1969

The Metabolic Behavior of Inorganic Elements in Calves With Subclinical Ostertagiasis.

Lester Barrett Waymack

*Louisiana State University and Agricultural & Mechanical College*

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WAYMACK, Lester Barrett, 1937-
THE METABOLIC BEHAVIOR OF INORGANIC ELEMENTS IN CALVES WITH SUBCLINICAL OSTERTAGIASIS.

The Louisiana State University and Agricultural and Mechanical College, Ph.D., 1969
Agriculture, animal culture

University Microfilms, Inc., Ann Arbor, Michigan
THE METABOLIC BEHAVIOR OF INORGANIC ELEMENTS IN CALVES WITH SUBCLINICAL OSTERTAGIASIS

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Animal Science

by

Lester Barrett Waymack
B.S., University of Arkansas, 1960
M.S., Mississippi State University, 1963
August, 1969
ACKNOWLEDGEMENT

The author would like to express gratitude to Drs. S. L. Hansard and R. H. Klett for their advice and assistance throughout the course of these studies.

He also wishes to express appreciation to the following: Miss Virginia Gordon for her assistance in the laboratory; Mr. Lawrence Ned, Jr. in caring for experimental animals; Dr. Prentiss Schilling in aiding with the statistical treatments; the LSU Department of Veterinary Science for permission to use the experimental data; Drs. C. K. Vincent and T. E. Patrick for serving as committee members; Miss Lynn Pizzuto for assistance in compiling data and Mrs. Roy Smith and Mrs. Carolyn Pulaski for typing the manuscript.

Very special thanks are due Mrs. Betty J. Torbert, without whose tireless efforts and vigorous interest in every aspect of the study, much of the data collection would have been impossible. The concern and encouragement of Dr. George Robertson was a vital stimulus for the completion of this graduate study program.

The author is indebted to: his father, for his willingness to sacrifice; his mother, for her undying faith; his wife, for her patience and understanding and his daughter, for giving it all purpose.
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ABSTRACT

The effect of medium stomach worms on the utilization of calcium, phosphorus, and magnesium in weanling Holstein bull calves was studied using single experimental infections of cultured larvae of the parasitic nematode Ostertagia spp. Established infections were classed as subclinical based on moderate increases in fecal moisture patterns and changes in hematocrit values. A worm load of 65,000 infective larvae resulted in a noticeable impairment of phosphorus utilization while an infection of \( \frac{9}{75} \) 000 larvae caused a transient decrease in the phosphorus balance. With the heavier parasite burden, there was a decrease in plasma inorganic phosphorus which appeared to reciprocate an increase in the bound fraction. The changes observed in plasma bound phosphorus were reflected by similar changes in the total plasma phosphorus. The possibility of chelation was postulated. At both infection levels there was a decline in blood calcium. During the lighter infection, a hypercalcemia preceded the drop in blood calcium. Fecal excretion of calcium increased with time post-infection and the calcium balance decreased. Plasma magnesium maintained values between 23 to 25 ppm in both infection levels. Fecal and urinary magnesium excretion increased with time after infection, but was not reflected by pronounced changes in balance ratios. Results of the initial studies indicated that subclinical ostertagiasis adversely affected calcium and phosphorus utilization, but had no significant effect on magnesium utilization in weanling
calves. Several mechanisms were postulated and formed the basis of subsequent investigations. Radiocalcium (Ca-45) and radiophosphorus (P-32) were employed using comparative balance methods to ascertain the influence of the parasite on endogenous metabolism. Although calcium balance ratios decreased and the secretion of the intravenous tracer dose increased after inoculation, there was no detectable adverse effects on endogenous calcium metabolism. Phosphorus balance showed a substantial decline in one trial where fecal egg counts were high, and the rate of secretion of the intravenous dose of P-32 was consistently lower in calves before they became parasitized. An attempt to create a diarrhetic condition in calves similar to that caused by parasitic infections was not totally successful using cascara segrata parenterally. The desired degree of diarrhea could not be satisfactorily maintained, and the calves appeared to become refractory to the drug. The efficiency of mineral utilization generally increased during drug treatment. Results of these studies did not support the postulation that oster-tagiasis adversely affected the permeability of the abomasal mucosa. Preliminary observations suggested that dietary calcium level had an effect on the development of the nematode within the host. Calves on 0.03% dietary calcium level showed decreases in number of nematode ova voided in the feces, average number of mature larvae in the abomasum and calcium concentration in the tissues of mature and immature larvae.
CHAPTER I

INTRODUCTION

Economic losses due to stomach worms have been recognized for several years as a serious problem to the livestock producer. One of the aspects of gastro-intestinal parasitism that frequently goes unnoticed is the long-term residual effect. This may be prolonged, causing unthriftiness lasting many months, so that full recovery from an infection may not be complete until several weeks after the worm has been removed from the host.

_Ostertagia spp._ appear to be among the most important species associated with morbidity and mortality when field cases of bovine helminthisms are encountered in Louisiana. The exact mode of the detrimental action to the host has yet to be fully elucidated. The nature of the damage can only be speculated upon by observing changes produced in the host. Clinical cases reported from the field attest to the various syndromes which as yet remain unexplained.

Most of the research involving host-parasite relationships has been done on sheep with a lesser amount being done on cattle and goats. Little work has been done specifically with _Ostertagia spp._ Considerably less work has been done on the influence of the parasite on bovine mineral metabolism. This study was designed to examine the effects on the mineral metabolism of calves which had received single infections of gastro-intestinal parasites. Specifically it was desired to determine if abomasal dysfunction alters absorption and subsequent balance of the various inorganic minerals necessary for maintenance and growth.
Stewart (1933) gave a comprehensive outline of various theories of how parasites exerted their adverse effects on the host. He stated that the very inconclusive opinions which existed stimulated his studies on the effects of nematode infestations on the metabolism of the host. He correlated fluctuations in fecal ova numbers to changes in digestion coefficients of crude protein, ether extract and nitrogen-free extract (NFE) using lambs infected with a mixture of parasitic nematodes. *Haemonchus contortus* was the predominant species of the mixed infection. It was observed that as the worm infestation decreased, the digestibility of crude protein increased. As far as could be discerned, ether extract and NFE bore little relationship to the degree of nematode infestation.

Shearer and Stewart (1933) studied the effects of a mixed infestation of nematodes on the mineral metabolism of lambs. These workers reported a very noticeable inverse relationship between the number of nematode eggs in the feces and the balance of calcium and phosphorus. From this it was concluded that nematode infestations interfered with the calcium and phosphorus metabolism. They further stated that a substance had been extracted from the nematodes of sheep which was shown by *in vitro* experiments to inhibit the action of pepsin.

Bosworth and Stewart (1933) investigated the influence of heavy parasite infestations on bone growth and density in sheep of Northern England. Four young sheep which were very debilitated and heavily infected with parasites were divided into two groups. One group was
killed immediately. The second group was drenched with copper sulfate and placed on pasture. Four months later they were killed and their bones were compared with those of the first group. It was found that in the second group there had been a corresponding improvement in the condition of all the bones which had become more dense and appeared macroscopically to be practically normal. Changes in the bones of the infected group were described as atrophy and osteoporosis due to diminished activity on the part of the bone-forming elements.

Neal (1931) found that different planes of nutrition induced changes in the skeletal structure of cattle, and that phosphorus deficiency caused a decrease in the amount of phosphate in the skeleton. Moreover, although they could not definitely prove that a calcium deficiency could also cause a decrease in the phosphate content of the bones, they were inclined to believe that this was so.

The effects of the nematode Cooperia curticei on the nutrition of lambs was studied by Andrews (1938). The results did not confirm the finding of Stewart (1933) that there was a depression of the digestibility of crude protein. Andrews (1938) also reported no evidence to support the contention of Shearer and Stewart (1933) that there was a decrease in the ability of the infested lambs to store calcium and phosphorus. There was further discord with earlier theories that parasites elaborate an anti-enzyme in quantities sufficient to interfere with the digestive processes of the host. However, the investigation showed that infestations with relatively non-pathogenic nematodes decreased the ability of the infested lambs to convert their feed into gain in weight, even when
the lambs were in excellent nutritional condition and showed no clinical symptoms of parasitic infestation.

According to Andrews (1938), evidence was presented indicating that decreased efficiency of nutrient utilization in parasitic infections could be attributed to increased energy metabolism. The increase in energy metabolism was accounted for by nervous excitation of the host due to irritation of the intestinal mucosa by the worms.

In later work by Andrews et al. (1944) it was reported that "The presence of Trichostrongylus colubriformis, even in considerable numbers, did not produce symptoms of gastrointestinal parasitism or alter the digestibility coefficients of the ingested feed; it did affect the ability of the host to utilize the feed economically."

In one lamb where nematodes were present in sufficient numbers to produce severe and prolonged diarrhea, they caused a decrease in the digestibility coefficients and absorption of the different constituents of the feed; this was brought about because the vitality of the infected lamb was reduced to the point where the physiological processes, necessary for life, could no longer operate effectively.

Franklin et al. (1946) carried out metabolism trials on infected and control sheep in which the feed intake was kept at the same level. They found that moderate infections of Trichostrongylus colubriformis resulted in a slower live-weight gain, a depression of the digestibility coefficients for crude protein and a relatively large drain of calcium and phosphorus through the feces. Even when the intake of the two minerals was raised, the net absorption by parasitized animals was appreciably less than in controls. The difference in
mineral utilization was also seen in the blood picture in that declines in serum calcium, magnesium and phosphorus occurred and these were not attributable to a lowered feed intake. These workers considered it possible for death to have been accelerated by the mild-to-severe hypocalcemia observed at the terminal stages of the disease.

Spedding (1954) studied the effects of a sub-clinical worm burden on the digestive efficiency of sheep artificially infected with Trichostrongylus axei. A significant depression in dry matter digestibility coefficient was found, and though the apparent digestibility of crude protein was significantly lowered, the crude fiber fraction appeared to be unaffected. Infected lambs showed an 8% depression of appetite and with the lowered digestive efficiency, this meant an infected animal absorbed 10% less food than a worm-free lamb. The administration of 48,200 T. axei larvae induced a peak egg output of approximately 500 eggs per gram of feces. It was pointed out that although there was a measurable effect on the digestive efficiency it was only slightly less than that in worm-free lambs.

Herlich (1962) collected data on calves experimentally infected with combinations of Ostertagia spp., Trichostrongylus spp. and Cooperia spp. nematodes. Infected calves developed a pronounced hypophosphatemia, hypoglycemia and hypoproteinemia. These were coincident with or occurred shortly before the onset of diarrhea and anorexia. In addition to the decrease in total serum protein, there was a drop in the albuminglobulin ratio because of a fall in albumin and a rise in alpha and gamma globulins. There was no evidence of
anemia or serum calcium disturbances. Calves became inappetent, passed mushy to fluid feces, and lost weight or gained at a poorer rate than the uninfected controls.

Shumard (1957) studied the effect of the nematode Trichostrongylus axei on the utilization and excretion of certain mineral elements by lambs. Twenty-four-hour consumptions and excretions (via urine and feces) of sodium, potassium, calcium, phosphorus, and protein nitrogen of control and infected lambs were tabulated for 26 days. Results indicated that the infected animals maintained a relatively high sodium level while protein nitrogen approached, and in some cases exceeded, consumption levels.

Physiological and nutritional changes in lambs infected with a combination of four gastrointestinal nematodes were investigated by Shumard et al. (1957). Feed consumption decreased as the infections progressed. Weight losses were rapid. Water consumption per pound of lamb did not appreciably decrease until debility occurred. Moisture content of the feces varied with the type and severity of the infection, although fecal pH did not vary greatly. Hyperglycemias with corresponding serum hypophosphotemias and depressions in total serum protein took place in the blood of all infected lambs. Digestibility of protein gradually diminished while digestibility of crude fiber fluctuated widely as the infection progressed.

Earlier studies by Shumard and Eveleth (1955) supported the blood patterns of phosphorus and glucose in lambs infected with mixed nematode infections. It was reported that inorganic phosphorus levels dropped more than 50% in all infected lambs, then
made slight recoveries. Blood glucose levels of the infected lambs fluctuated greatly with a tendency toward a higher level.

Mahrt et al. (1964) observed that calves experimentally infected with larvae of *Ostertagia ostertagi* showed a significant decrease in serum albumin with a concurrent rise in gamma globulin. The changes occurred about three weeks after infection. There was little change in the total serum protein, hemoglobin, packed cell volume or total leucocyte values. Infected calves gained less weight than controls and heavily infected calves killed 68 to 81 days after infection had a moderate gastritis.

Horak and Clark (1964) reported investigations into the pathological physiology of *Ostertagia circumcincta* infestation of lambs. The main findings were loss of appetite, an apparent decrease in nitrogen absorption, anemia, hypoproteinemia with a sharp drop in plasma albumin and gamma globulin. A sudden drop in plasma inorganic phosphate was also observed.

Experimental *Trichostrongylus colubriformis* infections in the 15-20 week old goat were studied by Fitzsimmons (1966). Clinical symptoms were acute inappetence and weakness. There was no anemia, but hemoconcentration and neutrophilia occurred before death with a decrease in serum protein, calcium, magnesium and phosphorus.

**LIFE CYCLE OF OSTERTAGIA spp.:**

The species of *Ostertagia* are parasites of the abomasum of sheep, goats, cattle and other ruminants. Because of their color, they are known collectively as the brown stomach worm (Todd et al.,
Medium stomach worm has also been used synonymously with *Ostertagia spp.* They are smaller than the twisted stomach worm (*Haemonchus spp.*) and different in structure (Olsen, 1962).

Eggs passed in the feces complete their development in moist situations. The 1st-stage larvae hatch, feed and molt to form 2nd-stage individuals. After a short period of feeding they molt, forming 3rd-stage larvae ensheathed in the cuticle of the 2nd stage. The loose-fitting sheath protects the infective larvae against desiccation. They are active climbers, ascending blades of grass during the dim light of mornings and evenings, and on overcast days. Infection of the host occurs when the larvae are ingested with forage.

While the larvae are passing through the forestomachs, the cuticle enclosing them is lost. Upon reaching the abomasum, they undertake a minor migration into the mucosa for a short period. About 12 hours after being swallowed the larvae appear on the surface of the mucosa, migration begins and after 36 hours most of the larvae are embedded in the mucosa at the level of the gastric pits. The 3rd molt takes place in the tissue, following which the 4th-stage larvae return to the surface of the mucosa. The final molt occurs and the worms develop to sexual maturity in about 17 to 20 days following entrance into the host. Eggs may be detected in the feces of the host in about 18 to 21 days after ingestion of the 3rd-stage larvae.
Figure 1 is somewhat illustrative of the establishment of a patent infection by observing the number of eggs being voided in the feces of the host. A few eggs may be detected earlier, but around the 21st day after inoculation a very marked increase in egg production may be noted. The height of fecal egg production and the manner in which it returns to an almost constant value (in experimental animals) depends on the severity of the infection. Severity in turn is influenced by the number and manner in which infective larvae are administered. A given number of larvae have a greater chance of maturing more adults if they are divided and given in several consecutive daily doses.
FIGURE 1. ESTABLISHMENT OF PATENT INFECTION OF OSTERTAGIA spp. ACCORDING TO EGGS PER GRAM FECES
GENERAL EXPERIMENTAL PROCEDURE

This study consisted of 5 phases. Each of the first 4 phases was composed of 2 trials in replicate. The trial in the 5th phase was not replicated.

Holstein bull calves were used throughout the course of this study. They were removed from the cow at or near 1 week of age and maintained on a milk supplement for at least 4 weeks. Subsequently the calves were weaned onto a basal ration containing 20% roughage as cottonseed hulls and 16% protein.

Each time a ration was mixed, it was given a code number. All calves selected for a given trial were assigned to a ration number and maintained on that ration for the duration of the trial. This was an effort to minimize the effects of possible ration differences within a particular trial. The same basal formulation was used in all trials (Appendix VII).

BALANCE PERIODS:

The period of confinement of calves to metabolism units for a given balance was 6 days in order to provide at least five 24-hour collections of excreta. At the end of the 6th day, animals were removed from units and allowed to exercise and rest in holding pens until the end of the 7th day. Short balance periods were used due to the fact that several balance periods were carried out in succession. By using shorter collection periods, interrupted by approximately 36 hours of rest, it was assumed that the animals would not become fatigued by their confinement.
Prior to each phase of the study in which balance data were to be collected, the animals were maintained in metabolism units for a 4 to 5-day conditioning period. Feeding of the experimental ration was initiated at least 2 weeks before data collection began in order to minimize any effects of an abrupt change in diet.

**MINERAL DETERMINATIONS:**

Details of phosphorus analyses are given in Appendix tables I and II. Inorganic (free) phosphorus in blood serum was determined by first precipitating the protein with trichloroacetic acid. The filtrate was then reacted with ammonium molybdate and ferrous sulfate and the subsequent blue color produced was measured photometrically on a Spectronic 20 (B & L) spectrophotometer.

Total phosphorus was determined employing the same principle involved in the reaction of phosphorus and molybdenum except the samples were completely ashed in a muffle furnace at 625°C then brought to a 10-ml volume with 6N HCl before analyses.

Bound phosphorus was calculated as the difference between total and free phosphorus.

Calcium and magnesium were determined (Appendix IV) by atomic absorption spectrophotometry (Perkin-Elmer 303). Sample source was from the same 10-ml ashed sample in 6N HCl from which total phosphorus was determined.

Peculiarities in mineral analyses are outlined in more detail in the procedures of each phase.
MAINTENANCE AND CULTURE OF NEMATODE LARVAE:

The source of the infective larvae used in these studies was a yearling Holstein steer (seed animal) harboring a pure infection of Ostertagia spp. To prepare an inoculum of infective larvae, feces which contained nematode ova from the seed animal were collected in a large metal tray beneath the elevated cage housing the steer. Each 24-hour manure collection was uniformly mixed with vermiculite, placed in suitable containers covered with perforated aluminum foil and incubated for 10 days at 27°C. The purpose of the vermiculite (particle size would pass through a U.S. Standard No. 8 sieve) was to increase surface area and air circulation through the culture.

After 10 days, larvae which had hatched from the eggs in the fecal cultures could be recovered by transferring the culture mixture to a cheesecloth bag. The size of the bag approximated 1 liter and the walls were triple layers of the cloth. A funnel, 12 inches in diameter, was filled with warm water. A pinch clamp at the rubber tip of the funnel prevented water loss. The bag with the culture was then submerged in the funnel of warm water for 6 to 8 hours.

The 3rd-stage larvae, activated by the temperature increase, worked their way through the walls of the cheesecloth bag and settled in the tip of the funnel above the pinch clamp. Recovery of the larvae was facilitated by opening the pinch cock and collecting 50 to 75 ml of liquid. Once the larvae had been recovered, the culture bag was discarded and the funnel was boiled in water for 30 minutes and stored until further use.
The media were cleared by transferring to a second funnel of warm water and recovering after 1 hour. This prevented stagnation from lowering the viability of the harvested larvae. All larvae prepared in this way were pooled in a refrigerated glass container until their concentration could be determined and at which time they were ready to be inoculated into some specified calf.

Concentration of infective larvae was determined by first stirring the worm-containing medium, withdrawing exactly 1 ml and counting under a dissecting microscope with a hand tally. If the 1st volume was too concentrated for accurate counting, a 1:10 dilution was prepared and a 1.0 ml aliquot of the dilution was counted. Once the concentration of the stock medium had been ascertained, the degree of infection for each dosed animal could be more closely approximated.

**DETERMINATION OF FECAL EGG COUNTS:**

Nematode eggs per gram of feces (EPG) were determined by a flotation method. Two to 4 grams of feces were placed in a small mixing cup and stirred with 10 ml water. Excess fiber was separated by pouring through a 24-mesh sieve. The fiber was discarded and the liquid was centrifuged for 10 minutes at 2000 rpm in a 15-ml conical tube. Following centrifugation the supernatant was discarded and 5 to 7 ml of sucrose syrup (Sp. Gr. 1.13) was added to the tube and thoroughly mixed on a cyclomixer via vortex motion. The tube was then filled level to the rim with the sucrose syrup; a cover slip
was placed on top of the tube in such a manner as to prevent the presence of air bubbles under the cover slip. After another 10-minute centrifugation at 1200 rpm, the cover slip containing the ova (a Sp. Gr. of 1.18 allows the debris to settle to the bottom of the tube while the lighter ova tend to rise) was carefully transferred to a slide for microscopic examination.

The magnification of the scope varied according to the concentration of eggs in a field. Larger numbers of eggs necessitated higher magnification thus reducing the number of ova per field. Recordings were made with a hand tally.

**STATISTICAL ANALYSES:**

Analyses of variance of the various regression coefficients are detailed in Appendix table VI.
CHAPTER II

THE EFFECT OF OSTERTAGIASIS ON MINERAL BALANCES
OF THE WEANLING CALF

A comprehensive review of nutritional host-helminth relationships was prepared by Hunter (1953). Since that time nutritional and physiological aspects of host-parasite relationships have been investigated only to a limited extent. Most of the data in the literature were collected from sheep and quite often the nematodes studied were of the genera *Trichostrongylus spp.* and *Haemonchus contortus*. A considerable amount of this work was performed in Australia, New Zealand, The British Isles and other foreign countries, with only limited work being reported by U.S. workers.

Little information is available concerning the effect of parasitism on mineral metabolism. This applies specifically to the nematode parasite *Ostertagia spp.* and its effect on mineral metabolism of its bovine host.

The purpose of this phase of investigation was to ascertain whether or not *Ostertagia spp.* could influence the utilization of calcium, phosphorus and magnesium of weanling calves.

EXPERIMENTAL PROCEDURE

Holstein bull calves were maintained on the basal ration (Appendix VII) after having been weaned from milk supplement. Once the calves had become adjusted to the ration, blood samples
were taken twice weekly for approximately 4 weeks. Hemoglobin levels were recorded each time, and after centrifuging and determining packed cell volume, the plasma from each sample was stored in a freezer until chemical analyses could be completed.

Details of the photometric analyses of total and inorganic phosphorus are outlined in Appendix tables I and II, respectively. Bound phosphorus was calculated as the difference between the total and free fractions.

The analyses of calcium and magnesium by atomic absorption spectrophotometry (Perkin-Elmer 303) is described in Appendix table IV.

When blood chemistry had established individual norms, calves were selected according to age, weight and blood values. These animals were placed in metabolism units and daily feed intake, urine and fecal excretions were recorded for 1 to 2 unit periods. A unit period consisted of 7 days. Six of these days were spent in the metabolism unit while on the 7th day the animals were transferred to larger holding pens for exercise and rest. Daily samples of urine and feces from each calf were stored for chemical analyses.

The balance periods prior to parasite infection served as a baseline or control value for each animal. The cultured larvae of Ostertagia were administered per os via dose syringe in equal portions on 2 successive days. Following larval infection, the calves were maintained for a prescribed number of unit periods in
the manner previously described. With exception of the fact that blood sampling was increased from 2 to 3 times weekly, sampling and sampling procedures remained unaltered throughout the remainder of the trial.

**Trial One.**

Each animal in the 1st trial was inoculated with 65,000 cultured larvae of *Ostertagia spp.* after establishment of a baseline balance. Following infection, data were collected for 5 consecutive unit periods.

**Trial Two.**

The 2nd trial of this phase was essentially a replication of trial 1, with certain modifications. Results of the 1st trial indicated the need to replicate the work and extend the data collection over a longer period. Also, additional observations were made to include patterns of the fecal moisture over the control and infected period. In this trial there were 2 unit periods in the baseline, followed by 7 unit periods after larval inoculation. Due to a low recovery of viable larvae from the cultures, only 45,000 larvae of *Ostertagia spp.* were administered to each calf.

**RESULTS AND DISCUSSION**

**Phosphorus.**

Much of the earlier work concerning the effect of parasitism on mineral disposition by the host was reported in terms of changes in the plasma inorganic phosphate fractions. Figure 2 shows the
FIGURE 2. DIURNAL PATTERNS OF BOUND AND FREE PHOSPHORUS IN THE BLOOD PLASMA OF WEANLING CALVES FOLLOWING INFECTION WITH 65,000 LARVAE OF OSTERTAGIA SPP.
plasma patterns of bound and free phosphorus over a 30-day infection period in trial 1. Free phosphorus declined with a decrease in the magnitude of the decline until the 12th day after which a rise, gaining in magnitude, increased to the end of the 30-day period. On the average, the patterns of plasma inorganic phosphate observed in trial 1 were strikingly similar to those reported by Shumard et al. (1957) for sheep. Further, even though Herlich (1962) noted a more dramatic change in this component in his parasitized sheep, the trend in behavior was the same as that for the calves in trial 1 of this study.

The three-fold increase in the plasma bound phosphorus at 21 days post-inoculum was highly significant (P < .01) as was the subsequent decrease (P < .01). After having reached a peak near the 21st day, the bound fractions declined to near baseline values in only one third the time required to reach the maximum.

It appears that the effects of parasitism on the entire plasma phosphorus picture was either overlooked in earlier studies or disregarded in the quest for knowledge concerning the inorganic portions. Granting that the most readily available phosphorus source in the plasma is that in the free or elemental state, it is rather surprising that decreases in plasma free phosphate reported by earlier investigators provoked such little concern as to the fate of that portion removed from circulation and its relation to the organic fractions present.
It is ironic that the behavior of total plasma phosphorus and its bound component received so little attention in previous investigations. The most marked changes recorded in the 1st trial were found in these 2 entities. The three-fold increase in the plasma-bound phosphorus created some doubt as to whether the observed decrease in level of free phosphorus was due to its leaving the blood circulation. Although more pronounced, the bound components increased during that phase of decline by the free form and initiated its descent shortly after the subsequent rise in the plasma inorganic phosphorus.

Figure 3 shows the patterns of total phosphorus in the blood plasma in young calves in the 1st trial after a single experimental infection of Ostertagia spp. Total plasma phosphorus was relatively unaltered for the 1st 10 days following infection. By the 12th day an increase was evident and continued to show a significant \( (P < .05) \) incline until the 23rd day. Afterwards, the decline in plasma levels was similar in magnitude and appearance to the rise up to that point; however, the negative slope of the curve was not statistically significant.

Virtually all the marked changes in the behavior of total plasma phosphorus in trial 1 were reflected in the bound component. In fact, the rise and fall in bound plasma phosphorus were both highly significant \( (P < .01) \), whereas only the observed increase in the total was significant and even then to a lesser degree \( (P < .05) \). The agreement of the inorganic data in the 1st trial with that of
DAYS POST INOCULUM

TOTAL PLASMA PHOSPHORUS

FIGURE 3. DIURNAL PATTERNS OF TOTAL PHOSPHORUS IN BLOOD PLASMA OF WEANLING CALVES FOLLOWING INFECTION WITH 65,000 LARVAE OF OSTERTAGIA SPP.
earlier reports should add to the validity of the observed changes in plasma bound phosphorus even though the latter were calculated from the total and free components.

The trends in fecal and urinary phosphorus during the infection period of the 1st trial is illustrated in Figure 4. The excretory patterns of phosphorus in the urine resembled the trends of total plasma phosphorus, but more closely paralleled the fluctuations in plasma bound fractions. The exact form of phosphorus excreted via the urine was not determined, therefore, it is not certain if the rise and fall in different plasma fractions were reflected by corresponding changes in the urine. Since the urine is a convenient mode of regulating elevated blood levels of various components, it would seem plausible to suspect that this type of adjustment might have occurred here. Admittedly, this is not the primary pathway of phosphorus excretion as evidenced by the five-fold difference in fecal and urinary concentration in these trials. If further studies support these patterns, the possibility of chelation should be investigated.

The behavior of fecal phosphorus during the 1st 14 days of infection (Figure 4) was somewhat erratic and as yet remains obscure. However, from this point until the remainder of the study, the excretion of phosphorus in the feces exhibited a distinct increase at an apparently accelerated rate. Coincidentally, at approximately this stage of development, the nematode emerges in vast numbers from the gastric mucosa of the host to molt and become
FIGURE 4. PLASMA, FECAL AND URINARY PHOSPHORUS PATTERNS OF WEANLING CALVES FOLLOWING *OSTERTAGIA* SPP. INFECTION
sexually mature. As this pronounced change in fecal phosphorus excretion apparently influenced the negative trend in the host's phosphorus balance (Figure 5), many avenues of speculation become opened as explanatory possibilities.

Figure 6 shows the average hemoglobin (Hb) and packed cell volumes (CPV) in the 2nd trial after infecting the calves with 45,000 larvae of the medium stomach worm. Hemoglobin values decreased from 11.2 gm % to 10.2 gm % over the 7 unit periods, which followed inoculation with the nematode. The packed cell volume also showed a slight decline toward the end of the trial. Since hemoconcentration is usually a characteristic of more severe cases of ostertagiasis, the decline in CPV in these animals was suggestive of a low-level of parasitism.

Although blood values indicated that a minimum number of worms were active within the host, there was a substantial production of nematode eggs voided in the feces of the calves as evidenced by Figure 7. Curves reflecting egg production by the female worms were very similar for all 3 calves and the peak of fecal egg concentration (EPG) was almost equal. Ova were first detected in the feces during the 3rd week of infection as expected, and EPG continued to increase through the 5th week before exhibiting signs of decreased production by the female nematodes.

Figure 8 shows the fecal, urinary and plasma phosphorus patterns over the course of the 9 balance periods. Fecal phosphorus excretion was virtually a linear increase during the first 5 periods after
Figure 5. Patterns of phosphorus balance ratios (intake/excretion) in calves following oral dose of 65,000 infective larvae of Ostertagia spp.
FIGURE 6. AVERAGE HEMATOCRIT OF CALVES EXPERIMENTALLY INFECTED WITH 45,000 CULTURED LARVAE OF OSTERTAGIA SPP.
UNIT PERIODS AFTER INFECTION

FIGURE 7. AVERAGE NUMBER OF NEMATODE EGGS PER GRAM FECES (EPG) IN THREE CALVES EXPERIMENTALLY INFECTED WITH 45,000 CULTURED LARVAE OF OSTERTAGIA SPP.
FIGURE 8. PATTERNS OF PLASMA TOTAL PHOSPHORUS LEVEL AND URINARY AND FECAL PHOSPHORUS EXCRETION IN CALVES EXPERIMENTALLY INFECTED WITH 45,000 CULTURED LARVAE OF OSTERTAGIA SPP.
infection. After the maximum excretion was attained at period 7 there was a sharp drop in excretion rate which, at the end of the trial, remained 2 times higher than the baseline values.

Urinary phosphorus was unchanged during the baseline periods and after a small increase to the 4th unit period it began a steady decrease to the 7th period. The subsequent rise in urinary phosphorus excretion was rather distinct in that the level attained was above the baseline for the remainder of the study. The urinary and fecal phosphorus trends appeared to reciprocate each other throughout the period of data collection. As will be seen later, a similar relationship was observed with the element calcium. Irving (1957) quoted work which suggested that if the kidney was unable to excrete calcium, the intestine would take over the function. These data indicate that such a relationship may also exist with respect to phosphorus.

Unlike the results of the 1st trial, total plasma phosphorus levels were moderately depressed from the 4th to the 7th unit period before returning to pre-infection levels by the end of the trial. Herlich (1962) reported that "coincident with or shortly before the onset of diarrhea and anorexia in infected calves, levels of serum phosphorus began to drop. The decline generally became evident about 15 days after inoculation and continued until calves either died or signs of parasitism abated. In the latter eventuality, the levels of serum phosphorus began to rise and in some instances, the values had returned nearly to preinoculation levels by the end
of the experiment." A similar trend in plasma phosphorus was observed in both trials of the phase reported here. The lowering of the plasma phosphorus in these calves appeared to have been a function of decreased absorption from the alimentary tract. This postulation is based on the increase in the excretion of fecal phosphorus and the decrease in urinary phosphorus excretion. A depression of absorption could be attributed to several factors such as, trauma to the absorbing membranes by the worms, or the unavailability of the dietary phosphorus due to organic binding or chelation in the tract. Anderson et al. (1965) reported an increase in abomasal pH in cattle suffering with ostertagiasis. Such a shift in alimentary pH could lower the availability of exogenous phosphorus by lowering the solubility.

Figure 9 shows the patterns of bound and free components of plasma phosphorus. Free plasma phosphorus exhibited a slight increase toward the end of the trial, but there was no other noticeable change in this fraction. Bound plasma phosphorus was almost in equilibrium with the free component initially. After the 4th unit period there was a decline in bound phosphorus which showed an increase during the last week of the study but did not return to baseline values. It appears that the observed decrease in the bound plasma phosphorus was reflected in the total plasma phosphorus (Figure 8).

Balance ratios and average feed intake per calf per unit period are shown in Figure 10. Feed intake increased steadily until the 7th period. Following a moderate decrease, ration consumption continued to increase and was near 8 kilograms (kg) at the end of the 9th period.
FIGURE 9. PATTERNS OF PLASMA BOUND AND FREE PHOSPHORUS (AVE/UNIT PERIOD) IN CALVES EXPERIMENTALLY INFECTED WITH 45,000 CULTURED LARVAE OF OSTERTAGIA SPP.
FIGURE 10. PHOSPHORUS BALANCE RATIOS AND FEED INTAKE IN CALVES EXPERIMENTALLY INFECTED WITH 45,000 CULTURED LARVAE OF OSTERTAGIA SPP.
Fecal egg counts (Figure 7) indicated the worm had reached sexual maturity in the host sometime during the 7th period. This may have accounted for the slight anorexia encountered. Herlich (1962) reported similar results with mixed infections and heavier larvae loads.

Balance ratio showed little fluctuation until the 7th period where it dropped to 1.35 compared to baseline values of more than 2.0. It is uncertain as to whether the decreased efficiency was due to the anorexia or some direct influence of the parasite. As was shown earlier (Figure 8) fecal phosphorus excretion maintained a steady increase and paralleled feed intake until the 7th period. Apparently the high rate of excretion of fecal phosphorus coupled with anorexia during the same period caused a reduction in the efficiency of phosphorus metabolism. After the 7th period fecal excretion subsided and balance ratios followed the trends in feed intake.

It appears that though the mechanics are not understood, the small larval load used in trial 2 was sufficient to adversely affect phosphorus metabolism in the young calf, but was not able to cause lasting effects on efficiency. It is postulated that the mechanism involved in lowering the efficiency of phosphorus utilization was concerned with decreased absorption as the excretion of phosphorus in the urine began to decline after the fecal excretion of the element maintained a persistent increase. It is in this manner that the body's homeostatic mechanisms attempt to diminish an increased efflux of nutrients from the system.
Calcium.

The effect of experimental infections of Ostertagia spp. on the excretion of calcium in the urine and feces and the levels maintained in the blood plasma are illustrated in Figures 11 and 12. As each animal served as its own control, the baseline data were collected before the animals were infected with parasitic larvae.

Data collected prior to the inoculation with the parasite established a baseline value of 100 parts per million (ppm) plasma calcium. For the 2nd trial, the baseline value was 96 ppm. In the 1st trial, plasma calcium showed a decline beginning shortly after the 1st unit period with a continued decrease until the 4th period. A sharp drop occurred between periods 4 and 5, but this was followed by a positive inclination to the 6th period (Figure 11).

Although the baseline values for plasma calcium were lower in the 2nd trial, there was a marked rise in the level during the 5th unit period which was near the 20th day post infection. Following this rise, the concentration declined almost as rapidly as it had climbed. This low and that which followed another elevation during period 8 were still higher than the baseline values. At the close of the trial, blood calcium levels were closer to the physiological value of 100 ppm.

The reason for the lack of consistency in the plasma calcium patterns of the 2 trials is vague. There were, however, a few similarities in the patterns even though the extent of certain deviations varied. Baseline values were near 100 ppm for both trials. Further, there was a drop in concentration after the 21st day post
FIGURE 11. FECAL, URINARY AND PLASMA CALCIUM BEHAVIOR IN CALVES EXPERIMENTALLY INFECTED WITH 65,000 CULTURED LARVAE OF OSTERTAGIA SPP.
Figure 12. FECAL, URINARY AND PLASMA CALCIUM BEHAVIOR IN CALVES EXPERIMENTALLY INFECTED WITH 45,000 LARVAE OF OSTERTAGIA SPP.
infection which reached a low near 29 days. The questionable positive inclination by the 35th day of the 1st experiment received some fortification in trial 2, which also inclined and peaked during the same period of infection. The second set of calves were 30 to 45 days older and were inoculated with fewer cultured larvae, though the average number of eggs voided in the feces was similar for both groups. Granting the possibility of certain variations between infections, a range from moderately hypocalcemic to considerably hypercalcemic is not consistent with data previously reported by other workers.

Herlich (1962) stated that serum calcium abnormalities are not unusual since the bone trabeculae constitute readily available calcium stores, and serum calcium levels may be maintained even though animals are given calcium-free diets for a long time. This thesis is not supported by the findings of Franklin et al. (1946) and Fitzsimmons (1966) who reported declines in serum calcium levels of sheep and goats. Since Herlich (1962) based his assumption on work with calves, and it does seem physiologically possible, this might indicate that bovine and ovine hosts react differently to infestations of gastrointestinal parasitisms.

The possible influence of the parathyroid glands should not be overlooked or ruled out in considering the foregoing discussion. Under normal conditions (i.e., when parasites are not involved) the parathyroids would come into play should the animal be subsisting on a deficient diet for an extended period. It does not seem unreasonable that the parasitic moiety could have secreted or
excreted some waste metabolite which could have had a direct bearing on the activity of the parathyroid glands. Just as some drugs exhibit opposite effects on the host at high and low concentrations, a product given off by the invading parasite may cause varied response in the parathyroids or even the thyroid. With both *Cooperia curticei* and *Trichostrongylus colubriformis* infestations, Andrews (1939) and Andrews *et al.* (1944) found that the energy metabolism of all infested lambs was increased significantly.

Two points favoring the observations of the 1st trial are:
(1) they were more in accord with the blood patterns reported by other workers (Fitzsimmons, 1966; Franklin *et al.* 1946; Hiromoto, 1939) and (2) fecal and urinary excretion trends were more in line with those observed in plasma.

The patterns of urine and fecal calcium excretion are also given in Figures 11 and 12 for both trials. The urinary excretion of calcium in both trials increased grossly with time after infection. Also in both trials the renal clearance showed a marked drop around 30 days post inoculum followed by a vigorous increase until the termination of the study. It is believed by some that urinary excretion is an index of intestinal absorption, however, as will be seen later, the balance ratios do not support this concept in this study. The reason for the erratic excretion of calcium in the urine is uncertain at this writing.

It is of interest that Irving (1957) quotes work which suggested that if the kidney was unable to excrete calcium, the intestine would take over this function. In the 1st of these 2 trials, the fecal
excretion of calcium exhibited a rather marked increase shortly after
the decline in the urinary excretion rate; and during the following
period when renal clearance rate began to rise, the rate of fecal
calcium excretion showed some depression. This relationship was
also apparent in the 2nd trial at certain periods, but was not as
pronounced as was illustrated in the initial experiment. Further,
the somewhat lower concentration of the mineral in the urine creates
a question as to how much contribution the difference in urinary
excretion could make to the overall fecal output.

In both trials of this phase, the rates of fecal calcium ex-
cretion showed substantial increases over the baseline values. Also,
these patterns tended to reciprocate the trends of the balance ratios
(Figure 13) more closely than any other entity observed.

Even with the apparent inverse relationship of the fecal excre-
tion and balance ratios (Intake/Excretion), the latter only showed
trends toward ultimate negative values in the initial study. In the
2nd trial there was an initial increase in balance ratios indicating
an increase in efficiency. The subsequent decline was distinctly
negative except for the fact that the ultimate values did not surpass
the baseline values. A possible explanation for this may have been
due to an increase in ration intake which allowed a larger amount of
the element to become involved in the overall biological phenomena.
In other words, the increased intake not only could have evoked an
increase in fecal excretion, but also have concomitantly off-set the
effect on the balance ratios. The combined effect of these 2 factors
FIGURE 13, THE EFFECT OF SUBCLINICAL OSTERTAGIASIS ON CALCIUM BALANCE RATIOS OF WEANLING HOLSTEIN BULL CALVES.
could have exerted a masking effect on any possible adverse influence the worms may have had on calcium metabolism.

Figure 14 illustrates the course of the fecal moisture levels of the calves used in the 2nd trial. Similar data were not collected during the 1st study. Baseline values for fecal moisture varied little from approximately 66%. During the infective period peak values were near 70%. This, along with the lack of noticeable variation in the hematocrits attest to the infections being subacute. By the end of the trial the moisture in the feces had almost returned to the baseline values.

Magnesium.

Figures 15 and 16 summarize the observations of fecal, urinary and plasma trends over the baseline and infective periods of both trials. As the figures illustrate, plasma magnesium appeared to vary little from a concentration of 23 ppm while fecal and urinary excretion rates were not so static.

For the 1st 28 days post-infection, both feces and urine appeared to parallel in magnesium excretion rate after which time they practically reverted to reciprocation. The urinary excretion of magnesium showed an overall increase in trial 1, but with the exception of a sharp rise during the 7th period, trial 2 yielded no net change in this factor.

Conversely, the excretion of magnesium in the feces showed a net increase in both studies and, like the calcium, showed a rather reciprocal relationship to the balance ratios (Figure 17).
FIGURE 14. FECAL MOISTURE PATTERNS IN WEANLING HOLSTEIN BULL CALVES FOLLOWING INOCULATION WITH 45,000 CULTURED LARVAE OF OSTERTAGIA SPP.
FECAL, URINARY AND PLASMA MAGNESIUM BEHAVIOR IN CALVES EXPERIMENTALLY INFECTED WITH 65,000 CULTURED LARVAE OF OSTERTAGIA SPP.
FIGURE 16. FECAL, URINARY AND PLASMA MAGNESIUM BEHAVIOR IN CALVES EXPERIMENTALLY INFECTED WITH 45,000 CULTURED LARVAE OF OSTERTAGIA SPP.
FIGURE 17. THE EFFECT OF SUBCLINICAL OSTERTAGIASIS ON MAGNESIUM BALANCE RATIOS OF WEANLING HOLSTEIN BULL CALVES.
The balance ratios in the 1st trial showed negative inclinations for 28 days after infection followed by a mild upward trend. The baseline ratios of the 2nd trial were lower than those of the initial trial, but showed an increase to values approximating the corresponding values of the 1st trial. Subsequent to this rise there was a negative inclination for the remainder of the trial. The ratios almost reached equilibrium during the last 3 periods. Neither of the observed decreases in the magnesium balance ratios were significant.

Neither of these studies with Ostertagia spp. indicated the hypomagnesemia observed by Fitzsimmons (1966) in goats harboring Trichostrongylus spp. infection. Franklin et al. (1946) considered the hypocalcemia and hypomagnesemia noted in their studies with sheep that were infected with Trichostrongylus spp. to be a secondary effect of the anorexia encountered. Since there was no marked decrease in feed intake in the studies reported here and no corresponding decrease in plasma magnesium, the postulation of Franklin et al. (1946) may possess some merit.
SUMMARY

Weanling calves were experimentally infected with cultured larvae of *Ostertagia* spp., and the effects on phosphorus, calcium and magnesium utilization were observed during 2 separate trials consisting of one 35-day and one 50-day post-infective period. Each calf served as its own control as a baseline period preceded each inoculation. Fecal moisture increased slightly after infection, and there was a slight depression of hemoglobin and packed cell volumes in the 2nd trial.

Larval load was higher in the 1st trial and phosphorus utilization was noticeably impaired. In the 2nd trial, where fewer infective larvae were introduced into the calves, phosphorus balance was decreased, but only in a transient manner. It was postulated that the reduction in balance ratios was due in part to a decreased absorption from the tract.

Following infection with the larvae in the 2nd trial there was a hypercalcemia preceding a hypocalcemia whereas in the 1st trial only a progressive hypocalcemia was recorded. Fecal excretion of calcium increased with time post-infection in both studies though the extent of the increase varied. Urinary calcium excretion exhibited elevations and depressions which not only varied in magnitude, but also with time post-infection. The calcium balance decreased with time in both trials except for the fact that the decline in trial 2 was preceded by an increase of corresponding amplitude.
Plasma magnesium maintained values of 23 to 25 ppm in both trials save a moderate elevation between days 14 and 26 of the infective period of the 2nd trial. Fecal and urinary excretion of magnesium increased with time after infection, but were not reflected by pronounced changes in balance ratios. The modest declines in magnesium balances were not statistically significant.

The author attributed the inconsistency of the 2 trials to differences in age of calves used along with initial increases in ration consumption resulting in premature elevations of certain physiological components coupled with a deficit in larval load per calf. More work is needed to clarify the effect parasitic nematodes exert on the mineral metabolism of the young bovine host.
CHAPTER III

ENDOGENOUS MINERAL EXCRETION IN CALVES HARBORING

OSTERTAGIA SPP.

Preliminary work at this station indicated that calves infected with Ostertagia spp. nematodes exhibited decreased mineral utilization; however the design of the earlier experiments did not elucidate the exact cause of the disease. The purpose of this phase was to investigate any anomalies in endogenous mineral metabolism due to parasitism and to fortify the data of previous work.

EXPERIMENTAL PROCEDURES

Five weanling Holstein bull calves were housed in metabolism units during the first experiment and 4 similar calves were used in the 2nd experiment. Each trial consisted of 2 periods, a baseline (control) period and an infection period. The baseline was an 8-day balance period before each animal was experimentally infected with a predetermined number of cultured larvae of the medium stomach worm (Ostertagia spp.). The infection period was a second 8-day balance period beginning on or near the 18th day after inoculation. This permitted the collection of data before and after the tentative 21st day post-inoculum during which the worms reach sexual maturity and begin producing eggs which can be detected in the feces. Baselines allowed each animal to serve as his own control, thereby eliminating between-animal variation in susceptibility to infection.
Calves were fed a conventional ration with 20% roughage and 16% protein (Appendix table VII). Feed consumption and fecal and urinary excretion were recorded daily, and samples were stored for radioassay and chemical analysis.

Endogenous calcium and phosphorus losses were determined using radiocalcium (Ca-45), radiophosphorus (P-32) and the calculations used in the "Comparative Balance" method described by Comar et al. (1953), (Appendix table V).

Two calves in each trial were designated to receive the isotopes orally via stomach tube inserted through the left nostril. In the 1st trial, 3 calves received intravenous tracer doses; and in the 2nd trial, 2 of the animals were dosed directly into the blood stream.

Radiophosphorus was determined by counting 4 milliliters (ml.) of either whole blood or an acid solution of ashed urine or feces. Calcium was precipitated into planchets with oxalic acid and counted on a separate scaler where the pulse height excluded interference by P-32. Activity was calculated on a per gram basis and extrapolated back to total excretion. Total activity excreted was then expressed as a percent of the total tracer dose administered.

Total phosphorus was determined photometrically by conversion to phosphomolybdate (Appendix table II).

Total calcium was calculated both volumetrically by titration with std. potassium permanganate (KMnO₄) and gravimetrically by precipitation with oxalic acid (Appendix table III).
Ostertagia spp. larvae were cultured by collecting manure from a "seed calf", mixing with equal parts vermiculite and incubating at 27°C for 10-20 days. Third stage larvae were recovered by wrapping the cultures in cheesecloth and emersing in warm water in a funnel equipped with a stopcock at the tip.

Larvae were administered to calves via drench syringe in equal portions on 2 successive days. Each calf in the 1st trial received 100,000 cultured larvae and each one in trial 2 received 120,000 larvae.

RESULTS AND DISCUSSION

Calcium.

The influence of the medium stomach worm on calcium balance (Intake/Excretion) in calf 15 is shown in table 1. The baseline and infective periods of each calf were compared and the average daily number of eggs per gram feces (EPG) during the balance period 15 also shown. Fecal egg count was at best a poor index of worm load, but is the most widely employed when necropsy is not practical.

Three of the 5 calves in the 1st trial showed decreases in efficiency between the baseline and infection periods. The differences in balance ratios in the 2nd trial were even greater with the exception of calf 41, which gave some slight indications of increased calcium utilization. The average ratios in both trials were lower during infection, especially during trial 2. As shown in table 1, the fecal egg counts were considerably higher in trial 2.
Table 1. The Influence of *Ostertagia* spp. on Calcium Balance of Calves

<table>
<thead>
<tr>
<th>Animal</th>
<th>Balance Ratios (^1/)</th>
<th>Ave EPG (^2/)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Infection</td>
</tr>
<tr>
<td>Trial ONE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
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<td>1.77</td>
</tr>
<tr>
<td>37</td>
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</tr>
<tr>
<td>40</td>
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</tr>
<tr>
<td>ave.</td>
<td>2.21</td>
<td>1.95</td>
</tr>
<tr>
<td>Trial TWO</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>1.54</td>
</tr>
<tr>
<td>41</td>
<td>1.63</td>
<td>1.81</td>
</tr>
<tr>
<td>42</td>
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</tr>
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<td>43</td>
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<td>1.49</td>
</tr>
<tr>
<td>ave.</td>
<td>2.15</td>
<td>1.67</td>
</tr>
</tbody>
</table>

\(^1/\) Balance Ratio = Intake/Excretion

\(^2/\) EPG = Number of nematode eggs per gram feces

The number of larvae administered per calf was greater in the 2nd trial, and the cultures in trial 1 remained in the incubator longer. These factors account for some of the variation in fecal egg counts between the 2 trials.
Table 2 illustrates the comparison of the endogenous calcium losses in the calves before and after parasitism by the nematode. There was virtually no change in the rate of endogenous calcium excretion in the 1st trial. According to the means, the number of grams of endogenous calcium excreted was exactly the same for baseline and infection periods. As evaluated by fecal egg counts (Table 1), however, calves in the 1st trial carried extremely small worm loads.

Table 2. Endogenous Calcium Losses of Calves Infected with Ostertagia spp.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
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<td>Trial ONE</td>
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<tr>
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<td>8.0</td>
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<td>ave.</td>
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<td>7.5</td>
</tr>
<tr>
<td>Trial TWO</td>
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</tr>
<tr>
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</tr>
<tr>
<td>ave.</td>
<td>19.4</td>
<td>14.8</td>
</tr>
</tbody>
</table>

1/ Values expressed in terms of grams excreted during a balance period.
In the 2nd trial, where EPG indicated heavier worm infections, the average endogenous loss was lower for the infection period than for the baseline. Although data reported here are too limited to draw definite conclusions, the results were somewhat the reverse of the hypothesis underlying the phase.

There was some speculation that previously observed decreases in mineral metabolism efficiency of infected calves could have been due to increases in the endogenous excretion of the elements. Hansard et al. (1951) reported that, in steers, source and quantity of excreted calcium was dependent directly upon both gut content and body reserves. In their work, calcium absorption and exchange were augmented when gut and body stores were low. The data in table 1 suggest that the observed decreases in balance ratios (Intake/Excretion) was attributable to some mechanism other than an increase in endogenous calcium metabolism.

Table 3 lists the percent I.V. dose of radiocalcium excreted in the feces of infected calves. In both trials, there was a larger amount of the I.V. dose excreted in the feces during the infection period than during the baseline period. Only 1 of the 4 animals deviated from this trend. The means of both trials were in close agreement. It would seem that an increase in secretion into the alimentary tract of an I.V. tracer dose of calcium would be paralleled by an increase in the endogenous excretion; however, such a relationship was not evident in this study. Hansard et al. (1952) discussed the rapid rate of disappearance from the blood of
Table 3. Percent of Intravenous Dose of Radiocalcium Excreted in Feces of Calves Infected with *Ostertagia* spp.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial ONE</strong></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>38</td>
<td>5.8</td>
<td>10.5</td>
</tr>
<tr>
<td>39</td>
<td>4.2</td>
<td>11.2</td>
</tr>
<tr>
<td>ave.</td>
<td>5.0</td>
<td>10.9</td>
</tr>
<tr>
<td><strong>Trial TWO</strong></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>42</td>
<td>6.6</td>
<td>14.2</td>
</tr>
<tr>
<td>43</td>
<td>5.8</td>
<td>5.3</td>
</tr>
<tr>
<td>ave.</td>
<td>6.2</td>
<td>9.8</td>
</tr>
</tbody>
</table>

* intravenously administered Ca-45. They reported that the rapid disappearance observed could not be considered as removal of excess calcium from the blood, since the same linear pattern was shown in studies where less than 3.0 mg of total calcium was administered.*

Harrison and Harrison (1950), along with Hansard *et al.* (1954) and Falkenheim *et al.* (1951), reported that, in general, the rate of exchange of calcium ions between blood and other tissues decreases significantly with increasing age. It seems unlikely that 6 weeks could be enough difference in age to provoke the changes observed in table 3. Possibly the adverse effect of the parasite on the
host's calcium metabolism may concern interference with the rate of exchange rather than with increasing endogenous loss. More work is needed to fully discern the effects of Ostertagia spp. on the endogenous calcium metabolism of the young bovine.

**Phosphorus.**

Table 4 summarizes the effects of the medium stomach worm on the phosphorus balance in calves. In the 1st trial, the general trend was toward increased efficiency of phosphorus metabolism as evidenced by the rise in balance ratios from baseline to infectious period. It is again recalled that fecal egg counts (EPG) in this trial indicated extremely mild infection of parasitism. Without exception, the calves in the initial phase exhibited increases in balance ratios, some as much as a two-fold increase. The mean ratios rose almost an integral point.

In trial 2, where EPG indicated a higher degree of infection, patterns of balance ratios were reversed in that all calves showed marked decreases between baseline and infection periods. The average loss was a 1.25 decrease in intake-outgo. These data from trial 2 support the conclusions of previous work that Ostertagia spp. do adversely influence phosphorus metabolism in the young bovine.

Table 5 shows the endogenous phosphorus losses of the calves before and after being infected with the parasitic nematode. In the 1st trial, endogenous phosphorus losses were higher during the control period than during infection. The reason for this is unclear
Table 4. The Influence of Ostertagia spp. on Phosphorus Balance in Calves

<table>
<thead>
<tr>
<th>Animal</th>
<th>Balance Ratios 1/</th>
<th>Ave. EPG 2/</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Infection</td>
</tr>
<tr>
<td>Trial ONE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>2.52</td>
<td>3.51</td>
</tr>
<tr>
<td>37</td>
<td>2.35</td>
<td>3.06</td>
</tr>
<tr>
<td>38</td>
<td>2.27</td>
<td>3.26</td>
</tr>
<tr>
<td>39</td>
<td>1.93</td>
<td>3.12</td>
</tr>
<tr>
<td>40</td>
<td>1.78</td>
<td>4.09</td>
</tr>
<tr>
<td>ave.</td>
<td>2.17</td>
<td>3.41</td>
</tr>
<tr>
<td>Trial TWO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.35</td>
<td>1.45</td>
</tr>
<tr>
<td>41</td>
<td>2.58</td>
<td>1.87</td>
</tr>
<tr>
<td>42</td>
<td>3.63</td>
<td>1.70</td>
</tr>
<tr>
<td>43</td>
<td>3.35</td>
<td>1.93</td>
</tr>
<tr>
<td>ave.</td>
<td>2.98</td>
<td>1.73</td>
</tr>
</tbody>
</table>

1/ Balance ratio = Intake/Excretion

2/ EPG = Number of nematode eggs per gram feces.
as this followed neither an increased endogenous loss due to age nor
a mobilization of body reserves by the parasite.

Table 5. Endogenous Phosphorus Losses ¹/ of Calves
Infected with Ostertagia spp.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial ONE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>10.7</td>
<td>9.0</td>
</tr>
<tr>
<td>37</td>
<td>12.4</td>
<td>7.0</td>
</tr>
<tr>
<td>ave.</td>
<td>11.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Trial TWO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.0</td>
<td>19.2</td>
</tr>
<tr>
<td>41</td>
<td>9.6</td>
<td>16.4</td>
</tr>
<tr>
<td>ave.</td>
<td>8.8</td>
<td>17.8</td>
</tr>
</tbody>
</table>

¹/ Values expressed in terms of grams excreted during a balance.

The results of the 2nd trial were opposite from the 1st in that
endogenous phosphorus losses were higher during the period of para­
sitism than during the control period. Despite the apparently higher
parasite burdens in the 2nd trial, the lack of agreement between the
2 trials prevents drawing definite conclusions from these data. The
stimulus for these investigations was the question as to whether or
not the parasite induced mobilization of the body's mineral reserves.
The author did not feel that the parasitism in the 1st trial was severe enough to cause detectable differences in the endogenous mineral metabolism of the host. Even the apparently higher worm load observed in trial 2 was considered subclinical.

Table 6 shows the percent of the I.V. dose of radiophosphorus excreted in the feces of calves during the control and infective period with *Ostertagia spp.* Like the calcium data, there was a higher rate of phosphorus secretion into the tract during the infectious period than during the control; however, only the 2nd study gave any indication that the body reserves were affected. The means of each phase were in very good agreement. The reason for this increase in rate of secretion into the tract is as yet obscure.

Table 6. Percent of Intravenous Dose of Radiophosphorus Excreted in Feces of Calves Infected with *Ostertagia spp.*

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial ONE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>5.8</td>
<td>10.5</td>
</tr>
<tr>
<td>39</td>
<td>4.2</td>
<td>11.2</td>
</tr>
<tr>
<td>ave.</td>
<td>5.0</td>
<td>10.9</td>
</tr>
<tr>
<td><strong>Trial TWO</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>6.6</td>
<td>14.2</td>
</tr>
<tr>
<td>43</td>
<td>5.8</td>
<td>5.3</td>
</tr>
<tr>
<td>ave.</td>
<td>6.2</td>
<td>9.8</td>
</tr>
</tbody>
</table>
The collection of this type of data is not only time consuming due to the chemical analysis, radioassay, and the fact that 2 animals are required to obtain 1 endogenous mineral value, but is also expensive. The cost of the isotopes coupled with that of the experimental animals, which have no salvage value, exaggerate the price of tracer data.

These results suggest the need to pursue further studies on the parasitic influence on endogenous phosphorus metabolism.

**SUMMARY**

Weanling Holstein bull calves were housed in metabolism units for 8-day balance periods before and after infection with *Ostertagia spp*. Radiocalcium (Ca$^{45}$) and radiophosphorus (P-32) were employed concurrently to calculate endogenous losses of the 2 minerals. The trials were replicated. Parasite burden appeared to be higher in the 2nd trial. Calcium balance ratios (Intake/Excretion) generally decreased after inoculation with the parasite. Although the secretion of the I.V. tracer dose increased during the infective period, there were no detectable adverse effects on endogenous calcium metabolism. Phosphorus balance ratios increased in 1 trial where the worm load was considered light, but exhibited a marked drop during the infective period with substantial fecal egg counts. The rate of secretion of the I.V. dose of P-32 was consistently lower before infection with the infective larvae; however, only 1 trial gave indications of an increased endogenous metabolism. The need for further work in this area is emphasized.
CHAPTER IV

MINERAL RETENTION AS AFFECTED BY RATE OF INGESTA FLOW IN WEANLING CALVES

That diarrhea frequently accompanies ostertagiasis, especially experimental infections, has been recognized for quite some time. As to what degree this condition could influence nutrient utilization in weanling calves has not been fully elucidated. Several reports in the literature have reported diarrhea and decreased digestion coefficients for various nutrients in experimentally and naturally parasitized animals. None of the reports discussed the relationship of the diarrhetic condition to the decreased nutrient utilization. The decreased efficiency of nutrient utilization was attributed to the helminth infection, but no explanation for a possible mode of action was cited.

In the 1st trial of the phase discussed in Chapter II, it was observed that experimental ostertagiasis caused a decrease in calcium and phosphorus retention in weanling calves. It was postulated that an increased rate of passage of ingesta through the alimentary tract may not have allowed the minerals adequate exposure time to the absorbing membranes.

The purpose of the following 2 trials was to create a diarrhetic condition in calves similar to that of parasite infection to observe the effects on calcium and phosphorus retention.
EXPERIMENTAL PROCEDURE

Cascara segrata (20% in aqueous glucose vehicle) was employed in both trials primarily because it can be administered parenterally and mediates its action by increasing peristalsis in the alimentary tract. There are 2 advantages of using injectable laxatives. The 1st is that its action is on the contraction rate of the muscularis and is not irritating to the mucosal lining. Secondly, oral laxatives, such as magnesium sulfate or even cascara segrata, may cause chelation of the minerals in question which could create difficulty in the interpretation of results.

Calves were preconditioned to the basal ration and metabolism units, as previously described, before beginning treatment with cascara. Sampling procedures and the collection of balance data were detailed in the general experimental procedure. The chemical analyses of calcium and phosphorus are explained in Appendix tables II and III respectively.

Trial One.

Three calves were housed in metabolism units for 10 consecutive days. Subcutaneous injections were started on the 2nd day of the trial and continued daily through day 7. The 1st dose consisted of a 10 ml. injection to each calf in the morning followed by a 5 ml. injection in the late afternoon. Each successive morning injection was increased by 5 ml. increments up to 40 ml. The afternoon injection remained constant at 5 ml per day.
Fecal moisture was determined by weighing a representative sample in a crucible and evaporating to dryness in a drying oven. The percent moisture in the feces was used as an index of the extent of the diarrhea produced.

Blood samples were taken on the 1st day and on alternate days thereafter. Hemoglobin and packed cell volume were determined on the whole blood before centrifuging and freezing the plasma.

**Trial Two.**

In the 2nd experiment a baseline period was recorded for 4 calves prior to a 2nd period of equal length (6-day balance) in which they were injected with cascara segrata. As with previous and subsequent trials the baseline was for the purpose of allowing each animal to serve as its own control.

Once the baseline had established normal balance ratios (Intake/Excretion) for each animal, cascara was injected on the 1st day of the treatment period at a rate of 15 ml. morning and evening. The following 4 days the rate of dose increased by 5 ml. per injection per calf per day until each was receiving a total of 50 ml. cascara per day.

Fecal moisture was not recorded in this trial. The ratios of I/E were compared for the controls and treated periods.

Blood samples were procured on alternate days as in trial 1 and the analyses of plasma calcium and phosphorus were not changed.
RESULTS AND DISCUSSION

Trial One.

The results of the 1st trial are summarized in Table 7. As indicated, the cascara injections (SC) began with 15 ml. on the 1st day and was progressively increased to 45 ml. on the 7th day of the trial.

The desired degree of diarrhea, as measured by fecal moisture, was never reached during the course of the 1st trial. As shown in Table 7, the level of moisture in the feces had increased from 67 to 80% by the 6th day and remained near 79% through the 7th day. These elevated values, however, were the average of a 24-hour composite of feces. It was observed that within 4 hours after the morning injection, which was the largest, feces of a more liquid consistency was voided, but subsequent stools were firmer and resembled normal excretions regardless of the follow-up treatment in the late afternoon. Some of the more liquid excreta were certainly in excess of 80% moisture, but when added to the remainder of the 24-hour composite, the overall level was lowered. This erratic response to the drug treatment was observed about midway of the trial and it was concluded that deviation from the linearity of the treatment would lead to difficulties in the interpretation of resultant data. Within 2 days after cessation of cascara injections fecal moisture levels were almost down to the original values.

Feed intake was not adversely changed until the 4th day when consumption was 125 grams below the previous day. Up to that point
Table 7. Effects of Progressive Levels of Cascara Segrata Injections (SC) on Calcium and Phosphorus Behavior in Weanling Holstein Bull Calves

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cascara Inj.</td>
<td>ml</td>
<td>15</td>
<td>20</td>
<td>25</td>
<td>30</td>
<td>35</td>
<td>40</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal Moisture</td>
<td>%</td>
<td>67</td>
<td>71</td>
<td>74</td>
<td>76</td>
<td>76</td>
<td>80</td>
<td>79</td>
<td>72</td>
<td>69</td>
</tr>
<tr>
<td>Feed Intake</td>
<td>gm</td>
<td>668</td>
<td>717</td>
<td>750</td>
<td>625</td>
<td>725</td>
<td>608</td>
<td>767</td>
<td>720</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>gm</td>
<td>128</td>
<td>146</td>
<td>109</td>
<td>140</td>
<td>153</td>
<td>131</td>
<td>109</td>
<td>184</td>
<td></td>
</tr>
<tr>
<td>Calcium Bal.</td>
<td>I/E</td>
<td>1.14</td>
<td>2.07</td>
<td>1.97</td>
<td>1.57</td>
<td>1.97</td>
<td>2.20</td>
<td>1.18</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>Fecal Cal.</td>
<td>gm</td>
<td>3.02</td>
<td>2.64</td>
<td>1.54</td>
<td>1.99</td>
<td>1.77</td>
<td>1.66</td>
<td>1.51</td>
<td>2.91</td>
<td>2.14</td>
</tr>
<tr>
<td>Urine Cal.</td>
<td>gm</td>
<td>.035</td>
<td>.019</td>
<td>.015</td>
<td>.019</td>
<td>.029</td>
<td>.014</td>
<td>.013</td>
<td>.011</td>
<td></td>
</tr>
<tr>
<td>Plasma Cal.</td>
<td>ppm</td>
<td>100</td>
<td>--</td>
<td>99</td>
<td>--</td>
<td>100</td>
<td>--</td>
<td>--</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Phos. Bal.</td>
<td>I/E</td>
<td>0.90</td>
<td>1.41</td>
<td>1.12</td>
<td>1.15</td>
<td>1.25</td>
<td>0.97</td>
<td>1.09</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Fecal Phos.</td>
<td>gm</td>
<td>0.91</td>
<td>1.50</td>
<td>0.74</td>
<td>1.26</td>
<td>1.30</td>
<td>1.17</td>
<td>0.86</td>
<td>1.84</td>
<td>1.90</td>
</tr>
<tr>
<td>Urine Phos.</td>
<td>gm</td>
<td>0.73</td>
<td>1.13</td>
<td>1.17</td>
<td>1.12</td>
<td>0.64</td>
<td>0.89</td>
<td>1.38</td>
<td>0.70</td>
<td>0.38</td>
</tr>
<tr>
<td>Plasma Phos.</td>
<td>ppm</td>
<td>180</td>
<td>--</td>
<td>160</td>
<td>--</td>
<td>140</td>
<td>--</td>
<td>--</td>
<td>140</td>
<td></td>
</tr>
</tbody>
</table>

1/  I/E = Intake/Excretion
2/  ppm = Parts per million
feed intake had shown a steady increase. The lowest consumption of ration was on the 6th day when the fecal moisture levels were highest.

Calcium balance ratios were near equilibrium at the beginning of the treatment period and steadily increased to the 6th day. The trends in calcium balance were virtually reciprocated by those of fecal calcium excretion. Urinary calcium excretion followed no pattern and appeared to be unrelated to any other data trends. Plasma calcium maintained a concentration of 100 ppm except for a drop to 83 ppm near the end of the trial.

Unlike calcium, the initial phosphorus balance ratios were negative and barely maintained a positive value until the 6th day where excretion exceeded intake and remained negative for the rest of the trial. Fecal phosphorus patterns did not appear to bear any direct relation to the balance ratios, but did seem to reciprocate the trends observed in urinary phosphorus. As was mentioned earlier, Irving (1957) quotes work which suggests that, in humans, if the kidney is unable to excrete calcium, the intestine takes over the function. The data reported in this trial suggest that a similar relationship may exist for phosphorus.

The initial plasma phosphorus levels were somewhat higher than expected and exhibited almost a linear decline to the 8th day of the trial. This observation was difficult to interpret as it was not obviously reflected in any other physiological process involving phosphorus.
Trial Two.

The effect of cascara segrata injections on calcium balance ratios in the 2nd trial is shown in Table 8. Quite obviously the efficiency of calcium utilization was greater during the balance period where cascara was administered. Whether the observed increase in calcium balance was due solely to the treatment is unclear. The inclusion of a placebo would have added refinement to the trial.

The effect of the injectable laxative treatment on phosphorus utilization (Table 9) was similar to that of calcium. The mean phosphorus balance ratio during the control period was 1.43 compared with 1.69 for the treated period. Again it is uncertain as to what contribution the drug had on the increase in balance ratios.

As indicated in both tables (8 and 9), feed intake was slightly decreased during the period of treatment, however, this showed no adverse effect on the balance of either calcium or phosphorus. It was postulated that the effect of the drug was an indirect one in that, by increasing peristalsis without irritation to the mucosa, gastric secretions were increased thereby lowering the intestinal pH and increasing the solubility and absorption of calcium and phosphorus.

Since the theory underlying the study was that increased rate of ingesta passage lowered absorption efficiency, the mechanism by which the laxative may have caused an increase in absorption is superfluous to the investigation. However, in view of the apparent lack of data in the literature relating intestinal absorption to
Table 8. The Effect of Cascara Segrata Injections on the Efficiency of Calcium Utilization by Weanling Calves

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline Feed Intake</th>
<th>Baseline Balance Ratio</th>
<th>Treatment Feed Intake</th>
<th>Treatment Balance Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>9351</td>
<td>7000</td>
<td>0.84</td>
<td>6975</td>
<td>1.40</td>
</tr>
<tr>
<td>9352</td>
<td>7000</td>
<td>0.92</td>
<td>6775</td>
<td>1.84</td>
</tr>
<tr>
<td>9353</td>
<td>7000</td>
<td>1.48</td>
<td>6950</td>
<td>1.49</td>
</tr>
<tr>
<td>9354</td>
<td>6800</td>
<td>0.85</td>
<td>6290</td>
<td>1.19</td>
</tr>
<tr>
<td>Mean</td>
<td>6950</td>
<td>1.03</td>
<td>6740</td>
<td>1.48</td>
</tr>
</tbody>
</table>

1/ Subcutaneous injections of cascara segrata

2/ Balance Ratio = Intake/Excretion

3/ Feed intake expressed in total grams
Table 9. The Effect of Cascara Segrata Injections on the Efficiency of Phosphorus Utilization by Weanling Calves

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline Feed Intake</th>
<th>Baseline Balance Ratio</th>
<th>Treatment Feed Intake</th>
<th>Treatment Balance Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>9351</td>
<td>7000</td>
<td>1.30</td>
<td>6975</td>
<td>1.83</td>
</tr>
<tr>
<td>9352</td>
<td>7000</td>
<td>1.47</td>
<td>6775</td>
<td>1.92</td>
</tr>
<tr>
<td>9353</td>
<td>7000</td>
<td>1.84</td>
<td>6950</td>
<td>1.71</td>
</tr>
<tr>
<td>9354</td>
<td>6800</td>
<td>1.13</td>
<td>6290</td>
<td>1.30</td>
</tr>
<tr>
<td>Mean</td>
<td>6950</td>
<td>1.43</td>
<td>6747</td>
<td>1.69</td>
</tr>
</tbody>
</table>

1/ Subcutaneous injections of cascara segrata

2/ Balance Ratio = Intake/Excretion

3/ Feed intake expressed in total grams
ingesta flow rates, further experiments should be designed to study this basic phenomena. Such information would be a valuable aid in the interpretation of data involving host-parasite relationships as well as other disciplines far removed.

SUMMARY

Weanling Holstein bull calves were given parenteral injections of cascara segrata to study the influence of rate of intestinal flow on mineral retention. An attempt to create a diarrhetic condition in calves similar to that caused by parasitic infection was not totally successful. The desired degree of diarrhea could not be satisfactorily maintained and the calves appeared to become refractory to the drug. The efficiency of mineral utilization generally increased during drug treatment.
CHAPTER V

THE INFLUENCE OF OSTERTAGIA SPP. ON
ABOMASAL PERMEABILITY

The traumatic effect of Ostertagia spp. on the abomasal mucosa of calves was described by Porter and Cauthen (1946) and discussed in more detail by Anderson et al. (1965).

Symons and Fairbairn (1962) observed congestion and thickening of the jejunum and upper ileum of white rats parasitized with Nippostrongylus braziliensis. These were irregularities and partial fusion of villi, hyperplasia of the mucosa and hypertrophy of the muscularis externa. It was reported that the changes in the mucosa brought about marked derangement of transport and absorption of fluid and certain ions. The influx into the intestine of water, sodium and chloride ions was essentially unchanged and the rate of efflux from the intestine was greatly reduced so that there was a net influx into the intestine.

This phase was initiated to investigate the influence of the medium stomach worm on the permeability of the abomasal mucosa to calcium and phosphorus in the weanling calf.

EXPERIMENTAL PROCEDURE

Five weanling Holstein bull calves were used in each of these 2 trials. One was a control while the other 4 calves had been previously infected with cultured larvae of Ostertagia spp. Infection
rate was 120,000 3rd-stage larvae in trial 1 and 150,000 3rd-stage larvae in the 2nd trial.

At 30 days after inoculation all calves in each trial were necropsied and the abomasum from each removed. Each abomasum was flushed with 0.9% saline (NaCl) and a ligature of strong cotton cord was positioned on the 2-inch segment of the duodenum remaining with the excised organ. Each preparation was checked for leakage by infusing 50 to 100 ml saline and applying moderate pressure.

Administration of the tracer dose of radiophosphorus (P-32) and radiocalcium (Ca-45) was accomplished by inserting a 6-inch piece of polyethylene tubing (Clay-Adams P.E. 260) through the anterior end of the abomasum after which a tight ligature was attached to said portion of the organ. Both tracers were introduced through the tube via 50-ml glass syringe and flushed with an additional 20 to 30 saline.

At this point each organ was suspended in 1000 ml 0.9% saline with approximately the posterior two-thirds of the abomasum submersed. The ligated anterior portion remained outside the liquid environment as it was attached to a support above the container via a suspending cord. The thermometer in each container was suspended in a similar manner.

The bathing medium of each preparation was constantly agitated by the magnetic stirrer supporting it. All 5 preparations were housed in a temperature-controlled incubator and a 4-ml aliquot from each suspending medium was withdrawn at a 60-minute intervals for radioassay. The incubation period was 8 hours at 37° C.
The vehicle for the tracers was 0.9% saline which was isotonic to that in which the abomasum were suspended. This was intended to minimize the possibility of a gradient other than that of the isotope mixture. Results were calculated as the percent dose in the environment as a function of time after dosing.

RESULTS AND DISCUSSION

Phosphorus.

Figure 18 shows the rates of radiophosphorus transport across the abomasal wall plotted as the percent dose per unit time in the 1st trial. At the end of 2 hours one of the preparations showed 1.3% of the total dose of tracer phosphorus to be present in the medium. By the 4th hour all preparations, including the control were registering radioactivity. At this point the range in total P-32 was from 1.4% for the control to 5.7% in the calf-42 preparation. Transport of radiophosphorus across the wall into the liquid environment was almost linear for all preparations from 4 to 8 hours after the tracer was infused into the abomasum. The control was consistently lower than any other preparation in quantity of P-32 dissipated into the environment throughout the course of the 1st trial. At the termination of the data collection, maximum radiophosphorus in the medium ranged from 3.5% for the control to 11% for calf-42 preparation.

In the 2nd trial (Figure 19), the control preparation exhibited little difference from those which had been damaged by the worms.
FIGURE 18. RELATIVE RATES OF TRANSPORT OF P-32 ACROSS THE ABOMASAL MUCOSA OF CALVES EXPERIMENTALLY INFECTED WITH 120,000 LARVAE OF OSTERTAGIA SPP.
FIGURE 19. RELATIVE RATES OF TRANSPORT OF P-32 ACROSS THE ABOMASAL MUCOSA OF CALVES EXPERIMENTALLY INFECTED WITH 150,000 LARVAE OF OSTERTAGIA SPP.
The primary deviation seemed to be that, for the control there appeared to be a closer conformance to linearity throughout the course of the trial. Preparations from the infected calves exhibited rather erratic patterns in the rate of P-32 transport across the abomasal wall; however, the maximum percent of tracer in the environment at 8 hours was in close proximity to the observations of the 1st trial. Radio-phosphorus in trial 2 was detectable earlier than in trial 1.

**Calcium.**

Figure 20 shows the rate of passage of radiocalcium (Ca-45) across the abomasal walls of calves in trial 1 which had been infected with *Ostertagia* spp. compared with a similar preparation from a noninfected control calf. Like the patterns of radiophosphorus in the 1st trial, little activity was detectable before 4 hours of incubation. However, the subsequent increases in passage rates was considerably more pronounced than radiophosphorus as one preparation recorded 52% of the Ca-45 in the environment at the end of an 8-hour incubation. The lowest tracer level for a preparation from an infected calf at the end of the incubation was 36% for calf 41. The control calf lagged behind considerably with only 13.8% transported by the end of the trial.

Passage rates of Ca-45 in the 2nd trial (Figure 21) increased at a more rapid rate and terminated with a larger portion of tracer calcium in the environment at 8 hours than trial 1. As was the case with radiophosphorus transport, the rate of calcium passage across the wall was essentially the same for control and parasitized organs.
FIGURE 20. RELATIVE RATES OF TRANSPORT OF CA-45 ACROSS THE ABOMASAL MUCOSA OF CALVES EXPERIMENTALLY INFECTED WITH 120,000 LARVAE OF OSTERTAGIA SPP.
FIGURE 21. RELATIVE RATES OF TRANSPORT OF CA-45 ACROSS THE ABOMASAL MUCOSA OF CALVES EXPERIMENTALLY INFECTED WITH 150,000 LARVAE OF OSTERTAGIA SPP.
It was of interest that, in the case of calcium, more than 50% of the tracer dose found its way across the mucosal barrier. This suggests the presence of some active mechanism other than simple diffusion. In the case of the latter process, rate patterns would have been expected to illicit an accelerated decrease as equilibrium was approached and plateau at 50% values. Since this was not the observed trend, the passage of more than half the tracer dose across the mucosa may be suggestive of an active transport phenomenon.

Rasmussen (1959) reported that in a medium containing physiological amounts of ionized calcium (1.25 mM) inverted sacs of rat small intestine were able to actively transport Ca-45 from mucosa to serosa against a concentration gradient. Schacter and Rosen (1959) made similar observations using Ca-45 and everted gut-sacs prepared from segments of the proximal small intestine of young rabbits, rats and guinea pigs. Vitamin D deprivation in rabbits and rats markedly impaired the capacity for active Ca-45 transport in vitro.

Wasserman et al. (1961) reported that in studies of the living rat, it was observed that ionic calcium was transferred against a concentration gradient and an electropotential gradient by the duodenal membrane. The active transport mechanism is limited in capacity, is dependent on oxidative phosphorylation, and appears to be relatively specific for calcium and magnesium (Schacter and Rosen, 1959).
SUMMARY AND CONCLUSIONS

The rate of transport of radiocalcium and radiophosphorus across the abomasal walls of parasitized calves was compared to uninfected controls using in vitro techniques. In the 1st trial both Ca-45 and P-32 passed out of the antrum of the infected organs at a more rapid rate than the uninfected controls. The 2nd trial did not confirm these results.

At the end of 8 hours of incubation the tracer calcium in both trials was found to be present in the liquid environment in excess of 50% of the initial tracer dose infused into the abomasum. This was interpreted as being indicative that calcium absorbed through the abomasal mucosa is accomplished by an active process.

Data failed to show conclusively that parasitism by Ostertagia spp. nematodes altered the rate of uptake of calcium and phosphorus by the abomasal mucosa.
CHAPTER VI

THE EFFECT OF DIETARY LEVEL ON DEVELOPMENT OF
OSTERTAGIASIS IN WEANLING CALVES

Among the several hypotheses put forth as to how the medium stomach worm may adversely influence bovine calcium and phosphorus metabolism, is the possibility that the nematode may have required the elements in its own metabolism. Such a case would indicate competition between host and parasite for dietary minerals. Investigations should first establish if the nematode does require certain dietary inorganic components and if so to what extent the host is taxed.

The investigation of Barnsdorf and Nyberg (cited by Nyberg 1963) have shown that the tapeworm Dibothriocephalus latus competes with its human host for vitamin B_{12}. Gibson (1963) suggested the possibility of a similar competition between animal hosts and their helminth parasites. Shumard and Herrick (1954) showed that H. contortus larvae cultured from eggs excreted by cobalt-supplemented lambs were of greater length than those cultured from eggs excreted by non-supplemented lambs. Threlkeld et al. (1956) concluded that the ability of H. contortus larvae to establish themselves within the host animal appeared to be dependent upon the presence of cobalt in the host's diet. The more recent work of Downey (1965) indicated that a low level of cobalt in the diet of the host was detrimental to the parasite H. contortus.
The purpose of this trial was to compare the development of experimental infections of *Ostertagia* spp. in 2 groups of weanling calves maintained on rations containing different levels of calcium.

**EXPERIMENTAL PROCEDURE**

The original design of the experiment stipulated that 12 calves were to be used. Six would be fed a ration low in calcium and 6 control calves fed a ration with calcium levels slightly in excess of NRC requirements. However, only 10 calves of similar history and appearance could be procured. Six of the 10 calves took severe diarrhea. Three died and the 3 which survived appeared to be too unthrifty to attempt to collect data. As a consequence, only 4 calves remained healthy enough to withstand an experimental infection of parasites.

The 2 experimental rations fed during the study are shown in Table 10. Feeding of the ration was started 8 days prior to inoculation with the cultured larvae of *Ostertagia* spp. This was intended to condition the calves' calcium metabolism to the dietary intake of calcium and also to establish the nematodes environment prior to his introduction into the host.

On the 8th day of the trial all calves were given an oral dose of 150,000 larvae of *Ostertagia* spp. For the following 30 days after infection calves were maintained on their respective diets at a rate ranging from 2.0 to 2.5% of their body weight per day. At the end of the 30-day period of infection, the calves were necropsied and the abomasums with contents were excised.
Table 10. Experimental Rations Containing Two Levels of Calcium

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>High Calcium</th>
<th>Low Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground Corn</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Rice Hulls</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Urea 281%</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Oyster Shell Flour</td>
<td>3</td>
<td>--</td>
</tr>
<tr>
<td>Salt</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Molasses</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Premix*</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Total Pounds</strong></td>
<td><strong>362.4</strong></td>
<td><strong>359.4</strong></td>
</tr>
</tbody>
</table>

* Premix contained 1.0 lb Aureomycin
  1,500,000 I. U. Vitamin A
  300,000 I. U. Vitamin D

Analyses: Crude Protein 14 14
          TDN 76 76
          Calcium % 0.35 0.03
          Phosphorus % 0.30 0.30

In the laboratory the contents were removed from the abomasums and the walls scoured with 0.9% saline to remove debris and residual nematodes left behind. The washings were included in the other ingesta. To this mixture was added a preservative solution made up of 70% alcohol, 10% formalin, glycerine and water. Immediately following a
thorough mixing a one-fifth aliquot was stored in the refrigerator until the numbers of nematodes present could be ascertained.

The everted abomasums were submerged in a 500 ml volume of pepsin digest solution (9.0 gm concentrated HCl, 5.0 gm pepsin USP in 1000 ml sterile water) and incubated overnight at approximately 30°C. The solution containing the digested mucosa and embedded nematode larvae were separated from the undigested muscularis and serosa. The digestion was terminated by denaturing the enzyme with ethyl alcohol and 10% formalin was added to the digest mixture and refrigerated for subsequent larval counts.

Total nematode population within a calf were determined by pouring a thin layer of the aliquot in a glass dish positioned atop a tracing light. The worms could then be seen with a dissecting microscope and recovered with a pair of small forceps and counted with a hand tally. Nematodes recovered in this manner were placed in a 15 ml vial and fixed in alcohol-formalin. Worms were then classified as either mature or immature and the total numbers of each multiplied by the aliquot to determine the total number of each composing the population.

The procedure for identifying a 4th-stage immature larvae of Ostertagia spp. was described and illustrated by Soulsby (1965). In essence, 4th-stage larvae measured 1.5 to 3.5 mm in length compared to 5.0 to 7.0 mm for adult worms. The male spicules and bursal rays were very underdeveloped being little more than noticeable. In the females, the ovaries were not distinct and the vulva
did not open to the exterior. Early 4th-stage larvae, especially males, had rather pointed tails which tended to become more blunt as the sexual characteristics developed.

After separating and counting the mature and immature larvae in an aliquot, the total of each group was transferred to a porcelain crucible, dried in an oven then ashed in a muffle furnace. The residue was adjusted to a 10 ml volume with 6N HCl and analyzed for calcium by the method described in Appendix table IV. Results were reported in terms of milligrams calcium per gram dry larvae.

The fecal output of ova was reported as eggs per gram feces (EPG). The procedure was described in the general experimental procedure.

RESULTS AND DISCUSSION

The effect of dietary calcium level on Ostertagia spp. development in the calf is shown in table 11. According to the number of eggs voided in the feces, the female nematodes were less productive in the lower calcium group. The average EPG was 7 times higher in the feces of the calves eating the ration containing 0.35% calcium. Bilkovich (1962) credited dietary corn with causing expulsion of stomach worms, *H. contortus* and noted a depressive effect of corn on nematode egg production in ruminants. Todd (1963) suggested that yellow corn lacks a substance essential for normal egg production by *Haemonchus spp.* in calves.
Table 11. The Effect of Dietary Calcium Level on *Ostertagia* spp. Development in the Young Bovine Host

<table>
<thead>
<tr>
<th>Ration Calf No.</th>
<th>0.35% Calcium</th>
<th>0.0% Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ave. EPG&lt;sup&gt;1/&lt;/sup&gt;</td>
<td>5,000</td>
<td>2,000</td>
</tr>
<tr>
<td>Mean</td>
<td>3,750</td>
<td>500</td>
</tr>
<tr>
<td>Mature Larvae</td>
<td>2,810</td>
<td>15,585</td>
</tr>
<tr>
<td>Mean</td>
<td>9,197</td>
<td>5,920</td>
</tr>
<tr>
<td>Immature Larvae</td>
<td>1,525</td>
<td>1,175</td>
</tr>
<tr>
<td>Mean</td>
<td>1,350</td>
<td>2,345</td>
</tr>
<tr>
<td>Mature Larvae Ca&lt;sup&gt;2/&lt;/sup&gt;</td>
<td>71</td>
<td>79</td>
</tr>
<tr>
<td>Mean</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>Immature Larvae Ca&lt;sup&gt;2/&lt;/sup&gt;</td>
<td>153</td>
<td>118</td>
</tr>
<tr>
<td>Mean</td>
<td>136</td>
<td>83</td>
</tr>
</tbody>
</table>

<sup>1/</sup> EPG = Eggs per gram feces

<sup>2/</sup> mg calcium per gram dry larvae

Vetter *et al.* (1963) found that lambs fed a semipurified diet showed greatly decreased worm egg production by *H. contortus* as compared with lambs fed an unpelleted natural diet. In a 2nd experiment worm egg production by the same parasite was significantly less when lambs were fed pelleted vs. nonpelleted natural diet. These investigators suggested that the natural diet supplied some substance required by the parasite for reproduction.
In further studies on the nutrient requirements of *H. contortus*, Theuer et al. (1965) reported that when hay-free rations of widely varying composition were fed to lambs, there was a consistent inhibitory effect on fertile egg production by the parasite which was overcome by alfalfa hay. The average calcium concentration in alfalfa hay is near 1.47% compared to 0.47% for average grass hays (Morrison, 1957). Possibly the difference in dietary calcium levels between alfalfa-supplemented and non-supplemented rations could have accounted for some of the observed variation in parasite egg production reported by Theuer et al. (1965).

Mature larvae recovered from the necropsied host averaged 5,920 for the low-calcium group compared with 9,197 for the high-calcium. There was more variation in the latter group of animals in this respect.

The recovery of immature larvae was in reverse of the mature populations. The average number of 4th-stage worms in the low-calcium calves was almost twice that of the controls and the variation in range was wider.

Chemical analyses showed the calcium concentration of the nematode tissue to be higher in the control animals. This was particularly noticeable in the mature worms where those from the control group averaged 3 times the amount of calcium per unit weight of dry tissue. Immature larvae in general were higher in tissue calcium than the mature worms and the concentration in the controls was nearly twice that of the low calcium.
One of the control calves matured more than 10% of the infective-larval dose, while both the low-calcium calves were near 4% maturity.

Gaafar and Ackert (1953) investigated mineral deficient diets as factors in resistance of fowls to parasitism. When chicks were given diets low in calcium and phosphorus, fewer and smaller *Ascaridia galli* nematodes were found. There was not the usual breakdown of resistance expected in chicks fed on a deficient diet, and it was supposed that the calcium and phosphorus were not sufficient to meet the worms needs. They reported that the effect of low-manganese diet on *A. galli* was too variable for any conclusions to be drawn.

Ross and Gordon (1933) reported that after being fed a ration which was rather low in protein and extremely low in calcium and phosphorus, for 50 to 71 days, aged sheep remained highly resistant to *Haemonchus contortus* infection, and more so than similar sheep on a normal diet.

Possibly the observed resistance mentioned by the foregoing authors (Ross and Gordon, 1933) was in actuality a reflection of the absence of the inorganic elements in the diet. If the dietary calcium and phosphorus were required in the parasited metabolic system, a source deficit could have prevented its normal growth and development.

Downy (1966) described experiments where lambs were reared under worm-free conditions and fed low cobalt diets. Results indicated that the effects of low cobalt intake and mild to moderate ostertagiasis were additive.
SUMMARY

Lowering the dietary level of calcium in the diets of calves infected with *Ostertagia spp.* showed decreases in: (1) the number of nematode ova voided in the feces, (2) average number of mature larvae in the abomasum, and (3) calcium concentration in the tissues of mature and immature larvae. The average number of immature larvae in the abomasum was higher in calves eating the ration deficient in calcium.

The data from this trial were too limited to draw definite conclusions; however, it is the author's opinion that the results of this preliminary trial justify further work in the area. If calcium is required in the metabolism of *Ostertagia spp.*, this may provide an avenue of approach for the development of an effective anthelmintic for these nematodes.
CHAPTER VII

FUTURE RESEARCH

The investigations described in the 1st 6 chapters of this manuscript can most accurately be considered preliminary studies. The 1st series of trials were directed toward determining whether or not the parasitic nematode, *Ostertagia spp.*, exerted any adverse effects on the utilization of calcium, phosphorus and magnesium in weanling Holstein calves. Subsequent experiments were designed to elucidate the mechanism by which the helminth prevent maximum utilization of these inorganic elements.

The concept of the production of toxins by worm parasites is not one of recent origin. Schwartz (1931) and Mönig (1937) published reviews covering the production of toxins by helminths. According to Gordon (1957), there does not appear to be any clear evidence as yet that worms do produce toxins and no toxin from a helminth has been isolated chemically and tested *in vivo*. Some of the so-called toxic effects observed may have been, in actuality, due to allergic reactions following the development of hypersensitivity.

It is the author's opinion that since helminths, in the case of *Ostertagia spp.*, actively feed on nutritive material in their surrounding environment, they must also rid themselves of metabolic by-products. These excreted (or secreted) waste materials, while not directly toxic to the host, may possess the capability to bind
or chelate certain inorganic nutrients of exogenous origin, thus making them unavailable to the host via intestinal absorption. By the same token, the absorption of such by-products into the bloodstream could tie-up the ionic form of the element thereby reducing the exchangeable fraction in the plasma and result in a form which is not readily reabsorbed by the renal convoluted tubules. An increase in the patterns of urinary calcium and phosphorus excretion was observed during several facets of this study. Also there was an increase in the bound phosphorus fractions in the blood plasma corresponding to a decrease in the free form. Such a trend is suggestive of phosphorus binding possibly by some organic compound. Since there are currently techniques available for culturing various nematodes in vitro, it should not be difficult to devise experiments to evaluate the feasibility of pursuing the idea further.

A decrease in balance ratios is indicative of a decline in nutrient utilization. In the case of minerals, this could be narrowed to either a decreased intestinal absorption or an increased mobilization of endogenous stores. There are many factors which could lead to an increased mineral loss from the body stores all of which are manifested by altering the plasma equilibrium. When physiological levels cannot be maintained from exogenous sources the difference is made up from endogenous sources.

Use of the comparative balance method (Comar et al. 1953) for the determination of endogenous calcium and phosphorus is one of
the more commonly used methods. This is partially due to the fact that it is not necessary to establish a "steady state" required in the isotope dilution method (Kleiber et al., 1951 and Visek et al., 1952) which helps to minimize the monetary expense of data collection. Both methods, however, are considerably expensive in terms of time required to acquire an endogenous value. The endogenous values reported in these studies were admittedly sparse, but in order to explore other possibilities with seemingly equal merit, the investigation was not carried beyond the scope which was discussed. The author feels that the influence of Ostertagia spp., as well as other species, on endogenous mineral metabolism in cattle should be continued with a revised and more intensive approach.

Future endeavors should relate the endogenous losses and blood levels of calcium and phosphorus to thyroid and parathyroid activity as well as histological examinations of the glands. The height of the follicular epithelium in the parathyroid glands is sometimes used to index the activity of the organ. According to Turner (1961), in hyperthyroid states there is increased mobilization of calcium from the skeleton and increased loss through the urine and feces. Andrews (1939) and Andrews et al. (1944) noticed an increase in energy metabolism of infected sheep; however, there was no evidence of decreased calcium and phosphorus absorption.

White et al. (1968) stated that "... as bone crystals dissolve, citrate is released and appears in the plasma." It would seem plausible that such information could serve as a valuable aid in supporting
endogenous calcium studies. Since citrate has the capacity to bind calcium this may have been the form of the element which showed an increased rate of urinary excretion.

Andrews (1939) and Andrews et al. (1944) also theorized that when diarrhea occurred, it was probably due to increased intestinal activity and to retention of guanidine as a result of the severe and prolonged water loss. The guanido residue is a strongly alkaline substance and could feasibly bind divalent ions such as calcium. The retention of excess guanidine in the blood stream could have been a factor in the elevated blood calcium values noted in some of the previous studies with *Ostertagia spp*.

In addition to increased endogenous excretion, the efficiency of mineral utilization can be lowered through decreased absorption from the gut. Several factors could contribute to decreased intestinal absorption, one of them being the traumatic effect of the worm on the abomasal mucosa of the host. It was originally believed that the permeability of the mucosa was mechanically altered by the parasite, and although the results of the 2 trials reported did not support the hypothesis, the idea is not completely without foundation. The alleged mechanism of alteration may have been more complex than the experimental design was capable of elucidating.

One of the prime concepts of decreased calcium and phosphorus absorption is thought to be concerned with a rise in pH of the gastrointestinal contents. Anderson (1965) reported that ostertagiasis is characterized by loss of weight and diarrhea along with an increase
in plasma pepsinogen and abomasal pH. Horak et al. (1965) used abomasal fistulas in sheep infected with *Ostertagia circumcincta* and observed that infected animals showed loss of appetite and weight with an increase in abomasal pH and a decrease in pepsin concentration. The accumulation of the enzyme precursor and a decline in the active principle suggests that the conversion reaction is being inhibited either by a blocking agent or in the absence of the necessary catalytic agent(s). Hydrochloric acid (HCl) is one of the catalysts involved in the conversion of pepsinogen to pepsin and is produced by the parietal cells of the abomasum. Coincidentally, *Ostertagia spp.* undergoes its histotrophic phase of development in that region of the true stomach where parietal cells are normally most active. There is evidence to suggest that the trend toward alimentary alkalinity associated with the parasite may be due to a decrease in the production of hydrochloric acid. Although it is possible that the diarrhetic condition may lower the concentration of HCl, either mechanism could lead to decreased availability of dietary calcium and phosphorus and both are worthy of further investigation.

Another aspect of decreased absorption which was investigated with unsatisfactory success was the possibility that the corresponding increase in rate of passage of ingesta, common to ostertagiasis, may shorten the time of exposure of the nutrients to the absorbing membranes of the alimentary tract. Gordon (1957) postulated that "The severe diarrhea which occurs in trichostrongylosis and in some of the trichostrongylidoses of cattle may also add to the effects of
malnutrition by rushing ingesta through the alimentary canal too fast for proper digestion and absorption. Massive injections of cascara segrata were ineffective in promoting scouring in weanling calves in the present studies. The search should be continued for a compound which can be administered parenterally and will bring about varied degrees of diarrhea. Subsequent studies could accomplish 2 aims. First, by simulating diarrhea associated with ostertagiasis, the influence of rate of ingesta flow due to parasitism could be ascertained. Should there be an adverse effect on nutrient absorption, such information could aid the practitioner in therapy of heavily infected animals. Since present anthelmintics are not very effective against helminths such as Ostertagia spp. and Oesophagostomum spp., which go through a histotrophic phase, the drain on the nutritional economy of the infected animal could be modified by alleviating the diarrhea. Secondly, data in the literature indexing intestinal absorption and rate of ingesta passage appears to be sparse if not completely nonexistent. Such information should be made available not only to nutritionists, but also to other investigators involved in metabolic studies.

In the line of decreasing availability of nutrients to the host animal, one might suspect from the fundamental definition of a parasite that there would be competition between the two for nutrients. Barnsdorf and Nyberg (cited by Nyberg 1963) demonstrated this to be true with the human tapeworm, Dibothriocephalus latum, in the case of vitamin B\textsubscript{12}. Gibson (1963) theorized similar competition between animal hosts and their helminth parasites.
Preliminary observations in these studies indicated *Ostertagia* spp. did not develop as well when the host was on a calcium deficient diet. There was some indication that the mature as well as the developing larvae tended to concentrate calcium in their tissues. The influence of host dietary calcium levels on the growth and development of *Ostertagia* spp. should be explored more intensively. Also, there should be a thorough investigation into the metabolic processes necessary for the worm's sustenance. If calcium, cobalt (Downey, 1965) or any other nutrient element could be "metabolically mapped" in the parasite, science would be able to take a positive step forward in the development of an anthelmintic which would be effective against the parasite regardless of the nature of its life cycle.
APPENDIX TABLE I

Inorganic Phosphorus Analysis

Reagents:

1. Trichloroacetic acid, 12% (w/v). Dissolve 120.0 gm. trichloroacetic acid (TCA) in water and dilute to exactly one liter.

2. Sulfuric acid, 10N. Add slowly to about 700 ml. distilled water 278 ml. concentrated H₂SO₄. Cool, and dilute to one liter with distilled water.

3. Ammonium molybdate, stock solution 10%. Add 50 gm. (NH₄)₆Mo₇O₂₄·4H₂O into a liter beaker and add 400 ml. of 10N sulfuric acid with constant stirring to prevent caking. When completely dissolved, transfer the solution to a 500 ml. volumetric flask and wash in quantitatively with 10N sulfuric acid to the mark.

4. Ferrous sulfate-ammonium molybdate reagent. Prepare just prior to using. Transfer 10.0 ml. of ammonium molybdate stock solution to a 100 ml. volumetric flask and dilute to about 70 ml. Add 5.0 gm. of FeSO₄·7H₂O, make up to volume with water and shake until the crystals are dissolved. Transfer to a brown glass bottle.

Procedure (Serum):

1. To a test tube containing 3.5 ml. of 12% TCA add 0.20 ml. of serum, mix, allow to stand for 10 minutes and then centrifuge rapidly for 10 minutes. Include a blank containing 0.20 ml. water and 3.0 ml. of 12% TCA.

2. Transfer 3.0 ml. of the clear supernatant filtrate to a cuvette and add 2.0 ml. of ferrous sulfate-molybdate reagent.

3. After 1.0 minute (or within 2 hours) read the unknown against the blank at a wavelength of 660 mu using the filter and a red-sensitive phototube.

4. Determine the concentration of phosphorus as mg. P/100 ml. serum from a standard curve.
APPENDIX TABLE II

Determination of Total Phosphorus

Reagents:

1. 1-amino,2-naphthol,4-sulfonic acid. To 340 ml. water add 60 gm. of Sodium meta-bisulfite. To this solution add 10 ml. of 10% Sodium Sulfite (9 ml. H2O / 1 gm Na2SO3). Now add 1 gm of the 1,2,4, amino-napthol-sulfonic acid which has had a small amount (2-4 ml.) of hot water poured on it. For this last step weigh the sulfonic acid on an aluminum dish and add the hot water to it. Date and store in refrigerator. Solution is stable for only four weeks.

2. Molybdic Acid Stock Solution: Dissolve 40 gm. Molybdic Oxide (MoO3) in 50 ml. 20% (by weight) NaOH. Dilute with H2O to 800 ml. Before using, dilute 1:1 with 10% H2SO4. (add few more ml. NaOH until MoO3 goes into solution).

3. Standard Phosphorus Stock Solution: (10 mg/100 ml) Dilute 0.4583 grams of Sodium biphosphate (Na2HPO4) to one liter with water. Dilute 10 ml. of stock solution to 100 ml. with water (10 ml. stock / 90 ml. H2O) to give a concentration of 10 ug P/ml.

Procedure:

1. Weigh sample in a crucible.

2. Ash at 600°C for 24 to 36 hours and make up to a known volume with 6N HCl.

3. Take 1.00 ml of the sample and dilute to 100.0 ml. with water. Then take an aliquot (1.0 ml) of the diluted sample and place in a tube having a 25.00 ml. mark.

4. Add 1.0 ml. of Molybdic acid / H2SO4 solution and allow to sit for 5 to 10 minutes.

5. Add 0.40 ml. of 1,2,4 amino-napthol-sulfonic acid. Shake, allow to stand for 10 min., then dilute to 25.00 ml. with water.

6. After 20 minutes read percent transmittance on a spectrophotometer at 700 mu wavelength. Use a conversion chart to change % T to optical density.
Appendix Table II (continued)

7. Prepare 3 standards by adding 20 ug P to a tube and following steps 4-6. (2.0 ml. of 1:10 dil. of stock P).

8. Prepare a blank by adding 1.0 ml. of triple distilled water to a tube and following steps 4-6. The blank is used to set the needle of the spectrophotometer on "zero."

Calculate:

\[
\frac{(\text{Aliquot})(\text{Mg P in Std})}{(\text{Ave. O.D. Std})} = K_p
\]

\[
\frac{(\text{O.D. of Sample})}{(\text{Sample weight})} \cdot K_p = \text{mg P/gm Sample}
\]
APPENDIX TABLE III

Determination of Total Calcium

Reagents:

1. Calcium Standard: (2.0 mg. Ca/ml.) Dissolve 5.0160 gm. CaCO$_3$ (dried in oven) in 50 ml. of 2N HCl. Dilute to 1.0 liter.

2. Ammonium Oxalate: 45 gm liter warm slightly

3. Wash Solution: 2N NH$_4$OH - saturated with Calcium Oxalate. 264 ml. conc. NH$_4$OH (58%), dilute to 2 liters then add Calcium oxalate beyond saturation point.


5. One volume water to one volume acetic acid.

6. One volume water to one volume ammonium hydroxide.

7. Stock Solution KMnO$_4$ (approximately 0.2N) 6.5 gm. KMnO$_4$ per liter.

   For 0.01N: 10 ml. per 200 ml.
   For 0.02N: 10 ml. per 100 ml.

8. Methyl red indicator: 100 mg. methyl red in 60 ml of alcohol diluted with 40 ml. distilled water.

Procedure for Titrimetric Determination:

1. Weigh sample in a crucible.

2. Ash the sample at 600°C for 2½ to 3½ hours and make up to a known volume with 6N HCl.

3. Pipette an aliquot (record) into a 40 ml. centrifuge tube (1.0 ml. for Feces -- 3.0 ml. for Urine).

4. Add one drop Methyl Red, 3 ml. Ammonium Oxalate, and bring to a faint pink color by adding first, 1:1 NH$_4$OH, then 1:1 acetic acid.
APPENDIX TABLE III (continued)

5. Allow to stand overnight.

6. Centrifuge at 3,000 RPM for 30 min. and decant.

7. Wash precipitate by spraying with wash solution then centrifuge for 10 minutes. Repeat this washing three times decanting supernatant each time.

8. Add 3.0 ml. of 10% H₂SO₄, heat in a 90°C water bath for 20 minutes or until all solids are dissolved.

9. Dilute stock solution of KMnO₄ to desired strength, filter, and use a bit to rinse the water from the micro-burette. Titrate to first pink color that lasts at least 15 seconds. After titrating the samples, rinse burette and fill with H₂O.

Procedure for Gravimetric Determination and radioassay of Ca-45:

1. Proceed according to steps 1-9 of the titrimetric determination.

2. Add 8 mg of carrier calcium (4 ml. of standard solution @ 2 mg/ml.). Add 2-4 drops of methyl red, then 3.0 ml. of ammonium oxalate. Adjust color to a faint pink by adding 1:1 NH₄OH or 1:1 acetic acid as needed.

3. Allow to stand overnight.

4. Centrifuge at 3,000 RPM for 30 min. and pour off supernatant.

5. Wash with solution No. 3 and transfer into the plastic cup assembly which has been previously weighed and prepared.

6. Centrifuge at 3,000 RPM, pour off supernatant, and remove the tube from the cup.

7. Dry in oven at 110°C or under a heat lamp. Caution - rapid drying will cause the precipitate to crack and peel.

8. Weigh and count. Record shelf number for sample and standard.
APPENDIX TABLE III (continued)

Calculations

Titration method:

\[
\left( \text{mg Ca in Std} \right) \left( \frac{\text{ml. KMnO}_4 \text{ for samp.}}{\text{fr. wt. of samp. gm.}} \right) \text{(aliquot factor)} = \frac{\text{mg. Ca}}{\text{gm. fr. wt.}}
\]

Gravimetric method:

\[
(0.2743) \text{(Aliquot factor)} \left( \frac{\text{wt. Ca Oxalate ppt.}}{\text{fr. wt. sample gm.}} \right) = \frac{\text{mg. Ca}}{\text{gm. fr. wt.}}
\]
APPENDIX TABLE IV

Analyses of Calcium and Magnesium by Atomic Absorption Spectrophotometry

Reagents:

1. Stock Lanthanum-TCA solution. Weigh 2.5 gm. La₂O₃ and add sufficient 6N HCl to put in solution (C.A. 25 ml.). To a funnel over a 500 ml. volumetric flask, add 100 gm. Trichloroacetic acid. Pour the Lanthanum-HCl sol. over the TCA and finish the dilution with distilled water.

Procedure (Serum):

1. Dilute 0.4 ml. serum and 2.0 ml. Lanthanum-TCA stock sol. to 10.0 ml. with distilled water. (Factor = 25X).

2. Centrifuge at 3,000 RPM for 10 minutes.

3. Decant supernatant and retain for exposure to the flame.

Procedure (Feces, Urine or other tissue):

1. Weigh sample in crucible, dry and ash at 600°C in a muffle furnace overnight.

2. Allow to cool slowly then wash sides down with 2-3 ml. 6N HCl. Wash contents of crucible into a test tube calibrated to 10.0 ml. and bring to volume with distilled water. Mix carefully but thoroughly on a vortex mixer for 5-10 seconds.

3. Pipet 1.0 ml. (for 1/100 dilution) of the supernatant into a 10.0 ml. volumetric flask (2.0 ml. for a 1/50 dilution).

4. Add 2.0 ml. Lanthanum-TCA stock solution and bring to volume with distilled water. Mix well by inversion. Store supernatant liquid in stoppered, identified tube. This solution is stable and ready for analyses by Atomic Absorption Spectrophotometry.

NOTE: Sample may require further dilution. Use appropriate lamp for element in question.
APPENDIX TABLE V

Calculation of Endogenous Fecal Calcium Using Radio-Calcium

And the Comparative Balance Method

\[
\text{Endogenous Calcium} = \frac{100 - \text{fecal Ca}^{45}}{1 - B} - (100 - \text{fecal calcium})
\]

where "B" = percent I.V. dose of tracer excreted in feces.

APPENDIX TABLE VI

Linear Regression of Various Factors on Time
Post Inoculum with Ostertagia spp.

Factors Regressed on Time:
1. Total Plasma Phosphorus
2. Bound Plasma Phosphorus
3. Phosphorus Balance Ratios
4. Plasma Calcium
5. Calcium Balance Ratios
6. Magnesium Balance Ratios

General Regression Equation:

\[ y = a + bX \]

where: \[ a = \bar{y} - b\bar{x} \]
\[ b = \frac{\Sigma xy}{\Sigma x^2} \]

In calculating these values, measurements were taken on four animals at each level of post inoculum time.

An analysis of Regression was conducted to test the null hypothesis that \( \beta = 0 \).
APPENDIX TABLE VI (continued)

Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>(SS_R)</td>
<td>(MS_R)</td>
<td>(F_R)</td>
</tr>
<tr>
<td>Deviation from Regression</td>
<td>(N-2)</td>
<td>(SS_E)</td>
<td>(MS_E)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>(N-1)</td>
<td>(SS_{tot})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[SS_{tot} = \Sigma y^2\]

\[SS_R = b \Sigma xy\]

\[SS_E = \Sigma y^2 - b \Sigma xy\]

\[MS_R = SS_R\]

\[MS_E = SS_E / (N-2)\]

\[F_R = MS_R / MS_E\] with 1 and \(N-2\) df
### APPENDIX TABLE VII

Composition of Experimental Ration

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Pounds per Ton</th>
</tr>
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<tbody>
<tr>
<td>Ground Yellow Corn</td>
<td>1,422</td>
</tr>
<tr>
<td>Urea (45% Nitrogen)</td>
<td>26</td>
</tr>
<tr>
<td>Cottonseed Hulls</td>
<td>400</td>
</tr>
<tr>
<td>Cane Molasses</td>
<td>100</td>
</tr>
<tr>
<td>Dicalcium Phosphate</td>
<td>12</td>
</tr>
<tr>
<td>Oyster Shell Flour</td>
<td>15</td>
</tr>
<tr>
<td>Salt</td>
<td>10</td>
</tr>
<tr>
<td>Premix*</td>
<td>15</td>
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#### Chemical Analysis:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>10</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>10</td>
</tr>
<tr>
<td>Total Digestible Nutrients (TDN)</td>
<td>67</td>
</tr>
<tr>
<td>Calcium</td>
<td>.53</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>.33</td>
</tr>
<tr>
<td>Magnesium</td>
<td>.42</td>
</tr>
</tbody>
</table>

* Premix contained 1.0 lb. Aureomycin
  1,500,000 I.U. Vitamin A
  300,000 I.U. Vitamin D
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VITA

The author was born November 22, 1937, in Pine Bluff, Arkansas, the son of Mona Barrett and Lester A. Waymack. He attended grammar school at Sam Taylor and was graduated from Watson Chapel High School in 1956. He enrolled in the College of Agriculture, University of Arkansas in September of the same year.

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Major Field:  Animal Science

Title of Thesis:  The Metabolic Behavior of Inorganic Elements in Calves with Subclinical Ostertagiasis

Approved:

R. Wallace Killett
Major Professor and Chairman

Max Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

E. Patrick

Donald M. Cressler

George L. Robertson

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Date of Examination:

July 21, 1969