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The Influence of Aluminium and Zinc upon the Absorption and Retention of Calcium and Phosphorus in Lambs

Alan Thompson

To the Graduate Council:

I am submitting herewith a thesis written by Alan Thompson entitled "The Influence of Aluminium and Zinc upon the Absorption and Retention of Calcium and Phosphorus in Lambs." 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:

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

July 10, 1957

To the Graduate Council:

I am submitting herewith a thesis written by Alan Thompson entitled "The Influence of Aluminium and Zinc 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:

Charles S. Hobbs

O. Glen Hall

Accepted for the Council:

Dale Trantham
Dean of the Graduate School

THE INFLUENCE OF ALUMINIUM AND ZINC 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

Alan Thompson

August 1957

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Alan Thompson

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CHAPTER I

INTRODUCTION

In recent years an increasing amount of attention has been given to the mineral fraction of feeds and to its importance in animal nutrition. As a result, it is now well-recognised that the value of a dietary source of an element depends not only upon its total content, but also upon the ability of the animal to extract that element and to retain it for use in metabolic processes. A considerable body of evidence now exists to show that the availability of the inorganic elements, to the animal, may differ widely in various livestock feeds and supplements.

Insufficient data are available, however, to allow any great improvement in the present system of recommending dietary allowances for minerals, which is based upon the total content of the element with no allowance being made for the non-available fraction. In addition, it is becoming apparent that the percentage availability or digestibility of an element is not a constant for a particular feed but varies with the species of animal, its age and past nutritional history and other factors.

The introduction of suitable radioisotope procedures has made possible a deeper understanding of the various metabolic processes associated with the ingestion and subsequent utilization and distribution of the inorganic elements. In addition, such methods have greatly helped in obtaining a more accurate estimate of the true digestibility of mineral elements in feeds under varying dietary and environmental conditions. Considerable data are now available from this type of study, particularly for the elements calcium and phosphorus

Several reports exist which strongly suggest that interactions between both the organic and inorganic constituents in the alimentary tract of an animal may increase or decrease calcium and phosphorus absorption. It would therefore seem pertinent to study some of these possible effects upon availability and to estimate their magnitude.

Little information is available regarding the effects of aluminium and zinc upon the availability of calcium and phosphorus in ruminants, though some does exist for laboratory animals. The element aluminium is of considerable interest in view of its suggested use as an alleviator in fluorine toxicity, while the increasing nutritional roles of zinc, and the suggestion of a relationship to calcium in parakeratosis, make it an element worthy of study.

The present investigation was conducted to determine the effects of dietary aluminium and zinc upon the absorption and retention of calcium and phosphorus in lambs. Conventional chemical and radiochemical balance experiments were made which allowed the calculation of data for the net absorption, endogenous fecal excretion and true digestibility of calcium and phosphorus.

CHAPTER II

REVIEW OF LITERATURE

Calcium and Phosphorus Availability Studies

The present literature review is primarily concerned with procedures for, and the results of, experiments to determine the absorption and excretion of feed calcium and phosphorus under varying dietary and environmental conditions. No attempt is made to review the vast literature dealing with the broader aspects of calcium and phosphorus metabolism. Such aspects are fully discussed in a number of extensive reviews (Duckworth and Hill, 1953; Glass, 1952; Greenberg, 1939; and others).

For the past fifty years, efforts have been made to measure the digestibility of calcium and phosphorus in human and animal feeds. In many such investigations, vegetable materials intended for human consumption were the subject of study (Shields et al., 1940; Kelly, 1943; Fairbanks and Mitchell, 1938), and in the majority, the rat has been the experimental animal. Calcium and phosphorus digestibility studies with farm animals are limited in number, thereby necessitating the extrapolation of the data from studies with laboratory animals.

Numerous procedures have been used for measuring the availability of calcium and phosphorus to the various animal species. Balance studies have been made on man by Mitchell and Curzon (1939) and on farm animals by Forbes et al. (1922), Lindsey and Archibald (1925), Weber et al. (1940), Mathur and Desia (1954), and others.

The use of small laboratory animals has made possible studies based

upon whole body analysis. Thus Tisdall and Drake (1938), Drake et al. (1949), Sur and Subrahamyan (1952), Armstrong and Thomas (1952), have all employed this procedure, using the rat as the experimental subject. It may be noted that such studies provide net retention, rather than digestibility data, based as they are upon the determination of stored calcium and phosphorus.

Partial body analysis, particularly of bones, is a further method of assessing the net retention of the two elements and has been employed by Wilcox et al. (1953) using chicks, and with rats by Barrentine et al. (1944) and Armstrong and Thomas (1952).

While most of the above-mentioned workers recognized the importance of the fecal endogenous excretion of the two elements, techniques were not available to distinguish between fecal calcium and phosphorus of food origin and that from body stores. As a consequence, most data were reported as the apparent digestibility or as net retention, rather than as the true digestibility, a term which requires a knowledge of the fecal endogenous excretion as a prerequisite to its calculation. Despite this and other limitations, such studies served to show, firstly, that animals cannot utilize all of the calcium and phosphorus in feeds (Forbes et al. 1922; Lindsey and Archibald, 1925; and others), and secondly, that feeds differ in the relative availabilities, to the animal, of their calcium and phosphorus contents (Turner et al., 1927; Williams et al., 1940 a and b; Armstrong and Thomas, 1952; and others).

Prior to the development of radioisotope techniques, some efforts had been made to determine the endogenous fecal excretion of calcium and phosphorus. Thus Mitchell et al. (1937), Longwell (1941) and Aylward and

Blackwood (1936), attempted to measure endogenous fecal calcium under fasting conditions or on diets free of, or low in, calcium. These techniques impose abnormal conditions and require the animal to be in a postabsorptive state, a condition difficult to achieve in the ruminant animal. Further, the data obtained under such conditions have limited application to normal feeding conditions even within the same animal (Benedict and Ritzman, 1935).

Mitchell and Curzon (1939), Steggerda and Mitchell (1951), and Hegsted et al. (1952), estimated the total endogenous calcium excretion at zero calcium intake in humans by extrapolating a regression line relating calcium uptake to calcium balance. Similar objections may be made to this method as to that of measuring the endogenous fecal element excreted on a calcium-free diet (Visek et al., 1953).

The Use of Radioactive Isotopes in Calcium and Phosphorus Availability Studies

Recent developments in radioactive isotope procedures have made possible the direct estimation of the fecal calcium and phosphorus of endogenous origin. An isotope dilution method has been used to determine endogenous fecal phosphorus by Kleiber et al. (1951), Lofgreen and Kleiber (1953) and Visek et al. (1953), whilst a method involving concurrent chemical and radiochemical balance studies has been proposed and used by Hansard et al. (1951 b) for calcium.

Comar et al. (1953) obtained excellent agreement for the endogenous fecal calcium excretion in cattle using both the isotope dilution and the comparative balance methods. These workers discussed the assumptions and limitations inherent in the two procedures and concluded that the greatest

limiting errors were those associated with any conventional balance trial.

The use of such procedures has added greatly to our knowledge, not only of the true digestibility to the animal of calcium and phosphorus from different dietary sources, but also of the fecal excretory mechanism under various dietary and physiological conditions. Hansard et al. (1954) have shown that the endogenous fecal calcium values were relatively constant for animals of the same species at the same age and nutritional status, and that from sexual maturity to maturity the effect of age was not great.

The importance of the age factor upon mineral element absorption cannot be over-emphasised. It is known that the rate of calcium absorption decreases with the progressive calcification of the skeleton (Fairbanks and Mitchell, 1936) such that when the adult stage is reached the net deposition is extremely low. At this point calcium excretion balances the intake. With advancing age the process goes further and increased catabolism leads to a loss in bone salts (McCay et al., 1935; Henry and Kon, 1947; and others).

Hansard et al. (1954), using calcium-45, clearly showed the relationship between age and calcium absorption. Only 3 per cent of an orally administered dose of calcium-45 was excreted in the feces of a young calf; this figure rose to 62 per cent by the age of 6 months, and to 83 per cent in an animal of 160 months of age. Similarly, the excretion of an intravenously administered dose of calcium-45, as indicative of the level of endogenous fecal excretion, rose from 8 per cent in the 6 month-old animal to 25 per cent at 160 months. Hansard et al. (1954) show the great effect of such age absorption and excretion patterns upon the results of availability experiments. Thus while at 10 days old, the apparent and true digestibilities of calcium for cattle were 93 and 98 per cent respectively, these values

were down to 14 and 34 per cent in one-year old animals and -28 and 22 per cent in aged animals. It is evident from these data that availability studies on different feeds can only have meaning when considered in relationship to the age of the animal, and cannot be thought of in terms of a constant attribute of the feed.

Yet a further difficulty in the interpretation of digestibility data for calcium and phosphorus in a particular feed are the variations caused by previous nutritional history. Hansard and Plumlee (1954) have reported that the current calcium intake had less influence upon fecal endogenous calcium losses in rats than had the calcium status of the animals at the time of measurement. In animals with low calcium body stores, the total absorption of calcium-45 was increased, while the endogenous fecal loss of the element was reduced, thus giving a greater net retention of calcium. However, under the same conditions, phosphorus-32 was absorbed but was subsequently re-excreted by way of the kidneys and so less was retained.

The Effects of Aluminium upon Calcium and Phosphorus Metabolism

While all plants and animals contain traces of aluminium, no evidence has so far been produced to show that it is an essential element in biological processes. Hove *et al.* (1938) concluded that if the rat needed any aluminium at all, then its requirement was satisfied by one microgram of the element per day.

Considerable attention has been directed to the possible toxic and deleterious effects of aluminium in view of its consumption, by man, in the form of baking powders and by solution from cooking utensils. Myers and

Mull (1928) fed rats, through four generations, on a diet which contained potassium aluminium sulphate in such a quantity as to give 240 p.p.m. of the element. They observed that the growth curves of such animals compared well with those of control rats and the treatment was without other effects.

McCollum et al. (1928) similarly found that a diet containing 600 p.p.m. of aluminium, fed to rats, exerted no deleterious action upon growth, reproduction or general well-being as judged by external appearance and autopsy. These workers concluded that aluminium was not absorbed out of the stomach or intestinal tract when present in the diet. Myers and Morrison (1928) confirmed these findings, using dogs as the experimental animals and diets containing 0.23 grams and 1.55 grams of aluminium per day. No marked increase in aluminium was found in the tissues, with the exception of the liver. They also concluded that little aluminium was retained by the tissue when the element was administered orally.

Mackenzie (1931) fed rats on a diet containing 0.1 per cent of aluminium and compared them with control animals fed a diet approximately free from the element. Urinary excretion of aluminium was found to be negligibly small. Almost all of the ingested element was recovered in the feces. Analysis of organs removed from rats from both groups revealed no evidence of any retention of aluminium.

The evidence cited strongly suggests that any deleterious effect which aluminium exerts is most probably confined to the alimentary tract, and its possible effect upon phosphorus absorption has received some study. However, even in this connection the evidence is somewhat conflicting. Thus, Mackenzie (1930) added aluminium to a swine ration in such quantity as to be stoichiometrically equivalent to 90 per cent of the phosphorus

content of the ration. This diet was fed for a total of 571 days without any effect on growth and metabolism. However, Cox et al. (1931) fed rations containing soluble aluminium (1400 p.p.m. of Al) in excess of the total phosphorus content (1350 p.p.m. of P) to guinea pigs and found that the blood phosphorus was lowered by 15 per cent and the ash, calcium and phosphorus contents of the skeleton were reduced to 70 per cent of normal in 12 weeks. Similar rations fed to rabbits produced an even more rapid lowering of the blood phosphorus level. The authors suggest that these effects were due to the precipitation of phosphorus in the alimentary tract, as aluminium phosphate.

Deobald and Elvehjem (1935) showed very dramatically the effects of feeding aluminium to day-old chicks. Sufficient of the element was added to a basal ration to unite with the total phosphorus in the ration. By the tenth day the chicks receiving the aluminium showed severe rickets, and all were dead at the end of the third week. While the authors concluded that rickets was caused by the precipitation of the phosphorus by the aluminium, the blood serum phosphorus data and the bone ash determinations can hardly be considered to give any real clear-cut indication of this.

Similar production of marked rickets with low blood inorganic phosphorus in rats fed a stock ration with high levels of aluminium sulphate, has been described by Jones (1938), while Street (1942) has presented further evidence on the inhibiting effect of aluminium sulphate on the availability of phosphorus to the rat.

Little data are available to indicate what effects, if any, aluminium may have upon calcium assimilation. Certain of the reports cited include reference to the calcium status of both blood and bones, but, in general, this appears to be regarded as representing merely a secondary effect from

the disturbed phosphorus metabolism.

The possible interference by aluminium in calcium and phosphorus metabolism has particular interest in view of the use of this element as an alleviator in fluorine poisoning in ruminants. Hobbs et al. (1954) have used 0.5 per cent aluminium sulphate in the rations of animals subject to fluorine levels known to be toxic. Other workers have confirmed the efficacy of such a treatment. It therefore becomes important to investigate the possible effects of aluminium on other body processes, and in particular its possible inhibition of the absorption of other minerals from the alimentary tract.

The Effects of Zinc upon Calcium and Phosphorus Metabolism

The early studies of Bertrand and Berzon (1922) indicated that the element, zinc, plays an essential role in animal nutrition. This finding has been confirmed and greatly extended and, at the present time, zinc has been assigned specific roles in several enzyme systems, particularly as an activator. Many of the biological properties of zinc have been reviewed by Hegsted et al. (1945).

Studies on the possible effects of excess amounts of zinc in rations are fewer in number than those concerned with aluminium. However, there appears to be agreement that little zinc is absorbed from the intestinal tract. Feaster et al. (1954) studied the absorption and tissue distribution of zinc in steers, using zinc-65. It was found that when given orally, 70 per cent of the dose was excreted in the feces and only 0.3 per cent in the urine. An intravenously administered dose resulted in a 20 per cent

excretion in the feces and 0.25 per cent in the urine.

Sheline *et al.* (1943), also using zinc-65, found similar results for the dog and the mouse, 50 per cent of the intravenously administered dose being excreted in the feces in 170 hours for the mouse, and 25 per cent in twelve to fourteen days in the case of the dog.

Several investigations have linked the effects of excessive zinc intake with reduced activity in enzyme systems. Van Reen (1953) found that for rats receiving 500 to 700 mg. of zinc per 100 grams of body weight, the catalase and cytochrome oxidase activities were considerably reduced.

There are few reports presenting data on the possible effects of zinc upon phosphorus and calcium metabolism. Sadasivan (1952) found that when rats were given high levels of zinc supplementation there resulted a decreased assimilation of phosphorus. The adverse effect of zinc did not appear to be due entirely to the precipitation of phosphorus in the alimentary tract by the zinc. Sadasivan noted that the intestinal phosphatase decreased as a result of the zinc treatment, while there was a concomitant increase in liver and kidney phosphatase.

Tucker and Salmon (1955) reported evidence that zinc deficiency is concerned in the skin disorder of swine, parakeratosis. These workers also found that the incidence of the disease increased when rations containing 1.5 per cent of calcium carbonate were fed. Similar findings by Hoekstra *et al.* (1956) have led to the suggestion that the two elements, calcium and zinc, exhibit antagonism.

CHAPTER III

EXPERIMENTAL PROCEDURES

General Plan of the Investigation

The investigation took the form of four conventional calcium and phosphorus balance trials using wether lambs as the experimental animals. In each trial, chemical and radiochemical data were collected for three groups of animals maintained on a control ration, the control ration plus added aluminium, and the control ration plus added zinc, respectively. In Trial I and II the levels of the added elements were twice those in Trial III and IV.

General Experimental Procedure

Twelve selected wether lambs averaging 37 kilograms weight were placed in dual-unit type metabolism stalls, described by Hansard et al. (1950) as being suitable for metabolism studies involving radioactive materials. These stalls had provision for the quantitative collection of urine via a metal grid in the floor, under which a funnel conducted the urine to glass storage jars. Feces was collected in paper-lined, removable aluminium boxes, held in position at floor level. This method of collecting feces and urine necessitated having the animals stanchioned at all times during the trial periods.

To accustom the animals to this close confinement as well as to the experimental rations, all twelve animals were fed the basal ration at the

rate of 2 pounds per day for a period of fourteen days. The composition of the ration, which was designed to supply maintenance levels of calcium and phosphorus, is shown in Table I. However, on analysis, the ration proved to contain 0.18 and 0.21 per cent of calcium and phosphorus respectively. The calcium content was therefore slightly lower and the phosphorus content slightly higher than those recommended by the National Research Council (1949) for the maintenance of such lambs.

At the end of the two-week period, the animals had become conditioned to both the metabolism stalls and the ration as evidenced by an improvement in the physical state of the feces and by fairly uniform feed consumption. When first given the ration in the stalls, certain animals had shown a marked tendency to scour and to lose their appetite. At the end of this conditioning period the lambs were weighed, randomly divided into three groups of four animals each and allocated to the three treatments for Trial I, namely, basal ration (Ration A), basal ration plus 1.0 per cent aluminium sulphate (Ration B), and basal ration plus 1.0 per cent zinc sulphate (Ration C). Ration B supplied 1,575 p.p.m. aluminium (as Al) and ration C, 2,280 p.p.m. zinc (as Zn). Detailed compositions of these rations are presented in Table I.

The animals were maintained on these rations for a seven-day preliminary period. During this time re-occurence of scouring and loss of appetite were noticed, particularly in the group receiving the added zinc. By the end of the preliminary period, however, all animals were again accustomed to their respective rations, although the zinc group still showed a tendency to refuse a small portion of their feed on a few occasions.

At the conclusion of this preliminary period the seven-day balance

TABLE I

COMPOSITION OF RATIONS

	Basal Ration(A)	High Aluminium Ration(B)	High Zinc Ration(C)
Trials I and II			
Concentrate Mixture[a]	200 lb.	196 lb.	196 lb.
Aluminium Sulphate -as $Al_2(SO_4)_3$	-	4 lb.	-
Zinc Sulphate -as $ZnSO_4.7H_2O$	-	-	4 lb.
	200 lb.	200 lb.	200 lb.
Trials III and IV			
Concentrate Mixture[a]	200 lb.	198 lb.	198 lb.
Aluminium Sulphate -as $Al_2(SO_4)_3$	-	2 lb.	-
Zinc Sulphate -as $ZnSO_4.7H_2O$	-	-	2 lb.
	200 lb.	200 lb.	200 lb.

[a] The concentrate mixture was composed of:

Cottonseed Hulls	200 lb.
Corn Meal	296 lb.
Soybean Oil Meal	60 lb.
Alfalfa Meal	40 lb.
Iodized Salt	4 lb.
	600 lb.
Vit A and D Supplement (containing 20,000 I.U. Vit A and 2,500 U.S.P. Vit D_2 per gram)	+ 50 grams

trial was commenced. Each animal received 1.0 pound of its respective feed at 8:00 a.m. and 3:00 p.m. each day. Any food residues were weighed daily prior to the 8:00 a.m. feed. An adequate supply of water was offered twice during the course of the day. Detailed records of feed intakes, food residues and fecal and urinary outputs were maintained. Feces and urine collections were made at approximately 10:30 a.m. each day, the exact time made to correspond with the initial time of dosing with calcium-45 and phosphorus-32.

Administration of Radioactive Calcium and Phosphorus

The administration of the calcium-45 and phosphorus-32 was made on the first day of the balance trial period and marked its commencement. Two of the four animals in each experimental group were given 500 microcuries of calcium-45 and a similar amount of phosphorus-32 administered intravenously into the jugular vein. The remaining six animals received the same quantities of the two isotopes administered orally by means of a stomach tube. The techniques used in dosing the animals were those described in detail by Hansard et al. (1951a). Aliquots of the calcium-45 and phosphorus-32 dosing solutions were transferred into 50 ml. graduated flasks using the same hypodermic syringes employed in measuring the doses. These solutions, after suitable dilution, were used as counting standards in all subsequent radioactivity measurements.

The Collection and Treatment of Samples

Feces samples, after weighing, were well mixed and portions of approximately 100 grams in weight were stored in screw-topped jars in a refrigerator pending the conclusion of the trial period. The urine samples

were similarly treated.

Duplicate samples of the three experimental diets were made at every feeding and these were bulked to give composite samples for the trial period. There was little evidence of settling out amongst the feed components and only slight preferential selection by those animals leaving food residues.

Blood samples were taken from all animals on the first day at approximately forty minutes after dosing intravenously and about three hours after dosing orally. Only three of the intravenously - and three of the orally - dosed animals were sampled on each of the three successive days of the trial. These latter blood samples were drawn primarily to provide information on the disappearance of the two administered nuclides from the blood and these data did not justify disturbing the animals unnecessarily by too-frequent bleedings. However, blood samples were taken from all animals on the last three days of the seven day balance period to provide blood equilibrium data for use in calculating the endogenous fecal loss of calcium and phosphorus by the isotope dilution procedure.

At all blood samplings approximately 15 ml. of blood from the jugular vein were collected in heparinised graduated centrifuge tubes. After centrifuging the samples for thirty minutes at a speed of 1500 revolutions per minute, the cell-plasma volume (hematocrit) was measured. The separated plasma was stored in a refrigerator pending the end of the trial period.

Experimental Procedures for Trial II, III and IV

At the conclusion of the balance period of Trial I the twelve sheep were removed from the metabolism stalls and during a resting period of ten days, all animals were maintained on the control ration.

Trial II was then commenced and followed an identical pattern to that of Trial I with the single exception that 1000 microcuries of each of calcium-45 and phosphorus-32 were administered. These higher levels of the isotopes increased the accuracy and ease of counting. The animals were re-randomised prior to the second trial.

Trials III and IV were carried out in a like manner to Trial II differing only in the amounts of zinc and aluminium being fed. The levels of these elements in the rations were reduced to 0.5 per cent as compared with 1.0 per cent in the first two trials. This reduction was made for two reasons; firstly, it was anticipated that more uniform feed consumption would result, particularly in those animals receiving zinc and secondly, by feeding these elements at two levels of intake it was hoped that a clearer picture of the metabolic effects upon calcium and phosphorus behavior would be obtained.

A period of inclement weather marked the duration of Trials II and III. Low temperatures, sufficient to cause freezing of the urine and feces, gave rise to some speculation as to the normalcy of physiological processes in the animals. Certainly, the animals suffered some discomfort and all exhibited signs of having colds in varying degrees of severity.

Chemical and Radiochemical Analysis

Methods of Analysis for Calcium

Calcium-45. In view of the low energy of the beta emissions from calcium-45 and the resulting high self-absorption, factors were necessary to correct for this loss. All measurements of calcium-45 were made against standards prepared from aliquots of the original dosing solutions evaporated to dryness in stainless steel cups. Such standards were assumed to have no mass, while the mass weights of the calcium oxalate from the samples provided the basis for the self-absorption corrections calculated by the method of Comar et al. (1951).

Blood plasma calcium. Whole blood was centrifuged for 30 minutes at 1500 revolutions per minute, and after the hematocrit reading was taken, the plasma was separated. A 3 ml. aliquot of the plasma was transferred to a 40 ml. centrifuge tube and the calcium determined by the method of Clark and Collip (1925). After titration with potassium permanganate, 8 mg. of carrier calcium and 3 ml. of saturated ammonium oxalate solution were added to each sample, and the pH adjusted to 6.0 using 50 per cent ammonium hydroxide and acetic acid. The samples were allowed to stand overnight and were then centrifuged, washed and collected in a plastic tube and metal cup assembly as described by Comar et al. (1951). The samples, after drying, were then counted for calcium-45 activity.

Fecal calcium. Duplicate samples of approximately 25 g. of fresh feces were dried at 100° C. and ashed in a furnace at 600° C. until a white ash was obtained. The latter was dissolved in 6N hydrochloric acid and made to a volume of 25 ml. After thorough mixing, the insoluble residue was allowed to settle and duplicate 3 ml. aliquots of the supernate were drawn for analysis. The calcium was precipitated in tared stainless steel cups as described for blood plasma calcium. The total calcium and the self-

absorption factor were calculated from the weight of the calcium oxalate in the dry cups.

Urinary calcium. Duplicate samples of 20 ml. of urine were evaporated to dryness and ashed at 600° C. The ash was dissolved in 6N hydrochloric acid and diluted to 25 ml. Duplicate 10 ml. samples were taken and the calcium and calcium-45 determined exactly as for blood plasma calcium.

Feed calcium. Quadruplicate samples of approximately 15 g. in weight were ashed, dissolved in 6N hydrochloric acid and diluted to 25 ml. Duplicate 0.5 ml. aliquots were used to determine the calcium as described for blood plasma calcium.

Methods for Analysis of Phosphorus

Phosphorus-32. In contrast to the low energy of the calcium-45 beta (0.26 Mev) that from phosphorus-32 has a high energy emission (1.71 Mev). This difference in energy provides a convenient basis for measuring the activity of the phosphorus-32 in the presence of calcium-45. Comar et al. (1951) showed that an aluminium absorber having a surface density of 55 mg. per square centimetre would absorb all the beta particles from the calcium-45 but would reduce the phosphorus-32 contribution by a factor of only 1.5. All samples and standards were therefore counted for phosphorus-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.

Blood plasma phosphorus. One ml. of plasma was added to 4 ml. of 5 per cent trichloracetic acid in a small centrifuge tube and thoroughly mixed. The solution was centrifuged and a 2 ml. aliquot of the supernate

was analysed for total inorganic phosphorus by the method of Fiske and Subbarow (1925).

Radioassay of phosphorus-32 in the plasma was conducted by counting a 3 to 5 ml. aliquot of the plasma contained in a small petri dish.

Fecal phosphorus. Duplicate 0.01 ml. aliquots of the same hydrochloric acid extract prepared for calcium analysis, were analysed for phosphorus by the method of Fiske and Subbarow (1925).

Radioassay of phosphorus-32 in the feces was performed on 10 ml. of the ash solution as described for plasma.

Urinary phosphorus. Phosphorus was determined on duplicate 0.2 ml. aliquots from the urine ash solution as prepared for calcium analysis by the procedure used for fecal phosphorus.

Radioassay of phosphorus-32 in the urine was made directly on duplicate 10 ml. aliquots of the fresh urine.

Methods for Calculation of Data

Isotope Dilution Procedure

Endogenous fecal phosphorus or calcium (E) in grams was determined from the mean specific activities for the feces (SA_f) and plasma (SA_p) on the last two days of the seven day balance trial:

$$E = \frac{SA_f}{SA_p} \times \text{daily fecal excretion}$$

Comparative Balance Procedure

The seven day isotope excretion data from animals receiving a single oral dose and that from similar animals receiving a single intravenous dose of the calcium-45 and the phosphorus-32 permitted calculation of the per-

centage of dietary element which was absorbed (A) as follows:

$$A = \frac{(100 - \%\ \text{fecal isotope from oral dose}) \times 100}{100 - \%\ \text{fecal isotope from intravenous dose}}$$

The fecal endogenous loss was then expressed as a percentage (P_e) of the food intake according to Hansard (1956):

$$P_e = A - (100 - \%\ \text{total fecal element})$$

The daily endogenous calcium or phosphorus was then calculated as follows:

$$E = \frac{P_e \times \text{daily intake of element}}{100}$$

The Calculation of True Digestibility

The two independent estimates of the endogenous fecal calcium or phosphorus obtained by the isotope dilution and the comparative balance procedures were used to correct the apparent digestibility data obtained from the conventional seven day balance trial as follows:

$$\text{Percentage true digestibility} =$$

$$= 100 - \frac{(\text{daily output of element (grams)} - E) \times 100}{\text{daily intake of element (grams)}}$$

It may be noted, however, that the percentage true digestibility of the dietary element may be obtained directly from the first calculation in the comparative procedure, it being identical with the net absorption (A), and therefore independent of the isotope dilution principle.

CHAPTER IV

RESULTS AND DISCUSSION

The Effects of Aluminium and Zinc upon Calcium Absorption and Retention

A summarized statement of results for calcium absorption and excretion is presented in Tables II and III. The chemical balance data showed a marked decrease in the net retention of calcium by animals receiving zinc as compared with the control animals. This lowered net retention of calcium was highly significant (P=0.01) at both levels of zinc intake. The effects of aluminium were less clearly defined. At the higher level of intake (Trials I and II) aluminium caused no signigicant difference in the net retention of calcium. At the lower level (Trials III and IV) however, there was a significant (P=0.05) decrease in calcium retention. Inconsistencies in the net retention data for the control animals, however, precluded any definite conclusion being reached as to the effects of level of administration of zinc and aluminium upon the net retention of calcium.

The apparent digestibilities of the calcium in the ration under the three treatments were consistent with the net retention data. The marked decrease in the apparent digestibility of the calcium in the high zinc ration was highly significant (P= 0.01) at both levels of zinc administration. The decrease occasioned by the aluminium treatment however failed to be significant. To some extent the depression in the apparent digestibility of the calcium, caused by the zinc, was a reflection of lowered calcium intakes on this treatment during the last few days of the balance trial

TABLE II

THE EFFECTS OF ALUMINIUM AND ZINC UPON CALCIUM ABSORPTION AND EXCRETION (MEAN DATA FOR TRIALS I AND II)

	Basal Ration	Aluminium Ration[a]	Zinc Ration[b]
Number of animals	8	8	8
Number of trials	2	2	2
Mean weight of animals (kg.)	35.6	33.4	33.7
Chemical balance data (g. daily):			
Intake of calcium	1.56	1.50	1.14
Output - fecal calcium	2.09	1.87	2.72
- urinary calcium	0.09	0.27	0.08
Net retention of calcium	-0.63	-0.65	-1.66[d]
Radiochemical data:			
Percent Ca-45 in feces;			
Intravenously administered	28.3	30.2	38.3
Orally administered	77.0	83.8	82.9
Fecal endogenous loss[c]:			
Isotope dilution technique	38.4	31.2	34.1
Comparative balance technique	27.4	24.2	57.7
Blood plasma calcium (mg./g.)	0.110	0.111	0.107
Apparent digestibility (%)	-33.8	-24.5	-140.9[d]
True digestibility (%):			
Isotope dilution technique	52.7	54.1	17.6
Comparative balance technique	31.2	32.7	24.8

[a] The aluminium ration contained 1,575 p.p.m. of aluminium (Al)

[b] The zinc ration contained 2,280 p.p.m. of zinc (Zn)

[c] Expressed as milligrams per kilogram of body weight per day

[d] $P < 0.01$

TABLE III

THE EFFECTS OF ALUMINIUM AND ZINC UPON CALCIUM ABSORPTION AND EXCRETION (MEAN DATA FOR TRIALS III AND IV)

	Basal Ration	Aluminium Ration[a]	Zinc Ration[b]
Number of animals	8	8	8
Number of trials	2	2	2
Mean weight of animals (kg.)	39.4	39.5	40.7
Chemical balance data (g. daily):			
Intake of calcium	1.57	1.53	1.50
Output - fecal calcium	1.67	1.89	2.29
- urinary calcium	0.03	0.10	0.12
Net retention of calcium	-0.13	-0.45[e]	-0.90[d]
Radiochemical data:			
Percent Ca-45 in feces;			
Intravenously administered	27.8	34.7	29.4
Orally administered	65.4	70.3	77.6
Fecal endogenous loss[c]:			
Isotope dilution technique	27.3	28.6	30.8
Comparative balance technique	19.2	29.6	36.9
Blood plasma calcium (mg./g.)	0.100	0.098	0.095
Apparent digestibility (%)	-6.4	-23.4	-51.0[d]
True digestibility (%):			
Isotope dilution technique	51.2	50.3	35.7
Comparative balance technique	48.5	46.2	34.6

[a] The aluminium ration contained 785 p.p.m. of aluminium (Al)

[b] The zinc ration contained 1,140 p.p.m. of zinc (Zn)

[c] Expressed as milligrams per kilogram of body weight per day

[d] $P < 0.01$

[e] $P < 0.05$

period. However, this by no means accounted for the total effect, for it may be noted that the total excretions of fecal calcium, irrespective of the intakes, were markedly higher when zinc was present in the ration.

No significant differences were found in the total plasma calcium levels of animals receiving any of the three rations, although a significant ($P = 0.05$) difference was obtained for blood calcium values in Trials I and II as compared with Trials III and IV.

The levels of calcium-45 in the blood of intravenously and orally dosed animals during the trial period are shown in Figure I. These data were calculated from the blood calcium-45 concentrations and the total blood volume, as described by Hansard et al. (1953).

The disappearance of intravenously administered calcium-45 from the blood follows a characteristic curve, and was similar to those for cattle presented by Hansard et al. (1954). The rapid initial fall corresponded to the period of transfer of the blood calcium-45 to the bones and soft tissues. Within about 24 hours this transfer was complete and equilibrium established between the blood, bone and gastro-intestinal calcium. Approximately 100 hours after administration of the dose, the rate of disappearance was sufficiently constant to allow the blood specific activity data and the corresponding data for the feces for this period, to be used in calculating the endogenous fecal calcium excretion. A high degree of reproducibility marked the blood calcium-45 disappearance data for all intravenously dosed animals, irrespective of treatment. No disturbance of the blood: bone: tissue, equilibrium could therefore be attributed to the zinc and aluminium treatments.

The appearance and subsequent disappearance curves for calcium-45

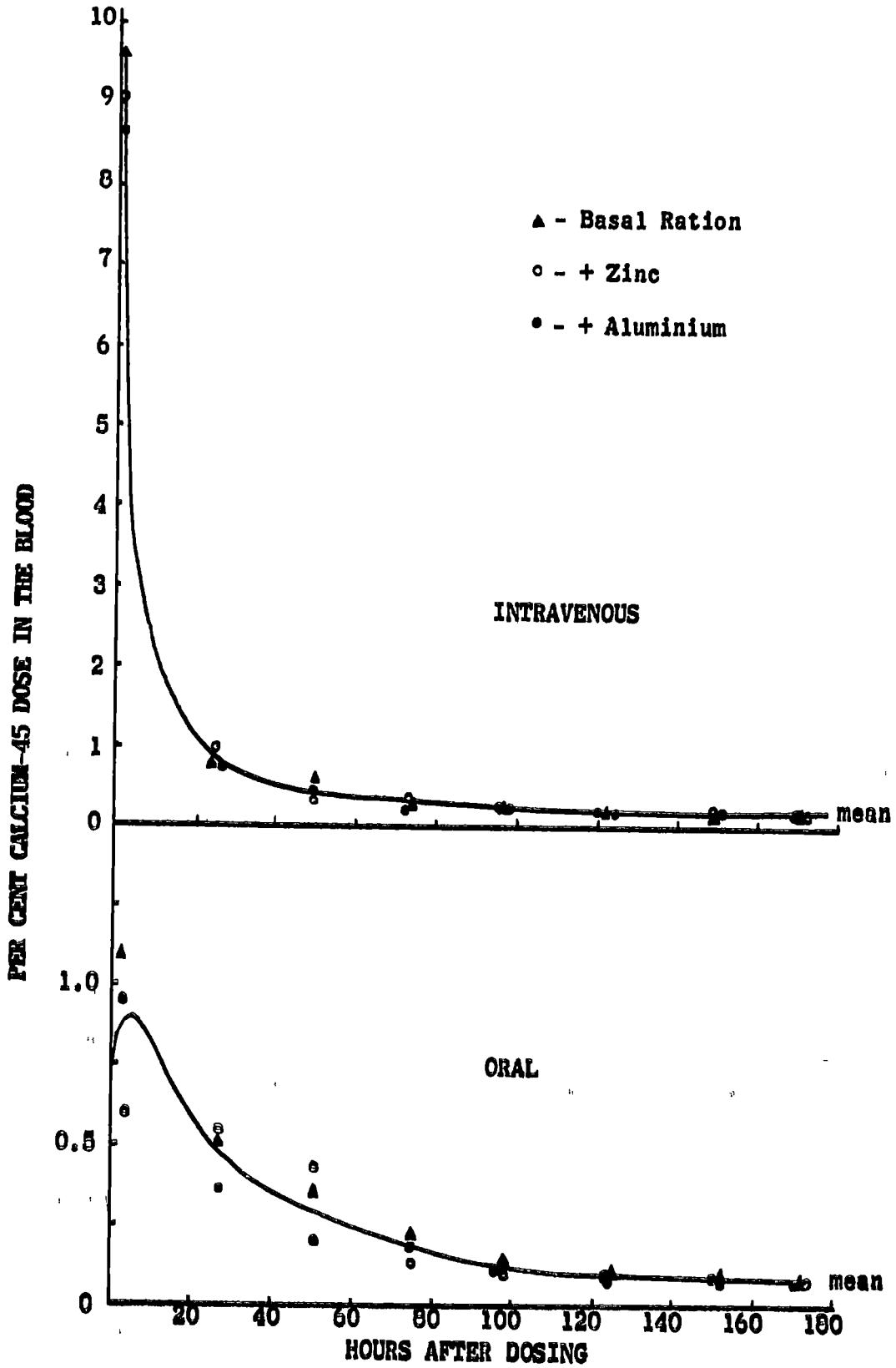

Figure 1. Calcium-45 in the blood plasma, following oral and intravenous administration

in the blood of orally dosed animals is also shown in Figure 1. Similar data has been presented for cattle by Hansard et al. (1954). In general, these curves show greater variability than those for the intravenously dosed animals. This is understandable in view of the complicating factor of gastro-intestinal transfer of calcium-45 to the blood with the concomitant transfer of the element to the bones and tissue. After approximately 100 hours, however, an equilibrium level was established almost identical with that for the intravenously dosed animals. By inspection, the present data suggested a lowered initial absorption of calcium-45 in those animals receiving zinc in their feed as compared with those on the basal diet. Less difference was observed in the curves for the aluminium-fed animals, however initial absorption was less than that of the basal group and disappearance from the blood was more rapid. After approximately 100 hours, no treatment differences could be detected in the final equilibrium level.

Figure 2 shows, graphically, the cumulative levels of fecal calcium-45 throughout the trial period for both orally and intravenously dosed animals. That for the orally treated animals primarily relates the cumulative excretion of non-absorbed calcium with time. The steep initial slope represents mainly unabsorbed calcium and after 70 hours the loss is largely endogenous calcium. From this graph it may be seen that some 65 per cent of the orally administered calcium-45 appeared in the feces during the first 72 hours with a total of 75 per cent being excreted after 7 days. This total cumulative percentage is compounded of the non-absorbed calcium-45 along with a smaller amount which may have been absorbed and re-excreted via the intestinal tract. Insignificant amounts were found to be excreted in the urine. The results suggest a reduced absorption of calcium-45 in those

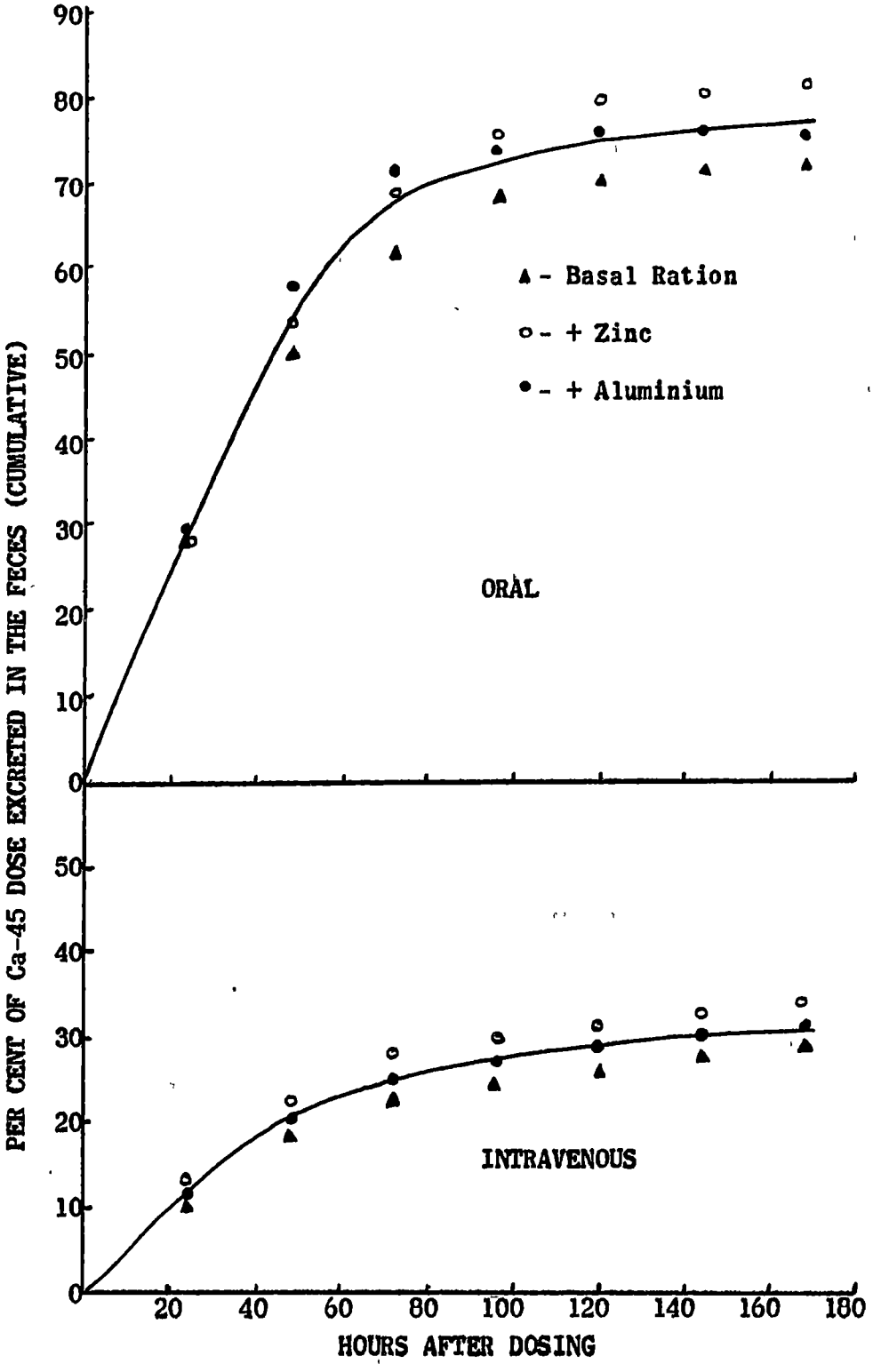

Figure 2. Cumulative fecal excretion of orally and intravenously administered calcium-45.

animals which received dietary zinc as compared to those on the control ration. The aluminium-fed animals also showed similar reduced absorption of calcium-45 but the change in slope towards the end of the absorption phase suggests also a slight reduction in re-excreted isotope.

Values for fecal levels of calcium-45 as a function of time after intravenous administration, are also presented in Figure 2. While the results suggested slight increases in the excretion of injected calcium-45 in the animals receiving zinc and aluminium as compared with the controls, it is doubtful whether much significance should be attached to this result, due to variation between individual animals.

The calculated radiochemical data, summarized in Tables II and III, show that the cumulative percentage of intravenously administered calcium-45 excreted in the feces averaged about 28 per cent for all animals, and about 32 per cent when calculated only for those receiving aluminium. This latter difference was not significant. At the higher level of zinc administration a significantly ($P=0.05$) greater amount of the calcium-45 appeared in the feces, namely 38.3 per cent. Of the orally dosed calcium-45, most of the animals receiving zinc or aluminium excreted more of the isotope in the feces than did the controls.

By combining the chemical and radiochemical data, fecal endogenous calcium excretion was calculated using the comparative balance procedure of Hansard et al. (1954), and by the isotope dilution method described by Hansard et al. (1957). Fecal endogenous calcium data, expressed as milligrams per kilogram of body weight per day, are shown in Table II and III. No significant differences were found in the endogenous fecal calcium excretions attributable to the zinc or aluminium, although there were

indications that aluminium had caused some reduction in the calcium loss from body stores. Neither was any significant difference noted between the results calculated by the two procedures. These findings justified the calculation of a total mean value for endogenous excretion. This was found to be 36 milligrams per kilogram of body weight per day, a level in general agreement with that found for similar lambs by Schroder (1957), namely, 42.5 milligrams per kilogram of body weight per day. While variations between individual animals precluded any significant differences being obtained between treatments, it would appear that the zinc-fed animals lost slightly more calcium from body sources than did the control animals.

The fecal endogenous calcium data for individual animals by the appropriate method of calculation, were used to calculate the true digestibilities of the dietary calcium, and the results are shown in Tables II and III. Despite the consistently lower true digestibilities obtained, using the comparative balance procedure, differences between the two methods did not attain significance. While the corrections for endogenous fecal calcium losses materially altered the absolute values of the apparent and true digestibilities, it did little to change the relative effects of the treatments. The feeding of aluminium did not appear to affect the true digestibility of the dietary calcium, however, zinc caused a highly significant ($P = 0.01$) depression. This decrease in the true digestibility of the dietary calcium was significantly ($P = 0.05$) greater at the higher levels of the zinc administration, indicating a possible accumulative effect.

Although the influence of zinc upon the true digestibility of the dietary calcium was most marked, the mechanism of this effect was less obvious.

The minor differences in endogenous fecal losses did not suggest that zinc caused any great change in the rate of loss of calcium from body stores. It must therefore be assumed that the effects of zinc were largely confined to the digestive tract. Feaster et al. (1954) presented data which indicated that most of an orally administered dose of zinc-65 was excreted in the feces, with relatively little being absorbed. It is therefore suggested that the feeding of zinc in the present study prevented or interfered with normal absorption of calcium, either by blocking the absorptive mechanism, or by direct antagonism between the two elements. Hoekstra et al. (1956), and others, have shown that diets high in calcium prevented the absorption of zinc by swine which resulted in a higher incidence of parakeratosis. This finding, together with the present data, suggest that the two elements may, in fact, exhibit some degree of antagonism.

The depression in the absorption of calcium caused by zinc was reflected in the blood calcium-45 appearance curve (Figure I) for orally administered isotope, however insufficient blood data during the critical first and second day period preclude any direct confirmation.

The Effects of Aluminium and Zinc upon Phosphorus Absorption and Retention

Data for phosphorus absorption and excretion in sheep, as influenced by the feeding of aluminium and zinc, are summarized in Tables IV and V. The results of the chemical balance showed a marked lowering of the net retention of phosphorus by animals receiving zinc. This result was highly significant ($P = 0.01$) at both the low and high levels of dietary zinc

TABLE IV

THE EFFECTS OF ALUMINIUM AND ZINC UPON PHOSPHORUS ABSORPTION AND EXCRETION (MEAN DATA FOR TRIALS I AND II)

	Basal Ration	Aluminium Ration[a]	Zinc Ration[b]
Number of animals	8	8	8
Number of trials	2	2	2
Mean weight of animals (kg.)	35.6	33.4	33.7
Chemical balance data (g. daily):			
Intake of phosphorus	2.01	1.95	1.49
Output - fecal phosphorus	2.06	2.07	1.92
- urinary phosphorus	0.05	0.04	0.07
Net retention of phosphorus	-0.10	-0.17	-0.50
Radiochemical data:			
Percent P-32 in feces;			
Intravenously administered	28.5	27.0	24.2
Orally administered	39.5	44.7	39.5
Fecal endogenous loss[c]:			
Isotope dilution technique	39.3	31.3	27.5
Comparative balance technique	50.3	53.3	50.7
Blood plasma phosphorus (mg./g.)	0.088	0.081	0.092
Apparent digestibility (%)	-3.03	-7.21	-35.5[d]
True digestibility (%):			
Isotope dilution technique	72.3	52.2	44.9[d]
Comparative balance technique	86.3	76.5	80.1

[a] The aluminium ration contained 1,575 p.p.m. of aluminium (Al)

[b] The zinc ration contained 2,280 p.p.m. of zinc (Zn)

[c] Expressed as milligrams per kilogram of body weight per day

[d] $P < 0.01$

TABLE V

THE EFFECTS OF ALUMINIUM AND ZINC UPON PHOSPHORUS ABSORPTION AND EXCRETION (MEAN DATA FOR TRIALS III AND IV)

	Basal Ration	Aluminium Ration[a]	Zinc Ration[b]
Number of animals	8	8	8
Number of trials	2	2	2
Mean weight of animals (kg.)	39.4	39.5	40.7
Chemical balance data (g. daily):			
Intake of phosphorus	1.76	1.72	1.69
Output - fecal phosphorus	1.64	1.80	2.09
- urinary phosphorus	0.04	0.03	0.05
Net retention of phosphorus	+0.08	-0.11	-0.44[d]
Radiochemical data:			
Percent P-32 in feces;			
Intravenously administered	26.1	28.7	31.3
Orally administered	31.8	37.2	39.0
Fecal endogenous loss[c]:			
Isotope dilution technique	23.2	28.3	26.3
Comparative balance technique	35.3	38.2	45.7
Blood plasma phosphorus (mg./g.)	0.076	0.076	0.071
Apparent digestibility (%)	+6.47	-6.78	-24.8[d]
True digestibility (%):			
Isotope dilution technique	53.6	53.9	37.0
Comparative balance technique	91.7	87.5	89.7

[a] The aluminium ration contained 785 p.p.m. of aluminium (Al)

[b] The zinc ration contained 1,140 p.p.m. of zinc (Zn)

[c] Expressed as milligrams per kilogram of body weight per day

[d] $P < 0.01$

intake when these were compared with their respective controls. The net retentions as between the two levels, however, showed no significant difference. No significant differences were obtained in the urinary excretion of phosphorus by any of the animals.

The apparent digestibilities of phosphorus in the different rations were consistent with the net absorption data. The marked decrease in the apparent digestibility caused by feeding zinc is highly significant ($P = 0.01$) at both levels of administration.

Figure 3 shows the blood phosphorus-32 levels in both intravenously and orally dosed animals during the trial period. The disappearance of intravenously administered phosphorus-32 from the blood followed the same characteristic curve as that of calcium-45. The phosphorus nuclide appeared to be removed from the blood at a slightly less rapid rate than was the calcium-45, an observation supported by the findings of Schroder (1957) for similar lambs.

The appearance and simultaneous disappearance rates of phosphorus-32 in the blood of orally dosed animals are also shown in Figure 3. The curve has a similar form to that for calcium. Relatively little significance may be attached to differences in phosphorus-32 uptake between the animals on the three treatments. The data for blood, sampled approximately 3 hours after dosing, would suggest, however, that slightly increased absorption of phosphorus-32 occured in those animals receiving zinc, and decreased absorption in those fed aluminium, as compared with controls. Within 24 hours after dosing the average disappearance rates of the phosphorus-32 were the same for all animals.

Figure 4 shows, graphically, the phosphorus-32 contents of the feces

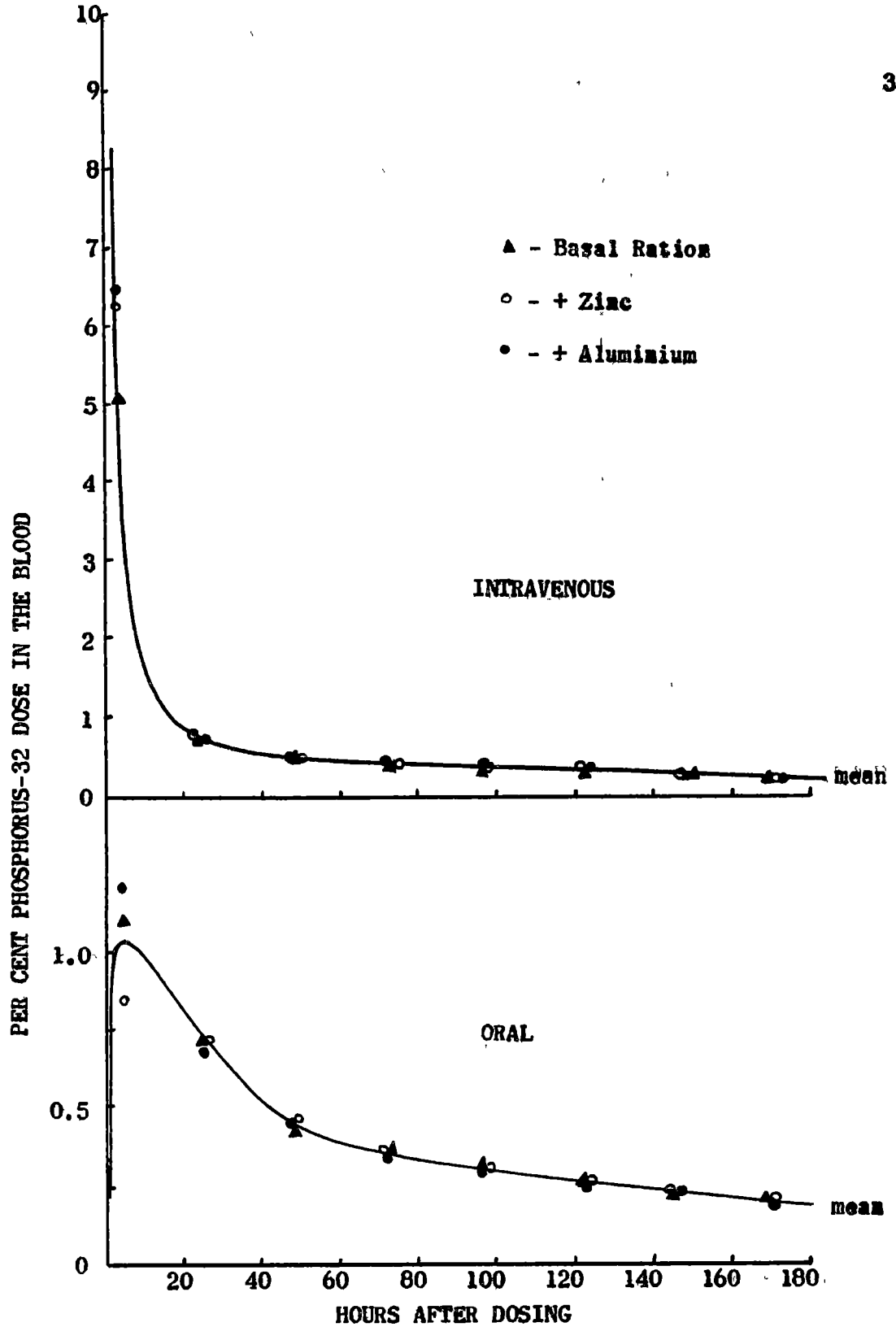

Figure 3. Phosphorus-32 in the blood plasma, following oral and intravenous administration.

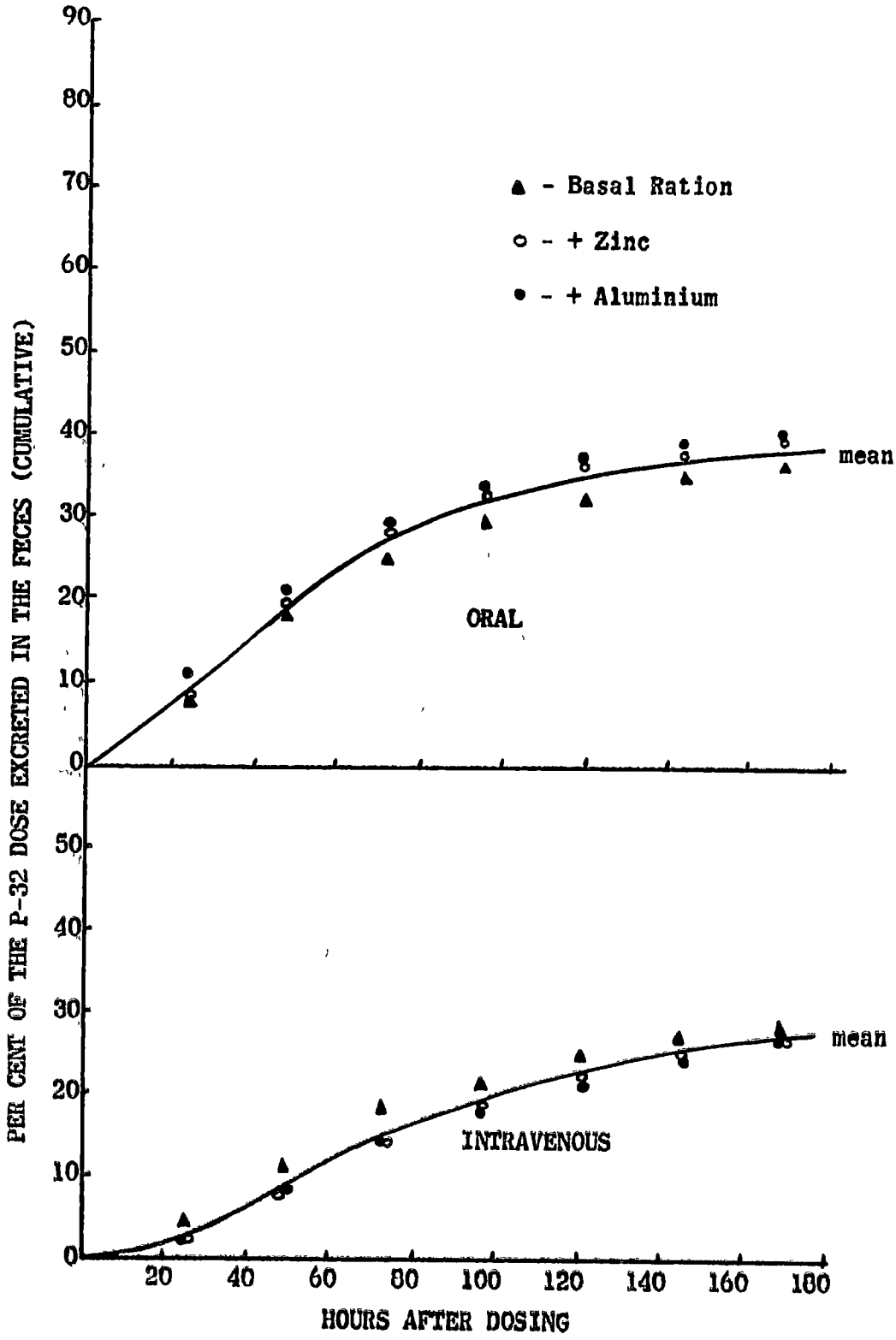

Figure 4. Cumulative fecal excretion of orally and intravenously administered phosphorus-32.

of orally dosed and intravenously dosed animals. By inspection, it may be seen that only some 40 per cent of the orally administered phosphorus-32 appeared in the feces. The first 60 hours on this curve primarily represents the non-absorbed phosphorus-32; after this time it mainly reflects that absorbed and then re-excreted into the gastrointestinal tract as endogenous excretion. These data would suggest a slightly greater excretion by those animals receiving zinc and aluminum than the controls. The intravenously injected phosphorus-32 appeared in the feces of the zinc - and aluminium-fed animals in slightly less quantities than it did in the control animals. These differences were, however, small and of little significance, except in that they reflect and substantiate the balance data.

The calculated radiochemical data summarized in Tables IV and V show that the mean cumulative percentage of the intravenously dosed phosphorus-32, recovered in the feces, was 27.6 per cent with no significant differences as between treatments or levels of feeding. Of the orally administered dose, the animals receiving the lower levels of zinc and aluminium appeared to excrete slightly more phosphorus-32 in the feces than did the control animals. However, at the higher zinc and aluminium intakes, only the aluminium-fed animals showed any increased excretion. None of these differences were significant however.

A discrepancy was noted between the results for the fecal endogenous phosphorus excretions, calculated by the comparative balance technique and by the isotope dilution procedure. In all cases the fecal endogenous phosphorus values (expressed as milligrams per kilogram of body weight per day) were greater when calculated using the former procedure. These differences between the two procedures were highly significant ($P = 0.01$) while dif-

ferences occasioned by the varying treatments were not.

The difference in method of estimation of the fecal endogenous phosphorus becomes even more marked when these data were used to correct the apparent digestibilities of the dietary phosphorus. The comparative balance procedure, for example, gave a mean true digestibility of the phosphorus in the ration of approximately 86 per cent, with no significant differences as between treatments. Whereas, the same data calculated by the isotope dilution method were lower, and with the exception of the true digestibility of the phosphorus for the basal ration in Trials I and II, averaged about 50 per cent. Further, the isotope dilution procedure showed a significant difference ($P = 0.01$) between the phosphorus digestibilities of the control ration and that of the zinc - containing diets; those for zinc being lower.

The fairly serious lack of agreement between the two techniques, as applied to the phosphorus-32 uptake and excretion data in the present study, warrants further discussion, particularly in view of the findings of previous workers showing satisfactory agreement between the two procedures. (Comar et al. 1953, and Schroder, 1957). This is made even more necessary by the fact that in the present study fairly close agreement was obtained for the calcium data calculated by the two procedures.

The chemical phosphorus balances show very clearly that dietary zinc had decreased the net retention of phosphorus and, as no significant differences were found between the endogenous fecal phosphorus excretions, it is reasonable to expect that the lowered net retentions caused by zinc would be reflected in the true digestibilities. This, the comparative balance data did not show and added support to the contention that the isotope

dilution procedure was giving the better estimate of the true digestibility.

In a comparative study of the two procedures for estimating the endogenous fecal calcium, the authors (Comar et al. 1953), state that the primary assumption in the comparative balance method is that the labelled calcium, given as soluble calcium chloride, and all the dietary calcium are absorbed to an equal degree. An alternative assumption, necessary before this method can give an estimate of the true digestibility of a dietary element, might be that the orally administered radioactive element and the stable form in the feed must reach complete equilibrium before independent absorption of the active element takes place. If this assumption is not valid, then much of the data will apply to the absorption of the particular form of the active element administered only, and not to the dietary form. The ease with which such an equilibrium may be attained will mainly depend upon physical conditions in the digestive tract and also upon the ease of exchange with the stable dietary element. In the latter connection it may be expected that any element may be present in any or all of three forms, namely, ionic, exchangeable and non-exchangeable. There is little reason to suppose that cations such as calcium are present in feeds as non-exchangeable forms, indeed it is highly probable that they exist largely in the ionic and exchangeable forms, thereby facilitating the establishment of equilibrium conditions with the administered calcium-45. However, in view of the many phosphorus-containing organic compounds present in biological materials it seems likely that some, at least, of the dietary phosphorus exists in forms not readily exchangeable with the administered phosphorus-32. If this were the case in the present study, then the values obtained for the true digestibility of phosphorus calculated by the comparative balance procedure appertained only

to the true digestibility of the exchangeable fraction of the dietary phosphorus and not to the dietary phosphorus as a whole. While this is a possible explanation of the consistently higher true digestibility data obtained using the comparative balance method, it offers little solution as to why this method fails to show any lowered digestibility of the phosphorus caused by feeding high levels of zinc. No obvious explanation is available from results of the present study and it can only be suggested that the mechanism by which the phosphorus was made less available to the animal extended also to rendering it less exchangeable with the administered phosphorus-32. This would result in true digestibility data which mainly referred to the availability of the administered phosphorus-32, which was in an ionic form, and not of the dietary phosphorus.

In the present investigation it was found that the feeding of 0.5 or 1.0 per cent of aluminium sulphate in the ration did not have any significant effect upon the true digestibility of the dietary calcium and caused only a slight decrease in that of the dietary phosphorus. These findings, particularly that relating to phosphorus, were surprising and at variance with a considerable body of evidence cited earlier. Cox et al. (1931) obtained a 15.0 per cent reduction in blood phosphorus in guinea pigs fed a ration containing 1,350 p.p.m. of soluble aluminium. The calcium and phosphorus in the bones of these animals were reduced to 70 per cent of normal in twelve weeks. In the present study no significant lowering of the blood phosphorus levels was noted which could be attributed to the feeding of aluminium. No satisfactory explanation of the discrepancy can be offered on the basis of the present data. However, it is of interest to note that all reported work showing a lowering of phosphorus availability by aluminum has

been conducted on non-ruminant animals, little is available reporting similar results for the ruminant. Struthers and Sieling (1950) have discussed the effects of organic anions upon phosphate precipitation. In the absence of such anions, aluminium phosphate is precipitated over the range pH 4.0-9.0. However, when such anions, particularly acids such as citric, are present the aluminium was complexed and was not precipitated as the phosphate at the above pH range. Small additions of citrate to already precipitated aluminium phosphate released 90 per cent of the 'unavailable' phosphorus. In view of the considerable quantities of such organic acids present in the rumen it may not be unreasonable to suggest that the same conditions for precipitation of phosphorus may not exist in the ruminant as they appear to do in the non-ruminant.

CHAPTER V

SUMMARY

The investigation was undertaken to study the effects of two levels of orally administered aluminium and zinc upon calcium and phosphorus retention in lambs. Twelve wether lambs were used in a series of four trials, in each of which, a basal ration, the basal ration plus added aluminium and the basal ration plus added zinc were fed. In Trials I and II the animals were given a ration containing 1.0 per cent of aluminium and zinc sulphates and in III and IV, 0.5 per cent.

Chemical and radio-chemical balance data for calcium and phosphorus, and calcium-45 and phosphorus-32 were collected, along with blood data for both the stable and radioactive nuclides. Calculations were made of the net retentions, the apparent digestibilities, the endogenous fecal excretions, and the true digestibilities of the dietary calcium and phosphorus.

In general, few significant differences were obtained between the two levels of administration of the zinc and aluminium with respect to their effects upon net retention, or upon the apparent and true digestibilities of calcium and phosphorus.

Very marked decreases in the net retention, the apparent and the true digestibilities of the calcium were found when zinc was fed but not when aluminium was administered. The endogenous fecal excretions on the three treatments showed no significant differences. This fact led to the conclusion that the effect of the zinc was largely confined to the alimentary tract and was a direct effect upon the absorption of calcium. No significant effects of aluminium upon the net retention, the apparent or

the true digestibilities of the calcium were found.

Zinc also seriously affected the retention of phosphorus, while the aluminium showed only a slight effect. Although the latter result was probably due to the precipitation of phosphorus as aluminium phosphate, the magnitude of the effect was very much smaller than that found in similar investigations by previous workers using non-ruminant animals. The lack of significance between the fecal endogenous phosphorus excretions by all animals on the three treatments again suggested that the slight depression in phosphorus retention attributable to aluminium and the much larger one due to the zinc were mainly the result of interference in the absorption processes in the alimentary tract.

Discrepancies were noted in the endogenous fecal phosphorus excretions as calculated by the isotope dilution technique as compared with those from the comparative balance method, and possible explanations of these discrepancies have been discussed.

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APPENDIX

TABLE VI

TOTAL CALCIUM AND PHOSPHORUS BALANCE DATA[a]
(TRIAL I)

Treatment	Animal Number	Body Weight Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
Basal Ration	730	39.7	1.70	2.06	2.44	2.66	0.19	0.02	2.63	2.68	-0.93	-0.62
	801	36.1	1.70	2.06	2.76	2.44	0.11	0.05	2.87	2.49	-1.17	-0.43
	689	32.4	1.70	2.06	1.90	2.54	0.20	0.05	2.10	2.59	-0.40	-0.53
	678	38.4	1.63	1.97	2.78	2.33	0.05	0.04	2.83	2.37	-1.20	-0.40
	Mean	36.6	1.68	2.04	2.47	2.49	0.14	0.04	2.61	2.53	-0.93	-0.49
Aluminium Ration	806	32.8	1.40	1.69	2.00	1.71	0.42	0.06	2.42	1.77	-1.02	-0.08
	825	26.1	1.21	1.46	1.62	1.83	0.05	0.04	1.67	1.87	-0.46	-0.41
	746	30.6	1.70	2.06	2.20	2.45	0.33	0.05	2.53	2.50	-0.83	-0.46
	733	37.2	1.70	2.06	2.57	2.47	0.50	0.04	3.07	2.51	-1.37	-0.45
	Mean	31.7	1.50	1.82	2.10	2.12	0.33	0.04	2.42	2.16	-0.92	-0.35
Zinc Ration	747	31.8	1.18	1.43	3.38	1.93	0.05	0.07	3.43	2.00	-2.25	-0.57
	724	32.9	0.72	0.87	1.54	1.31	0.11	0.06	1.65	1.37	-0.93	-0.50
	715	33.6	0.91	1.10	2.30	1.86	0.04	0.07	2.34	1.93	-1.43	-0.83
	739	36.0	1.28	1.55	3.46	2.31	0.09	0.08	3.55	2.39	-2.27	-0.72
	Mean	33.6	1.02	1.25	2.67	1.85	0.07	0.07	2.74	1.92	-1.72	-0.66

[a] Balance data expressed in grams per day

TABLE VII

TOTAL CALCIUM AND PHOSPHORUS BALANCE DATA[a] (TRIAL II)

Treatment	Animal Number	Body Weight Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
Basal Ration	746	32.4	1.53	2.12	1.71	1.85	0.04	0.04	1.75	1.89	-0.22	+0.23
	733	38.1	1.53	2.12	1.81	1.61	0.03	0.10	1.84	1.71	-0.31	+0.41
	739	36.1	1.53	2.12	1.83	1.41	0.03	0.04	1.86	1.45	-0.33	+0.67
	662	31.8	1.12	1.54	1.50	1.67	0.06	0.02	1.56	1.69	-0.44	-0.15
	Mean	34.6	1.43	1.98	1.71	1.64	0.04	0.05	1.75	1.69	-0.33	+0.29
Aluminium Ration	730	41.1	1.53	2.12	1.47	2.17	0.29	0.03	1.76	2.20	-0.23	-0.08
	724	34.7	1.41	1.95	1.69	2.05	0.34	0.04	2.03	2.09	-0.62	-0.14
	825	27.2	1.50	2.08	1.86	1.92	0.13	0.03	1.99	1.95	-0.49	+0.13
	801	37.0	1.53	2.12	1.54	1.94	0.12	0.03	1.66	1.97	-0.13	+0.15
	Mean	35.0	1.49	2.07	1.64	2.02	0.22	0.03	1.86	2.05	-0.37	+0.02
Zinc Ration	747	32.0	1.31	1.81	2.47	1.94	0.05	0.10	2.52	2.04	-1.21	-0.23
	678[b]	35.4	0.16	0.22	0.78	0.74	0.02	0.07	0.80	0.81	-0.64	-0.42
	689	32.9	1.16	1.60	2.70	1.84	0.07	0.07	2.77	1.91	-1.61	-0.31
	806	35.0	1.30	1.80	3.14	2.17	0.15	0.02	3.29	2.19	-1.99	-0.39
	Mean	33.8	1.26	1.74	2.77	1.98	0.09	0.06	2.86	2.04	-1.60	-0.30

[a] Balance data expressed in grams per day

[b] The data for this animal, which refused feed, are not included in the mean intakes, excretions and balances.

TABLE VIII

TOTAL CALCIUM AND PHOSPHORUS BALANCE DATA[a] (TRIAL III)

Treatment	Animal Number	Body Weight Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
Basal Ration	617	43.1	1.56	1.78	1.98	1.73	0.03	0.03	2.01	1.76	-0.45	+0.02
	733	40.8	1.56	1.78	1.51	1.81	0.02	0.07	1.53	1.88	+0.03	-0.10
	746	33.2	1.37	1.56	1.45	1.48	0.02	0.04	1.47	1.52	-0.10	+0.04
	724	38.2	1.56	1.78	1.68	1.56	0.05	0.05	1.73	1.61	-0.17	+0.17
	Mean	38.8	1.51	1.73	1.66	1.64	0.03	0.05	1.69	1.69	-0.17	+0.03
Aluminium Ration	641	38.2	1.56	1.78	1.93	1.97	0.03	0.02	1.96	1.99	-0.40	-0.21
	598	34.0	1.12	1.28	1.57	1.61	0.01	0.02	1.58	1.63	-0.46	-0.35
	659	34.5	1.56	1.78	2.20	1.71	0.04	0.02	2.24	1.73	-0.68	+0.05
	660	39.5	1.56	1.78	2.25	2.13	0.05	0.03	2.30	2.16	-0.74	-0.38
	Mean	36.6	1.45	1.66	1.99	1.86	0.03	0.02	2.02	1.88	-0.57	-0.22
Zinc Ration	730	43.1	1.56	1.78	1.62	2.04	0.03	0.06	1.65	2.10	-0.05	-0.32
	606	41.8	1.56	1.78	2.85	2.38	0.33	0.04	3.18	2.42	-1.62	-0.64
	801	39.5	1.56	1.78	2.47	1.95	0.04	0.17	2.51	2.12	-0.95	-0.34
	644	40.4	1.56	1.78	2.70	2.37	0.10	0.03	2.80	2.40	-1.24	-0.62
	Mean	41.2	1.56	1.78	2.41	2.19	0.12	0.07	2.54	2.26	-0.97	-0.48

[a] Balance data expressed in grams per day

TABLE IX

TOTAL CALCIUM AND PHOSPHORUS BALANCE DATA[a]
(TRIAL IV)

Treatment	Animal Number	Body Weight Kg.	Daily Intake Ca	Daily Intake P	Fecal Excretion Ca	Fecal Excretion P	Urinary Excretion Ca	Urinary Excretion P	Total Excretion Ca	Total Excretion P	Balance Ca	Balance P
Basal Ration	746	36.8	1.61	1.78	1.56	1.60	0.02	0.02	1.58	1.62	+0.03	+0.16
	659	34.7	1.61	1.78	2.19	1.79	0.02	0.02	2.21	1.81	-0.60	-0.03
	733	42.5	1.61	1.78	1.53	1.58	0.04	0.05	1.56	1.63	+0.05	+0.15
	730	45.8	1.61	1.78	1.41	1.55	0.03	0.02	1.44	1.57	+0.17	+0.21
	Mean	40.0	1.61	1.78	1.67	1.63	0.03	0.03	1.70	1.66	-0.09	+0.12
Aluminium Ration	617	44.0	1.61	1.78	1.82	1.82	0.18	0.02	2.00	1.84	-0.39	-0.06
	606	43.1	1.61	1.78	2.16	2.12	0.29	0.06	2.45	2.18	-0.84	-0.40
	801	41.1	1.61	1.78	1.14	1.35	0.03	0.04	1.17	1.39	+0.44	+0.39
	641	41.3	1.61	1.78	2.00	1.65	0.12	0.03	2.12	1.68	-0.51	+0.10
	Mean	42.4	1.61	1.78	1.78	1.74	0.16	0.04	1.94	1.77	-0.33	+0.01
Zinc Ration	724	35.8	0.97	1.07	1.53	1.51	0.02	0.02	1.55	1.53	-0.58	-0.46
	660	42.6	1.61	1.78	2.33	2.28	0.22	0.02	2.55	2.30	-0.94	-0.52
	678	40.6	1.61	1.78	2.12	1.93	0.16	0.02	2.28	1.95	-0.67	-0.17
	644	41.8	1.61	1.78	2.65	2.20	0.08	0.02	2.73	2.22	-1.12	-0.44
	Mean	40.2	1.45	1.60	2.16	1.98	0.12	0.02	2.28	2.00	-0.83	-0.40

[a] Balance data expressed in grams per day

TABLE X

CALCIUM-45 BALANCE DATA
(TRIAL I)

Treatment	Animal Number	Animal Weight Kg.	Method of Dosing	Percent Dose in Feces	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endogenous Iso.Dil.	Percent Fecal Endogenous Comp.Bal.	Milligrams of Fecal Endogenous[b] Iso.Dil.	Milligrams of Fecal Endogenous[b] Comp.Bal.
Basal Ration	730	39.7	I.V.	39.2	4.15	7.29	56.9		35.0	
	801	36.1	I.V.	37.5	3.86	6.81	56.7		43.2	
	689	32.4	O.S.	79.9				39.5		23.4
	678	38.4	O.S.	90.4				50.5		36.5
	Mean	36.6					56.8	45.0	39.1	30.0
Aluminium Ration	806	32.8	I.V.	37.6	6.05	9.08	66.5		42.3	
	825	26.1	I.V.	33.6	6.56	10.94	59.9		37.2	
	746	30.6	O.S.	89.6				35.2		25.2
	733	37.2	O.S.	90.0				43.9		30.4
	Mean	31.7					63.2	39.6	39.8	27.8
Zinc Ration	747	31.8	I.V.	56.0	5.50	8.29	59.6		46.2	
	724	32.9	I.V.	45.0	14.41	23.93	60.4		28.4	
	715	33.6	O.S.	74.2				76.2		52.0
	739	36.0	O.S.	84.8				68.9		65.8
	Mean	33.6					60.0	72.6	37.3	58.9

[a] Percent of the administered dose excreted during the 7-day balance period

[b] Expressed as milligrams per kilogram of body weight per day

TABLE XI

CALCIUM-45 BALANCE DATA
(TRIAL II)

Treatment	Animal Number	Animal Weight Kg.	Method of Dosing	Percent Dose in Feces[a]	Specific Activity		Percent Fecal Endogenous		Milligrams of Fecal Endogenous[b]	
					Feces	Plasma	Iso.Dil.	Comp.Bal.	Iso.Dil.	Comp.Bal.
Basal Ration	746	32.4	I.V.	20.6	3.74	4.89	75.3		39.7	
	733	38.1	I.V.	16.3	3.47	4.64	74.9		35.6	
	739	36.1	O.S.	78.3				38.3		19.4
	662	31.8	O.S.	59.4				63.1		30.0
	Mean	34.6					75.1	50.7	37.7	24.7
Aluminium Ration	730	41.1	I.V.	17.6	4.21	7.52	56.1		20.1	
	724	34.7	I.V.	32.1	6.84	13.40	51.1		24.9	
	825	27.2	O.S.	81.4				39.3		26.8
	801	37.0	O.S.	74.2				34.5		14.4
	Mean	35.0					53.6	36.9	22.5	20.6
Zinc Ration	747	32.0	I.V.	26.3	4.00	8.38	47.7		36.8	
	678[c]	35.4	I.V.	25.9	13.56	11.79	115.0		25.1	
	689	32.9	O.S.	87.8				64.2		52.5
	806	35.0	O.S.	84.8				67.2		60.2
	Mean	33.8					47.7	65.7	30.9	56.4

[a] Percent of the administered dose excreted during the 7-day balance period

[b] Expressed as milligrams per kilogram of body weight per day

[c] The data for this animal which refused feed, are not included in the mean endogenous fecal outputs.

TABLE XII

CALCIUM-45 BALANCE DATA (TRIAL III)

Treatment	Animal Number	Animal Weight Kg.	Method of Dosing	Percent Dose in Feces[a]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endogenous Iso.Dil.	Percent Fecal Endogenous Comp.Bal.	Milligrams of Fecal Endogenous[b] Iso.Dil.	Milligrams of Fecal Endogenous[b] Comp.Bal.
Basal Ration	617	43.1	I.V.	31.6	6.42	10.97	58.5		26.9	
	733	40.8	I.V.	18.7	4.86	11.54	42.2		15.5	
	746	33.2	O.S.	66.6				51.5		22.5
	724	38.2	O.S.	84.8				30.5		13.4
	Mean	38.8					50.3	41.0	21.2	18.0
Aluminium Ration	641	38.2	I.V.	36.7	8.12	16.14	50.3		25.4	
	598	34.0	I.V.	40.6	7.10	11.80	59.8		27.5	
	659	34.5	O.S.	72.0				62.9		39.9
	660	39.5	O.S.	83.7				52.0		29.7
	Mean	36.6					55.0	57.5	26.5	34.8
Zinc Ration	730	43.1	I.V.	18.3	4.80	11.38	42.2		15.9	
	606	41.8	I.V.	32.9	4.84	8.82	54.7		37.4	
	801	39.5	O.S.	52.7				70.1		43.8
	644	40.4	O.S.	65.0				71.5		47.5
	Mean	41.2					48.9	70.8	26.7	45.7

[a] Percent of the administered dose excreted during the 7-day balance period

[b] Expressed as milligrams per kilogram of body weight per day

TABLE XIII

CALCIUM-45 BALANCE DATA
(TRIAL IV)

Treatment	Animal Number	Animal Weight Kg.	Method of Dosing	Percent Dose in Feces[a]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endogenous Iso.Dil.	Percent Fecal Endogenous Comp.Bal.	Milligrams of Fecal Endogenous[b] Iso.Dil.	Milligrams of Fecal Endogenous[b] Comp.Bal.
Basal Ration	746	36.8	I.V.	22.7	5.41	8.74	61.9		26.2	
	659	34.7	I.V.	38.2	6.18	9.80	63.1		39.6	
	733	42.5	O.S.	57.1				59.1		21.3
	730	45.8	O.S.	53.1				62.2		19.2
	Mean	40.0					62.5	60.7	33.4	20.3
Aluminium Ration	617	44.0	I.V.	28.3	6.55	10.10	64.9		26.8	
	606	43.1	I.V.	35.1	6.46	9.40	68.6		34.4	
	801	41.1	O.S.	49.4				67.4		18.6
	641	41.3	O.S.	75.9				54.0		26.2
	Mean	42.4					66.8	60.7	30.6	22.4
Zinc Ration	724	35.8	I.V.	34.0	9.21	14.58	63.2		26.9	
	660	42.6	I.V.	32.3	4.66	7.73	60.3		32.8	
	678	40.6	O.S.	79.6				47.5		24.7
	644	41.8	O.S.	89.2				49.2		31.2
	Mean	40.2					61.8	48.4	34.8	28.0

[a] Percent of the administered dose excreted during the 7-day balance period

[b] Expressed as milligrams per kilogram of body weight per day

TABLE XIV

PHOSPHORUS-32 BALANCE DATA
(TRIAL I)

Treatment	Animal Number	Animal Weight Kg.	Method of Dosing	Percent Dose in Feces[a]	Specific Activity Feces Plasma	Percent Fecal Endogenous Iso.Dil.	Percent Fecal Endogenous Comp.Bal.	Milligrams of Fecal Endogenous[b] Iso.Dil.	Milligrams of Fecal Endogenous[b] Comp.Bal.
Basal Ration	730	39.7	I.V.	38.7	9.62 15.20	63.3		42.4	
	801	36.1	I.V.	34.2	9.43 13.24	71.2		50.6	
	689	32.4	O.S.	48.1			85.9		67.2
	678	38.4	O.S.	40.0			90.1		57.6
	Mean	36.6				67.3	88.0	46.5	62.4
Aluminium Ration	806	32.8	I.V.	36.1	10.62 21.07	50.3		26.9	
	825	26.1	I.V.	25.9	12.19 21.02	57.8		32.1	
	746	30.6	O.S.	40.6			83.4		70.8
	733	37.2	O.S.	62.7			61.6		42.3
	Mean	31.7				54.0	72.5	29.5	56.6
Zinc Ration	747	31.8	I.V.	28.9	13.98 21.25	65.8		39.9	
	724	32.9	I.V.	26.5	21.96 49.54	52.1		17.7	
	715	33.6	O.S.	43.7			88.0		48.2
	739	36.0	O.S.	42.5			86.0		55.3
	Mean	33.6				59.5	87.0	28.8	52.6

[a] Percent of the administered dose excreted during the 7-day balance period

[b] Expressed as milligrams per kilogram of body weight per day

TABLE XV

PHOSPHORUS-32 BALANCE DATA
(TRIAL II)

Treatment	Animal Number	Animal Weight Kg.	Method of Dosing	Percent Dose in Feces[a]	Specific Activity		Percent Fecal Endogenous		Milligrams of Fecal Endogenous[b]	
					Feces	Plasma	Iso.Dil.	Comp.Bal.	Iso.Dil.	Comp.Bal.
Basal Ration	746	32.4	I.V.	23.6	10.68	15.81	67.6		38.6	
	733	38.1	I.V.	17.4	9.21	15.31	60.2		25.5	
	739	36.1	O.S.	35.6				82.4		32.1
	662	31.8	O.S.	34.1				84.5		44.8
	Mean	34.6					63.9	83.5	32.0	38.5
Aluminium Ration	730	41.1	I.V.	22.7	9.46	17.84	52.9		28.0	
	724	34.7	I.V.	23.2	13.11	20.42	64.3		38.0	
	825	27.2	O.S.	40.0				78.4		55.4
	801	37.0	O.S.	35.7				84.5		44.2
	Mean	35.0					58.6	81.5	33.0	49.8
Zinc Ration	747	32.0	I.V.	25.6	20.06	26.06	56.9		34.6	
	678[c]	35.4	I.V.	15.9	34.99	40.89	85.5		17.8	
	689	32.9	O.S.	36.4				83.1		46.6
	806	35.0	O.S.	35.3				85.2		52.7
	Mean	33.8					56.9	84.2	34.6	54.6

[a] Percent of the administered dose excreted during the 7-day balance period

[b] Expressed as milligrams per kilogram of body weight per day

[c] The data for this animal, which refused feed, are not included in the mean endogenous fecal outputs

TABLE XVI

PHOSPHORUS-32 BALANCE DATA
(TRIAL III)

Treatment	Animal Number	Animal Weight Kg.	Method of Dosing	Percent Dose in Feces[a]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endogenous Iso.Dil.	Percent Fecal Endogenous Comp.Bal.	Milligrams of Fecal Endogenous[b] Iso.Dil.	Milligrams of Fecal Endogenous[b] Comp.Bal.
Basal Ration	617	43.1	I.V.	25.5	12.40	21.54	57.6		23.4	
	733	40.8	I.V.	21.3	11.10	19.46	57.0		24.7	
	746	33.2	O.S.	27.7				93.5		41.5
	724	38.2	O.S.	37.9				78.2		31.9
	Mean	38.8					57.3	85.9	24.0	36.7
Aluminium Ration	641	38.2	I.V.	31.0	13.60	20.53	66.2		34.1	
	598	34.0	I.V.	23.6	18.90	27.74	68.1		32.1	
	659	34.5	O.S.	41.9				91.4		45.4
	660	39.5	O.S.	41.5				84.5		45.4
	Mean	36.6					67.1	88.0	33.1	45.4
Zinc Ration	730	43.1	I.V.	29.5	12.00	23.02	52.1		24.6	
	606	41.8	I.V.	32.6	9.81	22.26	44.0		25.1	
	801	39.5	O.S.	28.8				98.8		44.7
	644	40.4	O.S.	37.1				94.6		54.5
	Mean	41.2					48.0	96.7	24.9	49.6

[a] Percent of the administered dose excreted during the 7-day balance period

[b] Expressed as milligrams per kilogram of body weight per day

TABLE XVII

PHOSPHORUS-32 BALANCE DATA (TRIAL IV)

Treatment	Animal Number	Animal Weight Kg.	Method of Dosing	Percent Dose in Feces[a]	Specific Activity Feces	Specific Activity Plasma	Percent Fecal Endogenous Iso.Dil.	Percent Fecal Endogenous Comp.Bal.	Milligrams of Fecal Endogenous[b] Iso.Dil.	Milligrams of Fecal Endogenous[b] Comp.Bal.
Basal Ration	746	36.8	I.V.	22.4	11.10	23.43	47.4		20.6	
	659	34.7	I.V.	35.2	15.00	32.20	46.5		24.1	
	733	42.5	O.S.	30.6				94.0		34.8
	730	45.8	O.S.	30.9				97.0		32.7
	Mean	40.0					46.9	95.5	22.4	33.8
Aluminium Ration	617	44.0	I.V.	29.2	15.80	26.90	55.4		24.3	
	606	43.1	I.V.	31.0	11.31	24.50	46.2		22.6	
	801	41.1	O.S.	23.7				90.0		29.4
	641	41.3	O.S.	42.0				81.5		32.6
	Mean	42.4					50.8	85.8	23.5	31.0
Zinc Ration	724	35.8	I.V.	31.4	21.10	35.82	58.9		24.7	
	660	42.6	I.V.	31.8	10.30	22.71	45.5		30.6	
	678	40.6	O.S.	43.8				84.2		40.0
	644	41.8	O.S.	46.5				83.0		43.6
	Mean	40.2					52.2	83.6	27.7	41.8

[a] Percent of the administered dose excreted during the 7-day balance period

[b] Expressed as milligrams per kilogram of body weight per day