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The influence of magnesium and oxalic acid upon the absorption and retention of calcium and phosphorus

K. N. Govindan Nagar

To the Graduate Council:

I am submitting herewith a thesis written by K. N. Govindan Nagar entitled "The influence of magnesium and oxalic acid upon the absorption and retention of calcium and phosphorus." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

M. C. Bell, Major Professor

We have read this thesis and recommend its acceptance:

R. L. Murphree, O. G. Hall, N. S. Hall

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 4, 1958

To the Graduate Council:

I am submitting herewith a thesis written by K. N. Govindan Nayar entitled "The Influence of Magnesium and Oxalic Acid Upon the Absorption and Retention of Calcium and Phosphorus in Lambs." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

M. C. Bell
Major Professor

We have read this thesis
and recommend its acceptance:

R. L. Murphree

O. Glen Hall

Nathan S. Hall

Accepted for the Council:

Dale Bentley
Dean of the Graduate School

THE INFLUENCE OF MAGNESIUM AND OXALIC ACID UPON THE ABSORPTION AND RETENTION OF CALCIUM AND PHOSPHORUS IN LAMBS

A THESIS

Submitted to
The Graduate Council
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

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by

K. N. Govindan Nayar

August 1958

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K. N. Govindan Nayar

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CHAPTER I

INTRODUCTION

While the physiological importance of some of the mineral elements contained in the body was recognized as early as one hundred years ago, our specific knowledge of their nutritional significance is due primarily to research carried out during the last thirty years. Further, it has come to be realized that interrelationships among certain minerals in the diet, as well as the actual amounts, govern both their usefulness and also their harmful effects.

In practical nutrition of farm animals apparently the only minerals that are ever deficient as listed by Dukes (1955) are sodium, chlorine, phosphorus, calcium, iodine, iron, copper, cobalt and manganese, with iron being a practical problem only during the suckling stage in pigs.

Of these, calcium may be said to be the most important inorganic element in the body as it occurs in it in the highest amount. Phosphorus is so closely related to calcium in metabolism and nutrition that it becomes as important as calcium and it is usual to discuss the calcium-phosphorus metabolism as one entity. Over 70 per cent of the ash of the body consists of calcium and phosphorus. As early as 1842 it became recognized through the work of Chossat, cited by Maynard and Loosli (1956), that an insufficient amount of calcium in the diet causes poor bone formation in many species of animals. During the next twenty years studies both in France and Germany showed that skeletal development in various species of farm animals was dependent upon the supply of calcium and phosphorus in the ration and that

deficiencies could be corrected by feeding bone meal and other sources of minerals. Discovery of Vitamin D in 1922 gave a great impetus to the studies of the metabolic processes involved.

Normal calcium-phosphorus nutrition depends primarily upon sufficient supply of each element, a suitable ratio between them and the presence of Vitamin D. Irrespective of the forms in which calcium and phosphorus are ingested their absorption is dependent upon their solubility at the point of contact with the absorbing membranes. The absorption of both calcium and phosphorus is thus favored by factors which operate to hold them in solution. Some minerals like iron, aluminum and magnesium have been considered to interfere with absorption of phosphorus and oxalic acid or oxalates present in spinach and other chenopodiaceae with calcium. However, much of the experimental work forming the basis of such general statements have been usually done using rats and the extrapolation of those results to ruminants is not justified.

The present work was undertaken to study the effect of excess of magnesium and oxalic acid on calcium-phosphorus utilization, retention and excretion when added to a normal balanced ration fed to lambs.

The conventional digestibility study involving striking the balance between the income as represented by the feed and the out-go in feces, usually employed for the proximate principles of foodstuffs in not adequate when studying the utilization of minerals because the feces contain in addition to the unabsorbed minerals, an endogenous fraction as well. Such an inherent error masks the true availability of minerals from livestock feeds unless the endogenous fecal loss is known. In the past, attempts

were made by various workers to estimate endogenous fecal excretion under fasting conditions, but this was found to be unsatisfactory as it imposes abnormal conditions on the animal. Advent of the use of radioactive isotopes has solved the problem and the accepted techniques with radio-isotopes have been employed in this study.

CHAPTER II

REVIEW OF LITERATURE

The importance of calcium-phosphorus metabolism has been recognized for over a hundred years and extensive work has been done on this subject. Consequently, a comprehensive review of literature on this topic presents certain special problems. A number of excellent reviews have appeared in scientific literature during the course of years, either on the whole or parts of the topic and each reviewer has attempted to view it only from the perspective of his special interest. Most of these have a bearing on clinical conditions and nutrition of man and in many instances one may search in vain through all these reviews for some specific information applicable to problems presented by animal nutrition. Probably the Annual Review type of article and a few available monographs are the best to keep abreast of progress.

Irving (1955) surveyed literature on calcium metabolism, weighed the various findings, and presented a summary based on his own conclusions from the published observations in the field. Most of his discussions apply to man and there is only limited reference to animal nutrition.

Howard (1957) reviewed certain current concepts about calcium metabolism, bones and calcium homeostasis.

Various authors reviewed the subject of mineral metabolism in the different volumes of the annual review of Biochemistry. Greenberg (1939) discussed the occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism as an extension of previous reviews

on the subject. He emphasized the development of new techniques particularly the use of radioactive isotopes as indicators of metabolism and the genesis of the then new concepts in the field of mineral metabolism. Maynard and Loosli (1942) and McCance and Widdowson (1944) presented general reviews on the subject. Sendroy (1945) considered developments bearing on the chemical and physiological activities not only of the metallic elements and salts but of certain acid-base metabolites. Maynard and Smith (1947) in their presentation emphasized the nutritional aspects. Cohn et al. (1942) on the other hand emphasized the clinical aspects. Davis and Loosli (1954) reviewed mineral metabolism in animals.

A symposium in two volumes was edited by McEllroy and Glass (1951). However, this contains more information on the intermediary metabolism of phosphates and little on the digestibility and absorption of phosphorus.

Though it is now recognized that interrelationships among certain minerals in the diet as well as actual amounts govern their usefulness or harmful effects, much less work appears to have been done on the effects of excesses of trace minerals on calcium-phosphorus metabolism, than on the effects of deficiency.

Tibbets and Aub (1937) found that ingestion of extra magnesium as magnesium lactate increased the urinary calcium in normal human subjects and also increased the calcium drain in both hyperparathyroidism and that caused by ammonium chloride. The loss in calcium produced by these agents could be checked by a large intake of sodium acid phosphate.

Talpatra et al. (1949) found that although it was known that calcium oxalate was not assimilated during digestion, the presence of oxalic acid

did not appear to interfere with the assimilation of calcium in ruminants. Oxalates of sodium and potassium appeared to be converted to bicarbonate or carbonate and if present in large quantities induced severe alkalosis.

Examination of the available literature on the subject has not revealed any other reference to work done to study the effects of excess of magnesium or oxalic acid added to the diet of ruminants. Evidently very little appears to have been done on this. Even the two studies referred to above, appear to have been made by the conventional gross balance methods and does not give any true picture of the details of the processes involved.

Precise methods for estimating endogenous calcium and phosphorus under varying conditions would be most helpful for calculation of true digestibility of these elements and for determinations of net as well as dietary requirements for functions such as maintenance, lactation or growth and for studying the effects of excess or deficiency of other factors in the diet on their utilization. Until a few years ago, endogenous fecal calcium and phosphorus had been determined under fasting conditions by various authors, but this is not satisfactory for obvious reasons. This and some other methods of study have now been replaced by methods employing radioisotopes. The older methods are at present only of historical interest and so are not reviewed here.

The availability of radioactive isotopes has provided investigators of metabolism in animals with techniques of unusual scope and flexibility. An element which occurs in the diet at a level of 0.05 ppm or less can still be followed from feed to ultimate location within a specific tissue

of an animal weighing 1000 pounds without increasing the amount of the element in the diet above the physiologic level. Davis (1957) has presented a summary of the use of radioactive micronutrients in metabolism in animals, and Comar and Wasserman (1957) of macronutrients. For principles of tracer methodology, reference may be made to Comar (1955) and Kamen (1957). Modifications of isotope dilution method for determination of fecal endogenous losses of phosphorus in livestock have been presented by Kleiber et al. (1951), Lofgreen et al. (1952) and Lofgreen and Kleiber (1953, 1954). Similar methods for calcium have been discussed by Comar et al. (1951, 1953) and Viseck et al. (1953). Comar and Wasserman (1956) have given a review on radioisotopes in the study of mineral metabolism.

CHAPTER III

EXPERIMENTAL PROCEDURE

The use of various species of farm animals for the study of nutritional problems, metabolism and radiation therapy is gaining increasing popularity. Boars, gilts, weanling pigs, ewes, wethers, steers, heifers and milk cows have been successfully employed. Metabolism cages and equipment for balance studies using radioactive isotopes should satisfy certain basic requirements, which have been described by Hansard (1951). He also summarized the pertinent literature and described adaptable metabolism units of simple design and operation that have through use been found entirely satisfactory for balance studies involving radioisotopes with either sex of the various species of farm animals and complete constructional and functional details are given.

Very important operations in metabolism investigations using radioisotopes are quantitative administration of isotopes intravenously, intreperitoneally or orally and taking blood samples. The usual precautions to be observed in handling radioisotopes have been given in Bureau of Standards Handbook 42, in the various U. S., A. E. C., Isotope Division Circulars and other publications. When large animals are used there are additional considerations which also have been discussed by Hansard (1951). The metabolism units employed, and techniques of quantitative administration of isotopes and collection of samples employed in this study were those described by Hansard (1951) and Hansard et al. (1951).

Animals

For the study, eighteen wether lambs were chosen from a group of forty-six animals recently obtained. These had been examined by a

veterinarian for freedom from any diseases and had been dewormed. The eighteen animals were chosen on the basis of apparent condition of the animals and uniformity of body weights. The body weights ranged from 61 pounds to 71 pounds and the average was 66.2 pounds.

These animals were divided into three groups of six animals and each group further subdivided into two subgroups of three animals in each. The different animals were assigned to the different groups and subgroups in such a way as to make the average weight per animal of each subgroup as nearly uniform as possible. Each animal had an ear tag to distinguish it from the rest. Group I was to be used as controls; Group II for treatment with magnesium and Group III for treatment with oxalic acid. One subgroup in each group (to be called subgroup a) was to be dosed with the radioisotope intravenously and the other subgroup (subgroup b) was dosed orally. Details of the groups are given in Tables I and II.

Experimental Ration

The animals were all fed a composite ration containing adequate amounts of dry matter, digestible protein and total digestible nutrients. The roughage was cottonseed hulls. A quantity of feed sufficient for a few days was made at one time, the ingredients being very thoroughly mixed in the feed mixer to produce a uniform distribution of all the ingredients throughout the quantity of feed mix. The composition of the basal ration is given in Table III.

Each animal was expected to eat 2 to 2.5 pounds of feed mix per day. Two and one-half pounds of the mixture contained 2.15 pounds of dry matter, 0.20 pound of digestible protein and 1.64 pounds of total digestible nutrients and was considered adequate for maintenance according to standards of

TABLE I

EXPERIMENTAL ANIMAL GROUPS

Trial I

	Animal number	Initial weight	Final weight
		lbs.	lbs.
Group I - Controls			
Subgroup (a)	1	68.0	68.0
	2	67.0	66.0
	3	64.0	63.0
Average		66.3	65.7
Subgroup (b)	4	63.0	60.0
	5	68.0	65.0
	6	68.0	65.0
Average		66.3	63.3
Group II - Magnesium treatment			
Subgroup (a)	7	66.0	63.0
	8	65.0	66.0
	9	68.0	65.0
Average		66.3	64.7
Subgroup (b)	10	68.0	69.0
	11	61.0	65.0
	12	68.0	65.0
Average		65.7	66.3
Group III - Oxalic acid treatment			
Subgroup (a)	13	65.0	63.0
	14	63.0	64.0
	15	71.0	68.0
Average		66.3	65.0
Subgroup (b)	16	66.0	65.0
	17	67.0	62.0
	18	66.0	62.0
Average		66.3	63.0

TABLE II

EXPERIMENTAL ANIMAL GROUPS

Trial II

	Animal number	Initial weight	Final weight
		lbs.	lbs.
Group I - Controls			
Subgroup (a)	1	80.0	81.0
	2	87.0	82.0
	3	70.0	74.0
Average		79.0	79.0
Subgroup (b)	4	70.0	72.0
	5	58.0	50.0
	6	78.0	77.0
Average		68.7	66.3
Group II - Magnesium treatment			
Subgroup (a)	7	83.0	78.0
	8	82.0	80.0
	9	72.0	74.0
Average		79.0	77.3
Subgroup (b)	10	84.0	85.0
	11	78.0	82.0
	12	61.0	66.0
Average		74.3	77.7
Group III - Oxalic acid treatment			
Subgroup (a)	13	78.0	81.0
	14	72.0	73.0
	15	82.0	81.0
Average		77.3	78.3
Subgroup (b)	16	66.0	73.0
	17	75.0	79.0
	18	75.0	77.0
Average		72.0	76.3

TABLE III

COMPOSITION OF BASAL RATION

	Pounds	Grams
Cottonseed hulls	200	
Corn meal	296	
Soybean oil meal	60	
Alfalfa meal	40	
Iodized salt	4	
Total	600	
Supplements added to the above quantity of feed mix:		
Calcium carbonate ($CaCo_3$)	1.2	
Vitamin A and D supplement (containing 20,000 I. U. Vitamin A and 2,500 U. S. P. units of Vitamin D_2 per gram		50

Morrison (1956). Phosphorus contained in the feed ingredients was adequate, but calcium was insufficient, so calcium carbonate had to be added. Two and one-half pounds of the final composite ration then supplied 0.007 pound calcium and 0.006 pound phosphorus.

Treatment with Magnesium

Magnesium was added in the form of magnesium carbonate. Magnesium was expected to combine with phosphate ions liberated during digestion and to produce magnesium phosphate. There are three magnesium phosphates, the monobasic, the dibasic and tribasic, in which the ratio of magnesium to phosphorus is 1:2, 1:1, and 3:2, respectively. Purely on the basis of their atomic weights, magnesium contained in 12 grams of magnesium carbonate is enough to convert all the phosphorus in the daily feed of each sheep to the insoluble tribasic magnesium phosphate. However, this amount of magnesium carbonate was expected to induce scouring which some animals show when put in metabolism units even without any such added laxative. Therefore it was decided to administer to each animal 6 grams of magnesium carbonate per day in 3 gram doses fed twice daily well mixed with the feed. It was considered that this amount was large enough to show its effect on utilization of calcium-phosphorus and at the same time small enough not to be a contributory factor to scouring.

Treatment with Oxalic Acid

It was considered that greater caution was needed in determining a suitable dose of oxalic acid, as in large doses it is considered to be a poison. If all the calcium in the diet was to be converted to calcium

oxalate, about 9 grams of oxalic acid would have been required. A safe maximum dose for sheep was not given in any of the literature consulted. Therefore, the dose was arbitrarily fixed at 6 grams per day, hoping that this was well within the toxic level. However, this assumption was verified by a preliminary feeding trial on two lambs, to whom gradually increasing doses from 1.5 grams to 3.0 grams of oxalic acid were given twice daily with each feed. The 6 gram dose was given to the experimental animals in 3 gram doses fed twice daily with each feed. The animals were watered twice daily ad libitum.

Preliminary Conditioning

All the animals were given the composite basal ration at the rate of 2 pounds per animal per day for a week to accustom them to the ration and they were then placed in dual unit type metabolism stalls described by Hansard et al. (1951), fed the same quantities of the ration and kept there for ten days to condition them to the restraint to which they are necessarily subjected. Within this period they became accustomed to these conditions as was evidenced by the general behavior of the animal and by satisfactory feed consumption. Then the animals were weighed, separated into the groups and subgroups and placed back in the metabolism stalls. The weights shown in Table I are the weights which were then recorded.

Trial Period I

At the end of the preliminary conditioning, Trial I was commenced. Each animal was dosed with 1 mc. Ca^{45} and 1 mc. P^{32}. The radioisotopes

were administered to the animals of the three subgroups a, intravenously and to the three subgroups b orally, and this marked the commencement of the trial period. Daily collection of urine and feces was started. Urine was filtered through two layers of cheese cloth tied over the mouth of the collection bottle, and feces was collected in paper lined metal containers. Weighed amounts of feed mixture was placed in the feed boxes at 8:00 a.m. and 3:00 p.m. each day and a well mixed sample of each day's feed mix was saved for later analysis. Any quantity of feed refused by the animal was weighed and recorded and kept apart for analysis. The daily samples of feed mix and the feed refused by each animal were pooled in individual paper bags.

Since the proper collection of samples is just as important as the accuracy with which the subsequent analysis is done, great care was exercised in collecting a representative sample. Feces was well mixed before sampling because successively passed excrete may differ widely in activity, especially at short times after administration of isotopes. Cross contamination was avoided to the most possible extent, because of the extremely high dilutions which were measured. The total feces and urine collected each day were weighed. Representative samples of the whole feces and urine were taken in Mason jars with tight-fitting lids, and refrigerated. Though it was preferable to take a sample for analysis immediately, inasmuch as this was not practicable, the procedure mentioned above had to be adopted to avoid moisture loss and putrefaction. About 30 ml. of blood was drawn from each animal into centrifuge tubes containing Heparin, 24, 96, 120, 144, and 168 hours after dosing. The blood was

centrifuged immediately and clear plasma collected into 15 ml. tapered bottom centrifuge tubes; it was centrifuged once again if necessary, tightly corked then stored in the refrigerator.

At the conclusion of the collection period of seven days, the animals were removed from the metabolism units, weighed again, and the animals of each group kept in a separate enclosure. All the animals were fed the same basal ration as before and the animals of Groups II and III continued to receive magnesium carbonate and oxalic acid, respectively, added to their diet. Magnesium carbonate was added to the diet of animals of Group II at the same rate of 6 grams per animal per day, but it was considered advisable to reduce the amount of oxalic acid in the diet of animals of Group III to 3 grams per animal per day to avoid deterioration in the general health of the animals. They received this treatment for a period of twenty-eight days. During this time the appetite of the animals improved and each was then given the basal ration at the rate of two and one-half pounds per animal per day.

Trial Period II

A week before the end of the above period of twenty-eight days, the animals were placed in the metabolism units once again to get themselves reconditioned to the units. They were then reweighed, placed back in the metabolism units, and all the procedure adopted during the Trial I period duplicated. At the end of the Trial II period of seven days, the animals were removed, weighed, and the animal experiment concluded.

Pre-treatment of Samples

Feces. The sample in each Mason jar was well mixed and a representative sample of about 10-12 grams taken into a clean dry weighed porcelain

crucible and the whole weighed again. The weighings were done on a gramatic balance to four decimal places and done without much loss of time to avoid loss of water during the process. The samples were placed in the drying oven set at 100° C. and left there overnight. Next, they were transferred to the Muffle furnace, the heat turned on and brought up to 600° C. and left there overnight, after which the heat was turned off, the furnace allowed to cool and the crucibles with the samples removed. To the ash was added 5 ml. of 6 N HCl and left for four hours to dissolve. The ash solution was then quantitatively washed into a test tube graduated at 25 ml. and the volume made up to 25 ml. As the dry matter digestibility was also being studied the dry weight of the feces was determined by weighing the crucible with the dry feces, before ashing.

Urine. Twenty ml. of well mixed urine were pipetted into a crucible, dried, ashed, dissolved in 5 ml. 6 N HCl and volume made up to 25 ml. as was done for feces. In the case of urine, 5 ml. of 6 N HCl were added before the urine was dried also as otherwise a highly insoluble ash resulted. One drop of octyl alcohol and addition of the acid gradually prevented loss of the sample by frothing. The dry samples were transferred to the cold furnace and temperature brought up very gradually as otherwise some samples would rise and a portion lost.

Plasma. Five to 10 ml. of plasma were pipetted into a crucible, dried and ashed, the temperature of the furnace being brought up gradually as in the case of urine and for the same reason. The ash was dissolved in 5 ml. of 6 N HCl and the volume made up to 25 ml.

Feed. The total of the daily samples of basal ration and the feed refused during the trial period by each individual animal were mixed

thoroughly in the individual bags and a representative sample of about 50 grams each taken into a large, clean, dried, weighed porcelain crucible and the whole weighed again. The feed was then dried at 100° C. overnight and the dry weight determined. It was then ashed, dissolved in 15 ml. of 6 N HCl, quantitatively transferred to a 250 ml. graduated flask and the volume made up to mark.

Chemical Analyses

Phosphorus. The method of Fiske and Subbarow (1925) was employed for the estimation of phosphorus. An appropriate aliquot of the ash solution was taken into a photometer tube graduated at 25 ml. The sides of the tube were washed down with about 5 ml. of water, 1 ml. of molybdic acid reagent and 0.4 ml. aminonaphtol sulphonic acid reagent were added and volume made up to 25 ml. with distilled water. An aliquot of a standard containing 40 mg. phosphorus was similarly treated with every batch of unknowns. The tubes were allowed to stand for ten minutes and the readings taken in the photometer within thirty minutes.

The light transmission value of the resulting solution at 720 mμ was determined in an Evelyn Photometer standardized to read 100 per cent transmission with a blank. The value for the standard was also read. The amount of phosphorus in the aliquot was found by referring to tables showing milligrams of phosphorus in the aliquot of the unknown for different light transmission values compared to that of the 40 mg. standard.

The amount of the aliquot of the unknown was so chosen as to give a transmission between 30 to 60 per cent as beyond this range Beer's Law was not followed. The 40 mg. standard gave a transmission of 37 to 40

per cent. Where the amount of the aliquot of the ash solution was less than 1 ml. the estimation was done in duplicate, or the ash solution was suitably diluted to give a larger aliquot.

The ash solutions contained some silica mostly from the crucibles. Silica interferes with color development if it is present in any appreciable amounts. In the case of feces and urine no difficulty was experienced as the amount of phosphorus being large only small aliquots were taken, thus reducing the amount of silica in the aliquot. But in the case of plasma, the phosphorus content was low and it was necessary to take a comparatively large aliquot to get a photometer reading within the desired range, with the consequent increase in silica as well. So with the ash solution of plasma samples it was found necessary to use the modification suggested by Fiske and Subbarow (1925a) in which 0.25 ml. of 10 N H_2SO_4 was added before the addition of other reagents and the photometer reading taken within five minutes. For the plasma samples obtained during Trial II, nickel crucibles were used instead of porcelain.

Calcium. Calcium in the ash solutions was estimated by the method of Clark and Collip (1925).

Feces. An aliquot of the ash solution, 3 to 4 ml., was taken in a tapered bottom 40 ml. centrifuge tube to which were successively added one drop of methyl red, 3 ml. ammonium oxalate, ammonium hydroxide until the color changed to yellow and acetic acid drop by drop until faint pink. It was left overnight, centrifuged, decanted, and washed twice with distilled water. The precipitate of calcium oxalate was thoroughly dispersed in about 4 ml. of water and transferred quantitatively to a plastic tube

and metal cup assembly. The cup had been previously cleaned, dried and weighed. This assembly was then centrifuged to pack the oxalate into the cup, the water decanted or syphoned off, the cup removed, dried and weighed. From the weight of calcium oxalate, the calcium content of the feces was calculated. The details of the process have been described by Comar (1955).

Urine and plasma. Ten to 15 ml. of the ash solution were pipetted into a 40 ml. tapered bottom centrifuge tube and the calcium precipitated as oxalate as described above. To the precipitate was added 3 ml. of 10 per cent H_2SO_4. It was then heated in a water bath at 90° C. for twenty minutes to dissolve. The solution of oxalic acid so produced was titrated with 0.02 N. potassium permanganate solution. A standard solution of calcium chloride containing 2 mg. calcium in the aliquot was treated similarly and titrated with the same potassium permanganate solution on each day the analysis was done. From the volume of permanganate solution required for 2 mg. of calcium, the calcium content of the aliquot of ash solution was calculated.

Feeds. The procedure was similar to that employed for feces. The calcium oxalate in the cup in the case of fecal ash or the titrated fluid in the centrifuge tube in the case of urinary ash or plasma was saved for radiochemical analysis.

Radiochemical Analysis

Radioactivity was measured by counting, using a Geiger Muller tube attached to a scaling unit. The counts were reduced to per cent of dose administered for purpose of calculations, a standard prepared from the dosing solution also being counted with every batch of samples in each

scaling unit under identical conditions.

Phosphorus - P^{32}. In contrast to the low energy of Ca^{45} beta emission (0.26 Mev), that from P^{32} has a high energy emission (1.71 Mev). This difference in energy provided a convenient basis for measuring the activity of P^{32} in the presence of Ca^{45}. An aluminum absorber having a surface density of 55 mg. per square centimeter would absorb all the beta particles from Ca^{45} but would reduce P^{32} contribution by a factor of only 1.5. All samples and standards were therefore counted for P^{32} activity through such an absorber. Samples and standards were counted as solutions, the necessity of correcting for self absorption being eliminated by using a volume of standard equal to that of the samples.

Feces. Ten ml. of the ash solution were pipetted into a petri dish and counted through the absorber. The dosing solution was suitably diluted and 10 ml. of this standard solution were pipetted into a petri dish and counted with every batch of samples.

Urine. Ten ml. of fresh urine were counted.

Plasma. Five ml. of fresh plasma were counted.

Calcium - Ca^{45}. The beta emissions from Ca^{45} are of low energy and this results in high self absorption. Calcium was precipitated as oxalate, washed and transferred to plastic tube and metal cup assembly as described by Comar et al. (1951) and the activity counted. All measurements of Ca^{45} were made against standards prepared from aliquots of the original dosing solutions suitably diluted and evaporated to dryness in metal cups. Such standards were assumed to have no mass, while the mass weights of the calcium oxalates from the samples provided the basis for the self absorption

corrections calculated by the method of Comar et al. (1951).

Feces. The calcium oxalate in the cup obtained during the chemical determination of calcium of the feces was counted.

Urine and plasma. To the contents in the centrifuge tube obtained after titration with potassium permanganate for the chemical determination of calcium was added a solution containing 8 mg. of carrier calcium and the total calcium reprecipitated as oxalate, cupped, dried and counted.

Calculations

1. Radioactivity. Radioactivity was calculated in terms of per cent of the dose administered. This value was obtained from the counts of the sample, counts of the standard and the fraction of dosing solution present in the volume of standard taken. A standard prepared from the same solution as was used for dosing was used for counting along-side the samples on each day, with each machine, so that the influence of all the variable factors in the technique was eliminated.

2. Specific activity. An expression to serve as an index of specific activity was obtained by dividing the per cent of the radioactivity administered present in an aliquot of a sample by the milligrams of total element present. The result usually being a small fraction was multiplied by a constant factor (10^4) to give a convenient expression.

3. Endogenous excretion of calcium and phosphorus. Description given below concerning calcium applies to phosphorus as well.

a) Isotope dilution method. If 100 units of calcium are ingested by the animal and A units of this 100 units are absorbed, 100 - A units of calcium will appear in feces. However, feces contains in addition,

the endogenous calcium excreted into the feces from blood. If this is represented by E. units, total fecal calcium = 100 - A + E units.

If plasma is labeled by injection of Ca^{45} and has a specific activity of Sp, the endogenous calcium excreted will also have the same specific activity, because the animal economy does not differentiate between stable calcium in plasma and Ca^{45} in the plasma and both will be excreted in the ratio in which they are present in plasma. In the feces, the endogenous calcium is diluted by the unabsorbed food calcium, with the result that the Ca^{45} specific activity of fecal calcium is less than that of the plasma calcium, the extent of the reduction in activity being directly proportional to the extent of dilution. If the specific activity of the calcium of the feces be Sf, then

$$\frac{Sf}{Sp} = \frac{\text{Endogenous Ca in feces}}{\text{Total fecal Ca}}$$

$$\text{or Endogenous Ca in feces} = \text{Total fecal Ca} \times \frac{Sf}{Sp}$$

b) Comparative balance method. Hansard et al. (1951, 1951a) have presented evidence that there was a greater retention of the part of ingested calcium that was absorbed, than of the calcium of the body stores. In other words the endogenous calcium of the feces comes primarily from the bone via the plasma.

The amount of radio-calcium that appears in the bone at a given time after oral administration will be governed first by the efficiency of absorption and second by processes that involve removal from the blood, such as deposition in the skeleton and excretion. Experience of many studies has shown that the internal factors, i.e. the fraction of plasma calcium that is deposited in the skeleton and the fraction that is excreted

will be similar between similar animals.

If 100 units of calcium are ingested by the animal and of these A units are absorbed, 100 - A units of ingested calcium will appear in the feces. The major fraction of the A units absorbed will be deposited or exchanged with the calcium of the bones and a smaller fraction excreted in the feces. If B represents the fraction of the calcium reaching the blood that is excreted into the feces, for A units of calcium absorbed into the plasma, BA units will be excreted.

Therefore, if 100 units of calcium are ingested,

$$\text{Fecal calcium} = 100 - A + BA$$

$$= 100 - (A - BA)$$

$$= 100 - A(1 - B)$$

$$\text{or } A(1 - B) = 100 - \text{Fecal Ca}$$

$$\text{or } A = \frac{100 - \text{Fecal Ca}}{1 - B} \quad \text{- - - - - - - (1)}$$

Therefore, the part of ingested calcium that appears in feces

$$= 100 - \frac{100 - \text{Fecal Ca}}{1 - B}$$

If E = units of endogenous Ca

$$E + 100 - \frac{100 - \text{Fecal Ca}}{1 - B} = \text{Fecal Ca}$$

$$\text{or } E = \text{Fecal Ca} - 100 + \frac{100 - \text{Fecal Ca}}{1 - B}$$

$$= \frac{100 - \text{Fecal Ca}}{1 - B} - (100 - \text{Fecal Ca}) \text{ - - - (2)}$$

E being the units of endogenous calcium for every 100 units of ingested calcium, the endogenous calcium is E per cent of the total ingested calcium. Therefore, endogenous calcium

$$= E \times \frac{1}{100} \text{ dietary intake - - - - - - - (3)}$$

Summarizing, if the amount of calcium in the food ingested by an animal, the amount in feces originating from this ingested calcium and the value for the fraction of the calcium reaching plasma that is excreted be known, digestibility and endogenous calcium excretion can be calculated from the equations (1), (2), and (3) above.

The necessary data were obtained as follows:

1. The amount of radioactive calcium appearing in the feces of animals of subgroups b was determined. As the animals of these subgroups had been administered 1 mc. of radiocalcium orally, the per cent of apparent digestibility (100 - Fecal Ca) was calculated from this.

2. To determine the fraction of absorbed calcium that was re-excreted, the amount of radiocalcium appearing in the feces of animals of subgroups a was determined. These animals had received the dose intravenously and so from the fecal excretion the value of the fraction B was calculated.

CHAPTER IV

RESULTS AND DISCUSSION

This study was primarily concerned with the influence of magnesium and oxalic acid upon the absorption and retention of calcium and phosphorus, but dry matter digestibility was also determined.

Analysis of the bulked daily samples of basic rations fed to the animals for the two trials showed that the two bulked samples varied in composition. The sample used for Trial I contained 1.123 grams calcium and 0.794 gram phosphorus per pound of ration, whereas that used for Trial II had 1.208 grams and 1.109 grams, respectively, of these two minerals. It would appear from this, that if the amounts of the minerals fed to the individual animals should be exactly equal, the different ingredients or at least those widely differing in weight per unit volume such as cotton-seed hulls and corn meal should be individually weighed for each animal.

Analysis of the feed refused by the animals showed that in many cases it differed in composition from the basal ration offered. Calcium varied from 1.033 grams to 1.725 grams per pound and phosphorus from 0.468 to 1.157 grams during Trial I. The figures for Trial II were 1.034 grams to 1.790 grams for calcium and 0.302 gram to 1.156 grams for phosphorus. The pattern of variations of the contents of calcium and phosphorus in the feeds refused by the different animals during the two trials showed some similarity. The phosphorus contents of the feeds left uneaten by animals No. 5 and No. 12 in both trials were much lower than the content of the basal ration, while in other samples higher. Calcium also was low in the

above two samples, but it was very much higher in others. The details showing these variations are given in Table IV.

Irrespective of whether this was due to the feeding habits or purposive selective feeding, it was clear that sheep could consume a feed which varied in composition with the individual animal when fed a feed mix composed of ingredients capable of easy physical separation.

The figures for dry matter digestibility are given in Table V. Digestibility varied from 61 per cent to 67 per cent. During Trial I, treatments with magnesium and oxalic acid did not show any marked decrease in digestibility. Magnesium treated animals actually showed an apparent slight improvement in digestibility probably due to the beneficient laxative action of the magnesium carbonate administered. Oxalic acid treatment appeared to effect a slight decrease. On the other hand continued treatments with magnesium and oxalic acid during the interval between trials and during Trial II showed a slightly greater effect of oxalic acid on dry matter digestibility. While the control group had a digestibility of 66 per cent and magnesium treated animals had 67 per cent, oxalic acid treated animals had a digestibility of only 62 per cent. However, the differences in digestibilities were not statistically significant. These and subsequent data were analyzed statistically by methods as described by Snedecor (1957). Talpatra et al. (1949) found that oxalates of sodium and potassium were converted into bicarbonates and if present in large amounts induced alkalosis. Watts (1957) thought that the main effect of large doses of oxalic acid was directly on the rumen, i.e. on the pH, and on the buffer mechanism of this organ, which was of great importance in bacterial digestion of cellulose.

TABLE IV

DRY MATTER, CALCIUM AND PHOSPHORUS CONTENTS OF FEEDS

	Average daily feed gms.	Per cent dry matter	Calcium per pound feed gms.	Phosphorus per pound feed gms.
Trial I				
Feed offered	908	82	1.123	0.794
Feed refused				
An. No. 5	143	81	1.122	0.482
12	182	82	1.033	0.468
13	58	81	1.617	1.080
14	123	82	1.224	0.991
15	26	81	1.719	1.157
16	65	79	1.725	1.145
17	32	80	1.404	1.154
18	136	81	1.588	1.119
Trial II				
Feed offered	1135	82	1.208	1.109
Feed refused				
An. No. 5	746	81	1.077	0.861
12	182	81	1.034	0.302
13	52	80	1.790	1.091
14	32	80	1.777	1.156
15	58	80	1.713	1.102
16	169	79	1.539	1.071
18	169	80	1.514	1.129

TABLE V

AVERAGE DRY MATTER DIGESTIBILITY

Group		D. M. consumed gms.	D. M. in feces gms.	Digestibility per cent	Group average per cent
			Trial I		
I	a	5236	1906	64	
	b	4966	1965	60	62
II	a	5236	1994	62	
	b	4890	1623	67	65
III	a	4842	2022	58	
	b	4805	1764	63	61
			Trial II		
I	a	6547	2300	65	
	b	5130	1689	67	66
II	a	6547	2297	65	
	b	6205	1951	69	67
III	a	6280	2443	61	
	b	5919	2159	63	62

In spite of the preliminary conditioning to the basal ration and metabolism units, during Trial I, each animal was consuming only about two pounds of the basal ration per day, whereas for maintenance by standards of Morrison (1956) each had to consume 2.5 pounds. The administration of radioactivity and collection of blood samples were also new conditions to which the animals were subjected during the trial period. These factors might have been responsible for the animals losing weight during the period of Trial I.

The feed consumption of the animals gradually went up during the interval of 28 days between the trials. The animals had by then also learned to tolerate dosing and blood sample collection. Consequently, the majority of the animals gained weight during the period of Trial II.

The dry matter digestibility also improved during Trial II, especially in the control group.

Radioactivity

The daily averages per animal of the specific activities of plasma or feces of each subgroup for the last three days of the trial periods were employed for calculations by isotope dilution method. For comparative balance method, the averages per animal for the trial period, of the percentages of the dose of activity administered that was excreted by animals of each subgroup was used. These data are given in Tables VI, VII, VIII, and IX.

Only just enough radiochemical data necessary to evaluate the calcium-phosphorus absorption and retention were obtained. Parellelism between the

TABLE VI

AVERAGE CA^{45} SPECIFIC ACTIVITIES (X 10^4) OF PLASMA AND FECES

	Day of trial	Group I a	Group I b	Group II a	Group II b	Group III a	Group III b
Trial I							
Feces	5	3.6	3.9	2.5	4.6	1.9	4.7
	6	1.8	1.6	1.8	1.6	1.9	1.8
	7	1.4	1.0	1.4	0.9	1.5	1.0
Average		2.3	2.2	1.9	2.4	1.8	2.5
Plasma	5	3.4	--[a]	3.2	1.6	3.8	1.9
	6	3.2	1.3	2.9	1.4	3.2	1.7
	7	2.5	1.1	2.0	0.8	2.3	1.3
Average		3.0	1.2	2.7	1.3	3.1	1.6
Trial II							
Feces	5	4.8	6.9	4.0	6.2	4.1	6.4
	6	3.5	3.8	3.4	3.2	2.8	3.1
	7	3.3	2.6	2.4	1.8	2.4	1.8
Average		3.9	4.4	3.3	3.7	3.1	3.8
Plasma	5	4.8	5.4	6.5	4.0	6.6	2.7
	6	4.9	3.8	5.8	3.3	5.5	3.1
	7	5.4	3.2	6.0	2.8	5.1	2.2
Average		5.0	4.1	6.1	3.4	5.7	2.7

[a]Sample lost.

TABLE VII

AVERAGE P^{32} SPECIFIC ACTIVITIES (X 10^4) OF PLASMA AND FECES

	Day of trial	Group I a	Group I b	Group II a	Group II b	Group III a	Group III b
Trial I							
Feces	5	16.1	13.8	15.8	16.8	11.9	13.5
	6	12.3	9.8	12.2	11.7	10.4	10.9
	7	12.0	8.0	8.5	10.4	9.4	9.0
Average		13.5	10.5	12.2	13.0	10.6	11.1
Plasma	5	18.6	16.9	19.8	13.6	18.7	20.4
	6	16.4	12.1	14.4	12.0	16.1	14.4
	7	14.5	11.1	15.0	9.3	14.6	11.7
Average		16.5	13.4	16.4	11.6	16.5	15.5
Trial II							
Feces	5	15.1	20.0	17.5	17.8	14.1	17.2
	6	12.1	12.9	10.2	10.8	10.8	10.4
	7	10.1	11.5	9.1	8.3	8.0	8.5
Average		12.4	14.8	12.3	12.3	11.0	12.0
Plasma	5	15.4	13.1	14.3	11.9	12.0	11.5
	6	12.7	12.1	14.1	11.6	13.3	11.6
	7	13.2	11.4	13.9	9.4	11.2	10.3
Average		13.8	12.2	14.1	11.0	12.2	11.1

TABLE VIII

AVERAGE CA^{45} PERCENTAGE OF DOSE EXCRETED IN FECES

Day of collection	Group I		Group II		Group III	
	a	b	a	b	a	b
			Trial I			
1	5.04	16.04	5.93	10.44	6.84	16.43
2	3.64	15.19	3.14	14.28	2.88	13.16
3	1.25	5.23	1.49	6.94	1.12	5.63
4	0.79	1.91	0.85	2.55	0.59	1.89
5	0.55	0.99	0.55	1.14	0.60	0.88
6	0.40	0.36	0.38	0.43	0.40	0.40
7	0.31	0.27	0.46	0.28	0.32	0.19
Total	11.98	39.99	12.80	36.06	12.75	38.58
			Trial II			
1	9.43	12.32	7.48	9.01	7.10	18.78
2	4.84	22.83	3.55	26.96	3.92	16.14
3	2.27	9.56	1.56	10.82	1.45	6.85
4	1.45	2.97	1.12	4.12	1.07	3.04
5	1.24	1.69	0.96	1.65	0.91	1.25
6	0.71	0.68	0.70	0.63	0.71	0.69
7	0.61	0.42	0.55	0.37	0.49	0.38
Total	20.54	50.47	15.92	53.56	15.65	47.13

TABLE IX

AVERAGE P^{32} PERCENTAGE OF DOSE EXCRETED IN FECES

Day of collection	Group I		Group II		Group III	
	a	b	a	b	a	b
			Trial I			
1	3.18	11.79	4.29	5.15	5.34	12.26
2	9.06	15.66	7.80	11.97	8.16	14.68
3	5.76	7.78	6.03	14.17	5.23	7.29
4	4.59	5.23	4.65	5.20	3.66	4.72
5	3.57	3.61	3.59	4.07	3.41	3.69
6	2.54	2.55	2.45	2.74	2.72	2.69
7	2.31	1.98	2.47	2.93	2.12	1.70
Total	31.01	48.60	31.28	46.23	30.64	47.03
			Trial II			
1	4.26	9.19	4.97	7.63	5.94	14.07
2	11.66	17.80	10.95	19.72	9.54	13.94
3	8.44	12.70	6.95	14.78	5.90	6.51
4	6.14	5.61	5.15	7.33	4.15	4.82
5	4.70	5.12	3.86	5.09	2.99	3.52
6	2.63	2.79	2.92	1.93	2.47	2.89
7	2.22	2.32	2.14	1.91	2.04	2.19
Total	40.05	55.53	36.94	58.39	33.03	47.94

specific activities of plasma and feces during the last three days of the trial period in individual animals, shown to exist by previous workers already quoted in the Review of Literature, had been assumed.

The total dose present in the feces of animals of subgroup a, was less than that in the feces of animals of subgroup b for the first five days of the trial period, but on the seventh day, the percentages of dose present were in most cases more in subgroup a. On the sixth day, the percentages of dose excreted in feces by all subgroups were nearly the same. This was due to the radioactivity in the feces of animals in subgroup b becoming less and less due to the unabsorbed oral dose and more and more due to the previously absorbed activity being re-excreted into the feces.

Balch (1950) found that a sample of stained hay appeared in feces twelve to twenty-four hours after feeding it with other feeds to dairy cows and 90 per cent was expelled in seventy to ninety hours. Then the rate of excretion flattened off, until excretion of all particles of stained hay was completed in seven to ten days after feeding. It was likely that removal of water soluble radioactivity administered orally was much more rapid. If further work would prove that radioactivity in feces ten days after the oral administration of a dose was all due to that previously absorbed and then re-excreted, there would be no need for intravenous administration of activity for determination of true digestibility by isotope dilution method, all that would be necessary being oral administration of probably a higher dose, collection of feces and plasma, probably on the 11th, 12th, and 13th days, determination of specific activities and making the necessary calculations.

The Effects of Magnesium and Oxalic Acid Upon Calcium Absorption and Retention

Summarized statement of results for calcium utilization is presented in Table X.

Trial I

Calcium balances were negative during the trial period, Group I having lost 0.55 gram, Group II, 1.51 grams, and Group III, 1.99 grams per animal. Thus both magnesium and oxalic acid apparently increased the loss of calcium, but the results were not statistically significant. The average plasma calcium levels for the three groups were 14.4 mg., 15.0 mg., and 14.1 mg. per 100 ml. of plasma and the calcium excreted in urine per animal during the period 0.51 gram., 0.51 gram., and 0.68 gram.

The percentage of true digestibility of calcium by isotope dilution method for Group Ia was 78, for Group IIa, 63, and for Group IIIa, 60. The amounts of endogenous calcium excreted into the feces per animal for the duration of the trial were for Group Ia, 11.10 grams, Group IIa, 12.11 grams, and Group IIIa, 9.95 grams. The difference from the controls and Group IIIa that had received oxalic acid treatment was significant ($P<.10$), the treatment having reduced true digestibility and thus the amount of calcium absorbed. However, this effect was partly compensated for by reduced endogenous excretion.

Trial II

All groups showed positive calcium balance, Group I having gained 5.08 grams, Group II, 5.29 grams, and Group III, 5.52 grams per animal

TABLE X

CALCIUM UTILIZATION

(Average per animal for trial period)

	Group I		Group II		Group III	
	a	b	a	b	a	b
Trial I						
Calcium consumed, gms.	15.72	14.90	15.72	14.76	14.23	13.80
Calcium in feces, gms.	14.56	17.15	17.15	16.35	15.72	16.28
Calcium, endogenous fecal, gms. (by isotope dilution)	11.10		12.11		9.95	
Food calcium absorbed, gms.	12.26		10.68		8.46	
Percentage true digestibility (by isotope dilution)	78		68		59[a]	
Percentage true digestibility (by comparative balance)	61		73		70	
Plasma calcium mg./100 ml.	14.4		15.0		14.1	
Urinary calcium, gms.	0.51		0.51		0.68	
Net retention, gms.	- 0.55		- 1.51		- 1.99	
Trial II						
Calcium consumed, gms.	21.14	17.01	21.14	20.23	19.85	18.49
Calcium in feces, gms.	15.56	12.43	14.90	15.82	13.97	13.34
Calcium, endogenous fecal, gms. (by isotope dilution)	12.06		8.52		7.67	
Food calcium absorbed, gms.	17.64		14.76		13.55	
Percentage true digestibility (by isotope dilution)	83		70		68[a]	
Percentage true digestibility (by comparative balance)	62		58		63	
Plasma calcium mg./100 ml.	11.0		7.7		10.9	
Urinary calcium, gms.	0.55		0.48		0.33	
Net retention, gms.	5.08		5.29		5.52	

[a]Significant at 10 per cent level or $P < .10$.

during the trial period. Magnesium and oxalic acid did not show the apparent decrease in net balance as in Trial I. The physiological mechanisms of digestion, absorption and endogenous excretion might have undergone an adaptation enabeling the animals to utilize calcium in spite of the treatments. Watts (1957) showed that rumen contents of sheep habituated to the intake of small quantities of oxalic acid were able to decompose considerably increased amounts of this substance. The plasma levels of calcium were for Group I, 11.0 mg., Group II, 7.7 mg., and Group III, 10.9 mg. for 100 ml. of plasma. Calcium excreted in urine was 0.55 gram for Group I, 0.48 gram for Group II, and 0.33 gram for Group III.

The percentages of true digestibilities by isotope dilution method were for Group Ia, 83, Group IIa, 70, and Group IIIa, 68. The endogenous calcium excreted into the feces per animal for the trial period was for Group Ia, 12.06 grams, for Group IIa, 8.52 grams, and Group IIIa, 7.67 grams. In this trial also, the difference in true digestibilities between the controls and those that had received oxalic acid treatment was significant ($P < .10$).

As in Trial I, the endogenous fecal excretion appeared to be proportional to the amount of calcium absorbed. The temptation was to postulate that, within limits, endogenous fecal calcium excretion was adjusted to the amount absorbed so that net retention is attempted to be maintained. Irving (1955) has reviewed the results of many studies on factors influencing endogenous excretion, and available evidence did not warrent such a generalization.

Percentages of true digestibilities obtained by the method of comparative balance did not agree with those obtained by isotope dilution

method. While a certain degree of difference in the absolute values could be anticipated, the same relative magnitudes of the values between the three groups were expected to be obtained. As reviewed by Comar et al. (1953) the primary assumption in this method was that labeled calcium usually given as soluble $Ca^{45}Cl_2$ and all the dietary calcium were absorbed to an equal extent from the intestinal tract. On the basis of the comparative results reported, there would seem to be little difference between absorption of calcium in normal plant material and that given as $Ca^{45}Cl_2$, but when magnesium carbonate or oxalic acid was administered along with the soluble radiocalcium, there was every possibility that the radiocalcium would be precipitated and made less digestible than food calcium which would be liberated only later after digestion. According to Comar in the reference quoted above, method of comparative balance should not be used if the extent of absorption of the dietary calcium was appreciably different from that of calcium^{45} administered separately.

The Effects of Magnesium and Oxalic Acid Upon Phosphorus Absorption and Retention

Summarized statement of results for phosphorus utilization is presented in Table XI.

Trial I

Phosphorus balances were negative during the trial, Group I having lost 4.82 grams, Group II, 4.16 grams, and Group III, 4.98 grams per animal. The average plasma phosphorus level for Group I was 8.8 mg., for Group II,

TABLE XI

PHOSPHORUS UTILIZATION

(Average per animal for trial period)

	Group I		Group II		Group III	
	a	b	a	b	a	b
Trial I						
Phosphorus consumed, gms.	11.12	10.77	11.12	10.68	10.02	9.76
Phosphorus in feces, gms.	14.44	17.08	15.23	14.89	14.89	14.84
Phosphorus, endogenous fecal, gms. (by isotope dilution)	11.87		11.09		9.67	
Food phosphorus absorbed, gms.	8.55		6.98		4.80	
Percentage true digestibility (by isotope dilution)	77		63		48[a]	
Percentage true digestibility (by comparative balance)	74		80		76	
Plasma phosphorus mg./100 ml.	8.8		8.9		10.0	
Urinary phosphorus, gms.	0.11		0.11		0.13	
Net retention, gms.	- 4.82		- 4.16		- 4.98	
Trial II						
Phosphorus consumed, gms.	19.41	16.11	19.41	19.13	18.60	17.50
Phosphorus in feces, gms.	17.63	14.63	16.75	17.07	16.30	14.67
Phosphorus, endogenous fecal, gms. (by isotope dilution)	15.77		14.55		14.72	
Food phosphorus absorbed, gms.	17.55		17.21		17.02	
Percentage true digestibility (by isotope dilution)	90		89		92	
Percentage true digestibility (by comparative balance)	75		66		81	
Plasma phosphorus mg./100 ml.	9.6		9.7		11.3	
Urinary phosphorus, gms.	0.11		0.11		0.31	
Net retention, gms.	1.63		2.36		2.57	

[a]Highly significant or $P < .01$

8.9 mg., and Group III, 10.0 mg., per 100 ml. The average amount of phosphorus excreted in urine for Group I was 0.11 gram, Group II, 0.11 gram, and Group III, 0.13 gram.

The percentages of true digestibilities of phosphorus by isotope dilution method were for Group Ia, 77, for Group IIa, 63, and for Group IIIa, 48. The difference between Group Ia and Group IIa was not significant, but that between Group Ia and Group IIIa was highly significant ($P < .01$). The amounts of endogenous phosphorus excreted in feces were 11.87 grams in Group Ia, 11.09 grams in Group IIa, and 9.67 grams in Group IIIa. Thus as in the case of calcium utilization, decreased absorption of the mineral was followed by decreased endogenous fecal excretion, with the result that net retentions in all the three groups were practically the same.

Trial II

All the groups showed positive phosphorus balance during Trial II, Group I gaining 1.63 grams, Group II, 2.36 grams, and Group III, 2.57 grams per animal. The differences were not statistically significant. The plasma levels of phosphorus were for Group I, 9.6 mg., for Group II, 9.7 mg., and for Group III, 11.3 mg. per 100 ml. The amounts of phosphorus excreted in urine were 0.11 gram per animal for the trial period by Group I, 0.11 gram by Group II, and 0.31 gram by Group III.

Isotope dilution technique showed a true digestibility of 90 per cent in Group Ia, 89 per cent in Group IIa, and 92 per cent in Group IIIa. As in the case of calcium utilization, true digestibility was higher in Trial II. The amounts of endogenous phosphorus excreted in feces were on an average 15.77 grams for the period per animal of Group Ia, 14.55 grams

of Group IIa, and 14.72 grams of Group IIIa. The improved retention of phosphorus in Groups II and III was therefore due to decreased endogenous excretion which more than made up for the decreased absorption, with the result that the treated groups showed greater net retention than the controls.

Percentages of true digestibilities obtained by the method of comparative balance disagreed with those obtained by isotope dilution method both in absolute values as well as relative magnitudes. The probable causes may be the same as those causing a similar difference in the percentages of digestibilities of calcium by the two methods.

CHAPTER V

SUMMARY

This investigation was undertaken to study the influence of magnesium and oxalic acid upon the absorption and retention of calcium and phosphorus in lambs.

Eighteen wether lambs were chosen and divided into three groups of six animals in each and each group subdivided into two subgroups of three animals in each. The animals of as nearly the same weight as possible were chosen and so divided into subgroups as to make the average weight of each subgroup as nearly the same as possible.

The animals were fed an adequate basal ration and after the preliminary conditioning to the ration and metabolism stalls, Group I was used as controls, Group II to study the effect of magnesium and Group III to study the effect of oxalic acid. Six grams of magnesium carbonate were mixed with the daily ration of each animal of Group III, Group I being used as controls. Radioactivity was administered intravenously to one subgroup of each group and orally to the other. Chemical and radiochemical analysis of feed, feces, plasma and urine were made and the true digestibility, endogenous fecal excretion, absorption and net retention of the minerals determined. Both the isotope dilution and comparative balance methods were employed in the calculations.

After the first trial period of seven days, the animals were removed from the metabolism stalls and kept for twenty-eight days on the same basal ration and same treatments to the respective groups, the dose of oxalic acid

being halved. Then a second trial was conducted duplicating the procedure of the first trial.

The true digestibility and amount of calcium absorbed were apparently reduced by both magnesium and oxalic acid in both trials, the effects produced by oxalic acid only being statistically significant ($P < .10$). Continued feeding with oxalic acid apparently decreased its effects on calcium utilization. The amount of calcium excreted endogenously in the feces apparently varied in the same direction as the amount absorbed, with the result that changes in the net retention were minimized.

Magnesium and oxalic acid treatments appeared to reduce the true digestibility of phosphorus during the Trial I. The effect of magnesium was not statistically significant, but the effect of oxalic acid was highly significant ($P < .01$). During the Trial II both magnesium and oxalic acid had no effect on phosphorus utilization, showing that continued feeding apparently removed its effects. Endogenous excretion appeared to depend upon the amount absorbed so that changes in the net retention tended to be minimized.

Comparative balance method gave results different from those obtained with isotope dilution method. Probable causes of this difference have been discussed.

LITERATURE CITATION

Balch, C. C. 1950. Factors Affecting Utilization of Food by Dairy Cows. 1. The Rate of Passage of Food Through the Digestive Tract. Brit. Jour. Nutrition. 4:361.

Chossat, M. 1842. Quoted by Maynard, L. A., and J. K. Loosli, 1956. Animal Nutrition. McGraw-Hill.

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

Cohn, W. E., E. T. Cohn, and J. C. Aub. 1942. Calcium and Phosphorus Metabolism. Clinical Aspects. Annual Rev. Biochemistry. 11:415.

Comar, C. L., S. L. Hansard, S. L. Hood, M. P. Plumlee, and B. F. Barrentine. 1951. Use of Ca^{45} in Biological Studies. Nucleonics. 8:19.

Comar, C. L., R. A. Monroe, W. J. Viseck, and S. L. Hansard. 1953. Comparison of Two Methods for Determining Endogenous Fecal Calcium. Jour. Nutrition. 50:459.

Comar, C. L. 1955. Radioisotopes in Biology and Agriculture. Principles and Practice. McGraw-Hill.

Comar, C. L., and R. H. Wasserman. 1956. Radioisotopes in the Study of Mineral Metabolism. Progress in Nuclear Energy. Vol. 6:153. Pergmon Press, London.

Comar, C. L., and R. H. Wasserman. 1957. Macronutrient Metabolism. Atomic Energy and Agriculture. Am. Assn. for the Advancement of Science. Washington, D. C. page 249.

Davis, G. K. 1957. Radioactive Micro-nutrients in Animal Metabolism. Atomic Energy and Agriculture. Am. Assn. for the Advancement of Science. Washington, D. C. page 231.

Davis, G. K., and J. K. Loosli. 1954. Mineral Metabolism (animal). Annual Rev. Biochemistry. 23:459.

Dukes, H. H. 1955. The Physiology of Domestic Animals. Comstock.

Fiske, C. H., and Y. Subbarow. 1925. The Colorimetric Determination of Phosphorus. Jour. Biol. Chem. 66:375.

Fiske, C. H., and Y. Subbarow. 1925a. The Colorimetric Determination of Phosphorus. Jour. Biol. Chem. 66:385.

Greenberg, D. M. 1939. Mineral Metabolism. Calcium, Magnesium and Phosphorus. Annual Rev. Biochemistry. 8:269.

Hansard, S. L. 1951. Radioisotope Procedures with Farm Animals. Nucleonics 9:13.

Hansard, S. L., C. L. Comar, and M. P. Plumlee. 1951. Radioisotope Procedures with Farm Animals. Nucleonics. 9:38.

Hansard, S. L., C. L. Comar, M. P. Plumlee, and C. S. Hobbs. 1951a. Integration of Concurrent Chemical and Radioisotope Balance Trials for the Interpretation of Calcium Metabolism Studies. Jour. Dairy Sci. 34:508.

Howard, J. E. 1957. Calcium Metabolism, Bones, and Calcium Homeostasis. A Review of Certain Current Concepts. Jour. Clin. Endocrinology and Metabolism. 17:1105.

Irving, J. T. 1955. Calcium Metabolism. John Wiley.

Kamen, M. D. 1957. Isotopic Tracers in Biology: An Introduction to Tracer Methodology. Academic Press.

Kleiber, M., A. H. Smith, N. P. Ralston, and A. L. Black. 1951. Radiophosphorus (P^{32}) as Tracers for Measuring Endogenous Phosphorus in Cow's Feces. Jour. Nutrition. 45:253.

Lofgreen, G. P., M. Kleiber, and J. R. Luick. 1952. Metabolic Fecal Phosphorus Excretion of the Young Calf. Jour. Nutrition. 47:571.

Lofgreen, G. P., and M. Kleiber. 1953. The Availability of the Phosphorus in Alfalfa Hay. Jour. An. Sci. 12:366.

Lofgreen, G. P., and M. Kleiber. 1954. Further Studies on the Availability of Phosphorus in Alfalfa Hay. Jour. An. Sci. 13:258.

Maynard, L. A., and J. K. Loosli. 1943. Mineral Nutrition. Annual Rev. Biochemistry. 12:251.

Maynard, L. A., and S. E. Smith. 1947. Mineral Metabolism. Annual Rev. Biochemistry. 16:273.

McCance, R. A., and E. M. Widdowson. 1944. Mineral Metabolism. Annual Rev. Biochemistry. 13:315.

McElroy, W. D., and B. Glass. 1951. A Symposium on Phosphorus Metabolism Vols. I and II. John Hopkins Press.

Morrison, F. B. 1956. Feeds and Feeding. The Morrison Publishing Company.

Sendroy, Jr., J. 1945. Mineral Metabolism. Annual Rev. Biochemistry. 14:407.

Snedecor, W. 1957. Statistical Methods. The Iowa State College Press.

Talpatra, S, K., S. C. Ray, and K. C. Sen. 1948. Quoted by Davis and Loosli, 1954. Jour. Agr. Sci. 38:163.

Tibbetts, D. M., and J. C. Aub. 1937. Quoted by Greenberg, 1939.

Visek, W. J., R. A. Monroe, E. W. Swanson, and C. L. Comar. 1953. Determination of Endogenous Fecal Calcium in Cattle by a Simple Isotope Dilution Method. Jour. Nutrition. 50:23.

Watts, P. S. 1957. Decomposition of Oxalic Acid In Vitro by Rumen Contents. Aust. Jour. of Agr. Res. 8:266.