>
<tr>
<td>John Young</td>
<td>Ragersville</td>
<td>Tuscarawas</td>
<td>Holstein</td>
<td>4</td>
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</tbody>
</table>