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Phosphorus Metabolism of Lambs

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BIOGRAPHICAL.

The author received his early education in the schools of O'Brien County, Iowa. He attended the Iowa State College at Ames, Iowa, from 1900 to 1904, and received from that institution the degree of Bachelor of Science. During the two years following graduation, he was a teacher of the high-school sciences in the State of Nebraska. The school year of 1906-7 was spent in graduate study at the University of Nebraska. From 1907 till 1910 he was Head of the Science Department in the high school of Everett, Washington. Without interruption from June 1910 till June 1912 the author has pursued graduate study in chemistry at the University of Illinois. During the year 1911-12 he has held a fellowship in chemistry.

With Professor H. S. Grindley, he has published in the Journal of Biological Chemistry, an article entitled, "The Determination of Organic and Inorganic Phosphorus in Meats."
PHOSPHORUS METABOLISM OF LAMBS.

INTRODUCTION.

A. THE DEMAND OF THE ANIMAL BODY FOR PHOSPHORUS. All animals require a certain amount of phosphorus in their food.

The sources of the phosphorus which serve as food for animals are found in plant bodies, fruits, seeds, and animal tissues. Plant bodies, such as hays, grasses, fodders, and plant seeds, such as the cereals vary in richness, from 0.266 per cent for alfalfa hay to 1.537 per cent of phosphorus for wheat bran. As a rule the larger part of the phosphorus in these fruits and seeds, is in the organic form. The organic compounds of phosphorus which are found most commonly in the plant foods are phytin, nucleo-proteins and lecithins. Meats are somewhat poorer in phosphorus. According to Emmett and Grindley, the per cent of phosphorus in fresh meats varies from 0.168 per cent for veal breast to 0.345 for beef round. About 64 to 75 per cent of the phosphorus in raw beef is water soluble and of the soluble phosphorus from one-third to one-fourth is organic. The phosphorus in meat is found as nucleo-proteins, lecithins, and phosphates of potassium and sodium.

The amounts of phosphorus required for the normal growth of various animals is not known with any degree of certainty. The amount of phosphorus required daily by an average man is estimated by Siven as 0.7 to 0.8 grams, by Ehrstrom as 1 to 2 grams, and by Sherman, Mettler, and Sinclair 1.5 - 3.5 grams. A fifty pound pig, Hart, McCullom and Fuller claim, should receive about 3 grams of phosphorus daily. Forbes states that Kellner claims that a lamb
from 4 1-2 months to 1 1-2 years old should receive 4.95 grams of phosphorus daily.

As to the relative availability of organic and inorganic phosphorus, there is a wide difference of opinion among investigators. That phosphorus is absolutely necessary for life is proven by the experiments of Umikoff43 on rats and doves, which were fed on a phosphorus-free diet which failed to maintain life. This point is also emphasized by the experiments of Hart, McCullom and Fuller6 in causing extreme pathological conditions in pigs by feeding them very small amounts of phosphorus. Certain chemists have found in their experiments that the feeding of inorganic phosphorus has been beneficial. Cohn11 found that when calcium phosphate was added to the rations of two to three year-old colts a good effect was noticed. Wheeler12 showed that when bone-ash was added to the feed of chickens good results were obtained. Various investigators further claim that there is good evidence that inorganic phosphates are assimilated. Kohler13 claims that calcium phosphate is assimilated by lambs and the di- and tri-calcium phosphates are better assimilated than bone ash. In experiments with men, Hoppe-Seyler14 obtained evidence that calcium phosphate was absorbed from the digestive tract. Lehmann,16 Weildt,16 and Gohren17 claim that calcium-phosphate is absorbed from the intestines of certain small animals with which they worked. Other workers in this field claim to have shown that the feeding of inorganic phosphorus causes an increase in the inorganic phosphorus content of milk and bones. Hess and Schaffer18 and Passon19 show data on cows which indicate that an increase in the calcium-phosphate content of milk was caused by calcium-phosphate feeding. Hart, McCullom and Fuller6 have shown that pigs fed on 1.12 grams of phosphorus per day developed weak, small porous bones, while those pigs ingesting
additional phosphorus in the inorganic form developed large, strong, healthy bones. McCullum\textsuperscript{20} fed rats on rations differing in the amount of phosphorus and kind of phosphorus and showed that calcium-phosphate produced bones and muscles as high in phosphorus content as phosphorus in the organic form. Patterson\textsuperscript{21} fed rabbits on oat meal and corn meal, both low in phosphorus, and caused a loss of calcium from the bones of the animals. Weiske\textsuperscript{22} also working with rabbits, showed a very marked demineralization of the bones of the animals when fed on oats exclusively. D'Auchald\textsuperscript{23} added bone ash to the diet of chickens and caused an increase in the weight of the skeleton thereby.

In working with steers at Mockern\textsuperscript{24} they showed that calcium phosphate added to the ration was beneficial to the bones. In the case of fasting, O. Wellman\textsuperscript{25} has shown an elimination of calcium and phosphorus at the expense of the bones. Chalmers Watson\textsuperscript{26} shows that when animals are put on exclusive meat diet the bones present an appearance of delayed and imperfect ossification, with increased vascularity, or blood content, and an increase in the number of red corpuscles. He also states that the symptoms produced are similar to those of rickets in human beings but microscopic examination showed that they are not identical.

Considerable evidence has been brought forward indicating that phosphorus in organic combination is the important form for purposes of food. Rohmann\textsuperscript{27}, Marcuse\textsuperscript{28}, steinitz\textsuperscript{29}, Lupziger\textsuperscript{30}, Zadi\textsuperscript{1}, Ehrlich\textsuperscript{32}, and Gottstein\textsuperscript{33} consider that phosphorus in the simpler organic combinations as most easily absorbed. In experiments where lecithin was fed by Selensky\textsuperscript{34}, Serono\textsuperscript{35}, Desgrez, and Zaky\textsuperscript{36}, Gilbert and Fournier\textsuperscript{37}, Carriere\textsuperscript{38}, and Slaude and Zaky\textsuperscript{39} beneficial results were noted. Zaky\textsuperscript{40} in feeding guinea-pigs got beneficial re-
suits when lecithin and nucleins were added to the diet. Calcium-
phosphate fed to lambs by Hormeister failed to satisfy the demands
of the animals. The soft tissues of dogs and rabbits did not retain
their phosphorus content when inorganic salts of phosphorus was added
to their diets by Leclerc and Cook. Cronbein and Müller showed
the advantages of organic phosphorus over inorganic in experiments
with five infants and one boy. Umikoff in feeding rats and doves,
showed that lecithin was a more efficient compound for these animals
than nucleo-proteins. In feeding rabbits on lecithin, Franchini showed
that an increase in the lecithin content of the livers was pro-
duced by lecithin feeding and also that most of the lecithin ingested
was absorbed. Loewi showed that in certain cases, nucleo-proteins
were absorbed. Fingerling did experiments showing that phytin, le-
cithin, sodium-nucleate, and inorganic phosphates were absorbed to
different degrees, depending on the physical characteristics of the
accompanying food. In certain experiments carried out by H. Labbe organic compounds such as nucleins and lecithins were better absorbed
than were inorganic phosphorus salts. F.C. Cook found that rabbits
fed organic phosphorus stored more organic phosphorus in the brain
and nerves and slightly more in the bones than where only inorganic
phosphorus was added to the diet. Sherman, Mettler and Sinclair conclude that organic phosphorus is of greater nutritive value to man
than inorganic phosphorus.

B. FATE OF PHOSPHORUS COMPOUNDS ENTERING THE BODY. The
fate of phosphorus compounds which are ingested by animals, is affect-
ed by the presence of the minerals, calcium, magnesium, potassium,
and sodium. As E. B. Forbes has pointed out, when these bases are deficient, the phosphorus of the foods cause an acidosis which
brings forth pathological conditions and death in a short time. The
acidity or alkalinity of the intestine seems to have some influence on the avenue of excretion of phosphorus. F. C. Cook states that when the contents of the intestine is alkaline, the main amount of phosphorus is excreted through the intestine. The kind of animal also is a factor in determining the way in which the phosphorus is eliminated. According to the text-books on nutrition by Lusk and Sherman, herbivora excrete the majority of their phosphorus by way of the feces, while man and carnivora excrete the bulk of phosphorus by way of the urine. The condition of the animal is also a factor that influences the fate of ingested phosphorus. H.C. Sherman and Preysz have shown that a man taking vigorous exercise will excrete more phosphorus than when no exercise is taken.

1. Organic Phosphorus. The extent of transformation, absorption, and storage of organic compounds is very hard to determine, owing to the fact that all these processes take place practically at the same time, and in the same place, simultaneously with an excretion of phosphorus compounds from the intestinal wall. H.C. Sherman says that the feces do not consist entirely of undigested residues, but contain a relatively large amount of metabolic products. Mendell and Thacher claim that mineral compounds absorbed from the intestinal tract may reappear in the feces.

Considerable evidence has been brought forward tending to prove that organic-phosphorus compounds are transformed by the life processes without ever having been stored as a part of the living tissue. Loewi found that in his work nucleins were practically split in the intestine, the nitrogen part being absorbed and the phosphorus eliminated in the feces. Scofoni and Gracosa claim that ingested phytin is eliminated principally in inorganic combinations. Bergman showed that an injection of glycero-phosphoric acid into
sheep did not cause a urinary excretion of that compound but did cause an excretion of extra inorganic phosphates in the feces. Jordan in working with cows, shows that unused phytin and nucleo-proteins are excreted in the feces, in the inorganic form. He also stated that at no time was phytin found either in the milk or foods of the cows which were fed large amounts of phytin at times. Hart, McCullum, and Fuller who worked with pigs which were fed variable amounts of the different forms of phosphorus, state that since practically all of the phytin was eliminated in the feces as inorganic phosphorus, and that since there is no phytin splitting enzyme in the digestive tract, the phytin must have been absorbed, transformed, in the blood and liver, and excreted in the inorganic phosphorus form.

That organic phosphorus compounds are assimilated, as such, is claimed to be proved by many chemists. The proofs seem in every case to be indirect and open to possible errors. No investigator has yet been able to say that the animals with which he was working excreted in the feces and urine just so much of such and such compounds as metabolic products. Neither has any worker been able to say with any direct proof that when certain organic phosphorus compounds have been fed to animals, these compounds have either been assimilated unbroken, or been assimilated in parts, one being an inorganic phosphorus compound and the other organic. Until an investigator can measure directly the above mentioned factors, it seems that there can be no direct proof for the direct assimilation of organic-phosphorus compounds. Till that time, indirect proofs must be accepted. The fact that the urines of many animals which naturally ingest considerable amounts of organic phosphorus, do not contain any or at most very small quantities of organic phosphorus,
is a good indication that little if any organic phosphorus is absorbed. Patton, Jordan, and Hart\textsuperscript{58} found no organic phosphorus in the urine of cows. Leclerc and Cook\textsuperscript{42} found no organic phosphorus in the urines of dogs or rabbits. Ceconi\textsuperscript{53}, Jolly\textsuperscript{60}, Cortel\textsuperscript{61}, and Keller\textsuperscript{62} in their work on organic phosphorus in urines, do not emphasize the importance of the small quantities found. F. C. Cook\textsuperscript{49} after feeding rabbits for several months on organic phosphorus could not find any organic phosphorus in the urine. A number of chemists state directly that organic phosphorus is absorbed. Franchini\textsuperscript{45} states that most of the lecithin fed to rabbits was absorbed, for only a very small increase was noted in the feces. As mentioned above Rohmann\textsuperscript{27}, Marcuse\textsuperscript{28}, Steinitz\textsuperscript{29}, Leipsiger\textsuperscript{30}, Zadik\textsuperscript{31}, Ehrlich\textsuperscript{32}, and Gottstein\textsuperscript{33} hold that probably the organic phosphorus compounds of the body are built up of the simpler organic phosphorus compounds ingested. Loewi\textsuperscript{46} claims that nuclein is absorbed as such.

2. Inorganic Phosphorus. There are many claims that inorganic phosphorus may be absorbed, stored, and partially resynthesized into organic phosphorus compounds.

Weiske\textsuperscript{22}, Chalmers Watson\textsuperscript{26} and Hart, MCCullum and Fuller\textsuperscript{6} have shown that when insufficient phosphorus is found in food, the bones become drained of their calcium phosphate. O. Wellman\textsuperscript{26} has shown that during fasting, the bones lose calcium and phosphorus.

D'Auchald\textsuperscript{23}, Mochern\textsuperscript{25} and Hart, MCCullum and Fuller\textsuperscript{6} have proved that the addition of calcium phosphate to diets poor in phosphorus, caused an increase in the calcium phosphate of bones, of chickens, steers, and pigs respectively.

Hart, MCCullum and Fuller\textsuperscript{6} have proved that on feeding rations deficient in phosphorus, the soft parts of the body remain practically normal in their phosphorus content at the expense of the
bones. If such is true, and inorganic phosphorus will build up bone, why is that not evidence enough that inorganic phosphorus may be synthesized into the organic phosphorus combination?

Through experiments of Umikoff,\(^{44}\) on rats and doves, Kohler\(^ {13}\) on lambs, Gohren,\(^ {17}\) Weildt\(^ {18}\) and Lehmann\(^ {15}\) on small animals, Hart, McCullum and Fuller\(^ {6}\) on pigs, and Hoppe-Seyler\(^ {14}\) on man, each have concluded that inorganic phosphorus can be absorbed.

That nucleo-proteins are synthesized from inorganic phosphorus compounds by the animal body, is held by Ehrstrom\(^ {4}\) and Keller\(^ {3}\). Through experiments in which rats were fed varying amounts and kinds of phosphorus, McCullum\(^ {20}\) concluded that these animals must have synthesized nucleo-proteins from calcium-phosphate.
PURPOSE OF EXPERIMENT.

Protein has long been known as a necessary constituent of the food of animals. The amount necessary for the normal development of various animals has been the subject of many experiments and discussions. The effect of various amounts of ingested protein food upon the metabolism of other nutrients has received some attention. In this experiment, the metabolism of the different forms of phosphorus by lambs which were fed on three different rations, was made the object of study. The rations differed mainly in the amount of oil meal, which is a feed very rich in protein. From the data gathered in this experiment, it will be attempted in the following pages to show the influence of these rations upon the metabolism of the different forms of phosphorus. This will be done, first by indicating the quantities of the different forms of phosphorus ingested and excreted, and second by showing the causes for the variations in the amounts of different forms excreted. Indirectly from such a study, the degree to which the storage, digestibility, and transformability of the different forms of phosphorus may have been influenced, will be studied.

GENERAL PLAN OF EXPERIMENT.

A. ANIMALS USED. Six good grade Shropshire wether lambs were used in this experiment. They were about nine months old. They were chosen as the best representatives of three lots of lambs which had been fed for about three months exactly as the six were fed during this experiment. The three lots of lambs consisted of seven lambs in each lot. They were chosen, by competent sheep
judges, when the lambs were about two weeks old and were brought to the University Farm with their mothers. Special care of them was taken from that time, with the purpose in mind of preparing them for this experiment.

B. RATIONS. The rations of the three lots consisted of alfalfa hay, corn, and oil meal. The amount of alfalfa hay fed depended upon the appetite of the individual. The amount received by each animal was 105 per cent of that consumed during the preceding week.

The amount of concentrates in the rations for every 100 pounds of live weight of the animal, was 1.5 pounds. The concentrates of the ration for the high-protein lot consisted of equal parts of corn and oil meal, for the medium-protein lot the concentrates consisted of 75 per cent of corn and 25 per cent of oil meal, and for the low-protein lot 95 per cent of corn and 5 per cent of oil meal. Unlimited water supply was accessible at all times.

For a period of three months preceding this experiment, these animals were fed exactly as during the experiment. Also for about two and one-half months more before the experiment, the lambs were fed almost exactly the same rations as during the experiment.

C. CARE OF ANIMALS. December 17th each lamb was put in a metabolism cage which was large enough to allow the animal to turn around easily. These cages were set close together in an unheated barn. Practically the only different condition for the animals in the metabolism experiment was that not so much exercise was possible as when the lambs were in their pens.

The lambs were fed twice each day - at 7 a.m., and at 4 p.m. The grain was fed about twenty minutes before the hay. The
Grain orts were collected just before feeding the hay. The hay orts of the previous feeding were collected before each new feeding.

To each lamb was strapped a canvass bag in which the feces were collected. The bag was lined with oil cloth in order to prevent loss of moisture and solid material. The feces were collected from these bags at 2 p.m. daily.

Under the slat bottom of each cage was a zinc funnal which caught the urine as it was passed.

Data on the animals was collected from December 21 to January 2. This time was divided into three periods of four days each.

Collection of samples and non-chemical data.

The hay, corn, and oil meal were thoroughly sampled according to the common assaying methods. The corn and oil meal samples were finely ground and put into airtight glass jars. The hay was air dried at 60 degrees, ground finely, and stored in air tight glass jars.

The orts were collected and weighed for each individual for the four day periods. They were treated in the same way as the feeds and stored similarly.

The feces of each lamb for each period of four days, were collected in thymoled air-tight tin pails, and weighed. These were well mixed by a thorough kneading. The sample used for analysis was taken fresh.

The urines of each lamb were collected and composites for the periods were made. These were weighed, thoroughly mixed,
and stored in 2 1-2 liter reagent-bottles. The specific gravity of each sample was also determined. The sample used for analysis was taken when fresh.

The weights of the lambs were taken December 21, just before the experiment proper began, and January 4 just after the experiment was over.

The amount of water consumed was determined by the difference between the amounts offered and refused.

Obviously the weights of the different feeds offered to each animal, were taken and recorded.

COLLECTION OF CHEMICAL DATA.

The alfalfa hay, corn, oil meal, hay orts, grain orts, feces, and urines were analyzed for constituents which were considered as important for the accomplishment of the purpose of this experiment. The hay and hay orts were first air-dried at 60 degrees. The air dried hay and hay orts, corn, oil meal, grain orts, fresh feces, and fresh urines were analyzed for total moisture, total nitrogen, non-protein nitrogen, total phosphorus, total acid soluble phosphorus, and inorganic acid soluble phosphorus, except in the case of the urines which were not analyzed for total acid soluble or inorganic acid soluble phosphorus.

The data on the acid insoluble phosphorus, organic acid soluble phosphorus and protein-nitrogen was gotten by difference. The amount of acid-insoluble phosphorus was considered as the difference between the amount of total and acid-soluble phosphorus. The amount of organic acid-soluble phosphorus was taken as the difference between the amount of total acid-soluble and inorganic acid-
soluble phosphorus.

The protein-nitrogen was calculated by taking the difference between the total nitrogen and the non-protein nitrogen.

The content of the fresh hay and fresh hay orts in total nitrogen, non-protein nitrogen, and different forms of phosphorus, was calculated from the data on the amount of moisture lost upon air-drying the samples at 60 degrees C.

The data on the content of the water-free hay, corn, oil meal, hay orts, grain orts, and feces, was calculated from the data on the content of these things as analyzed and the data on the water content of them as analyzed.

A. METHOD OF AIR-DRYING. A weighed sample of about one kilogram of material was spread out in a weighed shallow granite pan, and kept at a temperature of 60 degrees C. for a period of three to fourteen days, depending upon the kind of material. Frequently the substance was stirred with a weighed stirring rod. When the substance had been dried as much as possible under this treatment, the pan and contents were put in a cupboard for one day. In the cupboard, the unwarmed and unaltered atmosphere existed. At the end of the day of exposure to the atmospheric conditions, the pan and contents were again weighed.

B. METHOD OF DETERMINING MOISTURE. (VACUUM METHOD). Two to five grams of the material were weighed out into cleaned and weighed lead caps. These samples were placed in small sulphuric acid dessicating which were later evacuated of air. The dessicators were then placed on a shaking machine which kept the acid in motion for a period of 36 to 48 hours. After this time, dry air was carefully and slowly allowed to run into the dessicators through a wash bottle containing concentrated chemically pure sul-
phuric acid. The dried samples were rapidly weighed. They were then returned to the dessicators, and treated as before until their weights became constant.

C. METHOD OF DETERMINING TOTAL ASH. Four to eight grams of the material were weighed out into cleaned, ignited, and weighed 3-inch porcelain dishes. The dishes were then placed in an oven at 110° C. till the samples were dry. The samples were then charred on a sand bath at a temperature lower than red heat. When the material was completely charred it was digested with water for an hour. At the end of this time the liquids were filtered through 11 cm. quantitative filter papers into 150 cc. beakers. The residues on the filters were washed five times with boiling distilled water. Then the filter papers were transferred to their respective dishes and placed in an air oven and dried thoroughly. The dishes were placed over Bunsen flames until the carbon was completely oxidized. After the dishes had cooled, each extract was returned to its respective dish. The water from each was evaporated off on a water-bath and the drying was completed in an air-bath which was heated to about 100° C. After the drying, they were ignited at a temperature lower than red heat, over the Bunsen flame. When the ashes became white, the dishes were placed in dessicators to cool. After having cooled, about one hour, the dishes and contents were rapidly weighed. Then the dishes were heated and weighed repeatedly till the weights were constant.

D. METHOD OF MAKING 0.2 PER CENT HCL EXTRACT. A sample of suitable size (about 100 grams of feces or 50 grams of feed) was weighed about equally into two 500 cc. centrifuge bottles. A pinch of powdered thymol was put into each bottle. Into
each bottle was pipetted 300 cc. of 0.2 per cent HCl. The bottles were then closed by wiring on the rubber stoppers. Then the bottle was shaken till the contents was homogenous. The bottles were wired spoke-wise to a bicycle wheel which turned on a horizontal axis at the rate of about 38 revolutions per minute. The centrifuge bottles were allowed to be shaken in this manner from 12 to 14 hours.

The bottles were then opened, the sides of each bottle were washed with a 25 cc. pipette-full of the acid solution and then these were placed in the centrifuge. The centrifuge running at the rate of about 1700 revolutions per minute, was allowed to run ten minutes. The clear solutions in the bottles was carefully poured into a 3-liter measuring flash. Then 100 cc. of the acid solution was added from a pipette to each bottle. The bottle was shaken till the contents was homogenous and then the sides of the bottles again washed with 25 cc. of the solution. Centrifugation and pouring off was again carried out. These processes of washing were repeated till 9 centrifugations were done. The solution in the measuring flask was then made up to the mark with the 0.2per cent HCl solution and thoroughly mixed. If the solution in the flask was quite thick with sediment, it was allowed to settle before decanting off the liquid into the 10 inch good qualitative filters. The filtering was repeated till the filtrate was perfectly clear. Generally a tenth extraction was made, which was tested qualita-

e. METHOD OF DETERMINING TOTAL NITROGEN. Of the material 1.2 to 1.5 grams were taken in triplicate in Kjeldahl flasks of 500 cc. capacity. To transfer the sample to the flask, 11 cm. filter paper was used. Each sample was treated with 25 cc. of concen-
trated sulphuric acid, in such a manner that the material was thoroughly soaked with the acid. They were heated gently on the digester until the forthing ceased, and then were allowed to cool. Five grams of powdered potassium-sulphate and 0.7 grams of mercury were added to each flask. They were heated again until the liquid became clear. Boiling was continued for two hours after this, and then the flames were turned out. As soon as the flasks became cool the sides were washed with water and the contents boiled for half an hour longer. After the flask had cooled again, 250 cc. of ammonia-free water was added to each flask. The necessary amount of standard acid, colored with Congo-red, was measured out into bottles which were then placed under the condensers of the distilling apparatus. After adding to each flask a pinch of ignited powdered pumice-stone, 80 cc. of a mixture of sodium-hydroxide and potassium sulphide (containing 600 grams of Greenbank alkali and 12.5 grams of potassium sulphide per liter of distilled water), was carefully added to each Kjeldahl flask. The flasks were then connected to the condensers and shaken. The burners under the flasks were lighted immediately and so adjusted that about 200 cc. was distilled over in about 30 or 40 minutes. The delivery tubes were taken from the distillates and washed in the usual manner. Several drops more of the indicator were added to each distillate and each distillate titrated with standard ammonia. The ammonia was standardized each day.

F. METHOD OF DETERMINING NON-PROTEIN NITROGEN. Triplicate samples of about 2 1-2 grams of the material were weighed into 200 cc. measuring flasks. About 150 cc. of cold water was added to each flask and shaken frequently for half an hour. Then to each flask 2 cc. of a saturated solution of potassium alum were added.
Following this, each solution was treated with about 9 cc. of Stutzer's reagent and shaken. The flasks were filled to the mark by adding water. The contents were well mixed and allowed to stand overnight. Then these were filtered through 11 cm. B. and A. quantitative filters. Total nitrogen was determined by the Kjeldahl method on 100 cc. samples of this filtrate. Blank determinations on the reagents used were run according to the above method.

G. GENERAL METHOD OF DETERMINING TOTAL PHOSPHORUS. The residues from the ash determinations were treated with 20 to 30 cc. of concentrated chemically pure nitric acid and covered with watchglasses. The dishes were set on a steam-bath and digestion at a low temperature was carried on for a period of at least four hours. After the digestion each sample was diluted with distilled water and filtered through 11 cm. quantitative filters into 400 cc. beakers. The filters and dishes were washed four to six times with hot distilled water. To each filtrate 15 cc. of ammonia (sp.gr. 0.90) was added, and then the solutions were made acid to litmus paper with nitric acid. The solutions were then diluted with distilled water to about 150 cc. each, and heated on a water bath to 60° C. The phosphorus was precipitated by slowly adding, while stirring, 100 cc. of acid ammonium molybdate solution, consisting of approximately 6 per cent of molybdic acid. The solutions were reheated to about 76° C. and maintained at that temperature for 15 minutes. During the digestion the solutions were stirred every minute or so. They were allowed to stand three hours before filtering through 11 cm. quantitative filter papers. The beakers, precipitates, and filters were washed three to five times with ten per cent solution of ammonium nitrate, made slightly acid with 1 cc. of chemically pure concentrated nitric acid per liter. After the washing with acidified ammonium nitrate
solution was completed, clean 150 cc. beakers were put under the funnels and the precipitate dissolved through the filters by washing repeatedly with 2 1-2 per cent ammonia solution and hot water. The beakers in which the precipitations were made, were washed into the filters in the same way. The solutions were neutralized with concentrated chemically pure hydrochloric acid to the point where the solutions had a slight ammoniacal odor. When the solutions had cooled, the phosphorus was again precipitated by the addition, slowly drop by drop, of ten cc. of magnesium mixture containing 6 per cent of crystallized magnesium chloride. While the magnesium mixture was being added the solution was continually stirred with a small glass rod, care being taken not to strike the sides of the beaker. After the solutions had stood for about 15 minutes 30 cc. of ammonia (sp.gr. 0.90) was added to each beaker. The solutions were again stirred, covered, and set away for about 12 hours. At the end of this time, the magnesium ammonium phosphate was filtered out on 9 cm. Blue Ribbon S. and S. filter papers and washed about five times with 2 1-2 per cent ammonia solution. The beakers were cleaned of the clinging precipitates by the aid of a policeman and 2 1-2 per cent ammonia solution. The filters and precipitates were placed, apex upward, in ignited and weighted No. 00 Royal Berlin porcelain crucibles, and then placed to dry in an air oven at 100° C. After drying, the precipitates in the crucibles were placed in a red-hot muffle furnace. The muffle doors were left partially open to admit abundance of air, and as soon as the filters had completed glowing, the muffle was cooled and the precipitates ground with a large stirring rod. The crucibles were then returned to the muffle furnace and heated at the maximum muffle temperature for four hours. The crucibles were cooled and allowed to stand in a dessi-
cator for one hour and then weighed.

H. METHOD OF DETERMINING TOTAL ACID-SOLUBLE ASH AND PHOSPHORUS. Triplicate samples of 100 cc. each of the clear 0.2 per cent HCl extract were taken in weighed ignited 3-inch porcelain dishes. The solutions were evaporated to dryness and ashed according to the method described above, except that after charring the soluble part was not removed temporarily with water. The ashes were then treated as in the method for the determination of total phosphorus.

I. METHOD OF DETERMINING INORGANIC ACID-SOLUBLE PHOSPHORUS. Triplicate samples of 150 cc. of the acid extract were each treated with 25 cc. of magnesia-mixture. This magnesia-mixture was added slowly, drop by drop, while the solution was being stirred. After the solutions had stood for 15 minutes, 20 cc. of ammonia (sp.gr. 0.90) were added to each beaker. The beakers were covered and allowed to stand for about 12 hours. At the end of this time, the solutions were filtered through double 11 cm. Blue Ribbon S. and S. filters. The beakers and precipitates were washed a number of times with 2 1-2 per cent ammonium solution. The inside filters and precipitates were then returned to their respective beakers and treated with 25 cc. of dilute nitric acid. With the aid of stirring-rods the filters were well shredded. Clean beakers were put under the funnels and the solutions were filtered through their respective funnels. The filters were washed repeatedly with boiling distilled water. To each solution, 15 cc. of ammonia (sp.gr. 0.90) were added. The solutions were then made slightly acid with pure nitric acid. The phosphorus was precipitated with acid ammonium molybdate and the determination continued.
as in the case of the determination of total phosphorus.

J. METHOD OF DETERMINING TOTAL PHOSPHORUS IN URINES.

Fifty cc. of urine samples were measured in triplicate by means of pipettes into beakers of 400 cc. capacity. To each portion were added 30 cc. of concentrated chemically pure nitric acid (specific gravity 1.42) and 5 cc. of concentrated chemically pure hydrochloric acid (specific gravity 1.20). The beakers and contents were then placed upon the steam bath and evaporated to dryness. The residues in the beakers were moistened with very little concentrated chemically pure nitric acid (specific gravity 1.53) and 2 to 3 cc. of concentrated chemically pure hydrochloric acid (specific gravity 1.20). The acid mixtures were then digested upon the steam bath for a period of 1 hr. If at the end of this time the solutions had completely evaporated, a small amount of distilled water was added to each of the dried residues and the digestion continued in order to bring all materials possible into solution. This digestion with distilled water was found to be a necessary detail to insure clear solutions. Ammonia water (specific gravity 0.90) was next added to each of the solutions to neutralize the remaining acids, together with 5 to 10 grams of crystalized ammonium nitrate. The solutions were made slightly but distinctly acid to litmus paper with chemically pure nitric acid and then heated upon the water-bath to a temperature of 60° C. From 100 to 150 cc. of an acid ammonium molybdate solution, containing 6 per cent of molybdic acid, were added to each of the acidified solutions to precipitate the phosphorus. The operation of the total phosphorus determination, were continued from this point as described under General Method of Determining Total Phosphorus.
A. MAKE UP OF RATIONS. Three rations, containing alfalfa hay, corn and oil meal, were desired. As far as possible there was no variation in any other nutrient than protein. As stated above, this object was partially accomplished by feeding hay to all lots according to appetite and by offering to the three lots, three grain-rations differing in make up and not in weight, relative to body-weight of lambs. Each individual of each lot received 1.5 pounds of grain per day for every hundred pounds of live weight. The grain ration for the low-protein lot was made up of 95 per cent of corn and 5 per cent of oil meal, the ration of the medium-protein lot consisted of 75 per cent of corn and 25 per cent of oil meal, and the ration of the high-protein lot contained equal parts of corn and oil meal. Each lamb received during each period 105 per cent as much hay as it consumed during the preceding period. The individuals were given as much water as they desired. According to this standard of feeding the following data was collected.
**TABLE 1. RATIONS OFFERED.**

Results expressed in grams per day.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Hay</th>
<th>Corn</th>
<th>Oilmeal</th>
<th>Total concentrate</th>
<th>Total roughage</th>
<th>Ratio roughage to concentrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1. Low Protein.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>468</td>
<td>599</td>
<td>672</td>
<td>35</td>
<td>707</td>
<td>1306</td>
<td>0.85</td>
</tr>
<tr>
<td>463</td>
<td>798</td>
<td>653</td>
<td>34</td>
<td>687</td>
<td>1485</td>
<td>1.16</td>
</tr>
<tr>
<td>Average</td>
<td>698</td>
<td>662</td>
<td>34</td>
<td>697</td>
<td>1395</td>
<td>1.00</td>
</tr>
<tr>
<td>Lot II. Medium Protein.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>462</td>
<td>762</td>
<td>569</td>
<td>190</td>
<td>759</td>
<td>1521</td>
<td>1.00</td>
</tr>
<tr>
<td>458</td>
<td>871</td>
<td>615</td>
<td>205</td>
<td>820</td>
<td>1691</td>
<td>1.06</td>
</tr>
<tr>
<td>Average</td>
<td>816</td>
<td>592</td>
<td>197</td>
<td>789</td>
<td>1606</td>
<td>1.03</td>
</tr>
<tr>
<td>Lot III. High Protein.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>453</td>
<td>835</td>
<td>367</td>
<td>368</td>
<td>735</td>
<td>1570</td>
<td>1.14</td>
</tr>
<tr>
<td>451</td>
<td>798</td>
<td>379</td>
<td>380</td>
<td>759</td>
<td>1557</td>
<td>1.05</td>
</tr>
<tr>
<td>Average</td>
<td>816</td>
<td>373</td>
<td>374</td>
<td>747</td>
<td>1563</td>
<td>1.09</td>
</tr>
</tbody>
</table>
B. WEIGHTS OF RATIONS OFFERED. From the data from Table 1 it is clear that the consumption of hay per individual was quite constant. The average daily consumption of Lot I was 698 grams and for the other two lots 816 grams each. From these figures, it would seem that the low protein lot differed considerably from the others, but on examination of the individual data, it is found that the cause lies in that the consumption for animal No. 468 was excessively low, while the other was very close to the average of the other lots. The cause of lamb No. 468 consuming so much less hay than any of the other animals, seems to have been due to an individual peculiarity, for at no time during the experiment was the animal in an abnormal condition.

The number of grams of roughage to one gram of grain in the feeds is called the roughage-concentrate ratio. This ratio for the rations offered to the three lots was quite constant. The small variation, however, is regular, increasing from Lot I to Lot III. The cause of this regularity in the slight variation is due to the fact that in the low protein lot both the maximum and minimum ratios are found, while in the high-protein lot animal No. 453 had a relatively very high ratio.

C. WEIGHTS OF RATIONS CONSUMED. The data from Table 2 shows that the different lots consumed of hay and concentrates about the same amounts as offered. The low protein lot consumed 89 per cent of the hay and 85 per cent of the grain offered. The lambs of the medium protein lot ingested 84 per cent of the hay and 85 per cent of the grain offered. The lambs of the medium protein lot ingested 84 per cent of the hay and 100 per cent of the grain offered. In the case of the high protein lot, the animals
consumed 84 per cent of the hay and 99 per cent of the concentrates offered. The slight irregularity which occurs here, is found in the low protein lot, where the percentage consumption of hay was rather high and the percentage consumption of grain was rather low. This high value for the hay consumption is directly traceable to lamb No. 463 which consumed 96 per cent of the hay offered while its mate consumed 82 per cent of the hay offered which is quite near the average value. The low value for the percentage of consumption of grain is also directly traceable to the same animal No. 463, which consumed only 81 per cent of the grain offered, while animal No. 468 consumed 89 per cent of the grain offered.

### TABLE 2. FEEDS CONSUMED.
Results expressed in grams per day.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hay.</strong></td>
<td><strong>Concentrates.</strong></td>
<td><strong>Hay.</strong></td>
</tr>
<tr>
<td>468</td>
<td>487.37</td>
<td>625.72</td>
</tr>
<tr>
<td>463</td>
<td>754.85</td>
<td>556.45</td>
</tr>
<tr>
<td>Average consumption</td>
<td>621.11</td>
<td>591.08</td>
</tr>
</tbody>
</table>
Since there were some relative differences between the amounts of hay and concentrates offered and consumed, and such small differences make relatively large differences in the roughage-concentrate ration, these ratios are recalculated for the rations consumed. The following table gives the data thereon.

Roughage Concentrate Ratio of Consumed Rations.

<table>
<thead>
<tr>
<th>Low Protein Lot.</th>
<th>Medium Protein Lot.</th>
<th>High Protein Lot.</th>
</tr>
</thead>
<tbody>
<tr>
<td>468 463 Average</td>
<td>462 458 Average</td>
<td>453 451 Average</td>
</tr>
<tr>
<td>Ratio - -</td>
<td>0.78 1.36 1.07</td>
<td>0.76 0.96 0.86</td>
</tr>
<tr>
<td></td>
<td>0.93 0.91 0.92</td>
<td></td>
</tr>
</tbody>
</table>

The difference between the roughage concentrate ratios for the consumed and offered rations is very marked in practically all cases. In the low protein lot, the ratio of lamb No. 468 was only 57 per cent of that of its lot mate. In the medium-protein lot, Lamb No. 462 had a ratio which was 79 per cent of that ratio for lamb No. 458. The ratios of the animals of the high protein lot were quite close to one another, one being 98 per cent of the other. The lot roughage concentrate ratios were also quite relatively different, those for the medium and high-protein lots being 80 and 86 per cent, respectively, of the ratio for the low protein lot.

D. NUTRIENTS OF THE CONSUMED RATIONS. From the analyses of the rations offered and those refused, the amounts of important nutrients ingested were determined. In Table 3 the data on this
point is given.

The dry matter daily consumed by the low protein lot averaged 1058.90 grams, lamb No. 468 consuming 976.31 grams and lamb No. 463, 1141.50 grams. The medium protein lot averaged 1292.83 grams daily, which was the result of lamb No. 462 consuming 1180.48 grams and lamb No. 458 consuming 1405.19 grams daily. The high protein lot averaged 1255.96 grams daily, resulting from a consumption of 1253.01 grams and 1258.91 grams daily by lambs Nos. 453 and 451, respectively. Compared with the lot average for the low protein lot, the lot average for the medium and high protein lots were 122 per cent and 119 per cent.

The amount of protein ingested daily by animal No. 468 was 112.46 grams and by animal No. 463 was 140.39 grams, making an average for the lot of 126.42 grams daily per individual. The protein ingested by animals Nos. 462 and 458 was 174.94 grams and 202.25 grams daily, respectively, which made an average of 188.59 grams. In the high protein lot, animal No. 453 ingested 225.30 grams and animal No. 451 ingested 230.95 grams daily, making a lot average of 228.12 grams per day per individual. There was a large and regular variation in the amount of protein consumed by the three lots. With reference to the value for the low-protein lot, the medium-protein lot consumed 149 per cent and the high-protein lot 180 per cent as much protein as the low protein lot.

The fat consumption of lambs Nos. 468 and 463 was 34.77 and 36.93 grams daily, respectively, making an average of 35.85 grams daily per animal for the low-protein lot. In the medium-protein lot, animal No. 462 consumed 45.67 grams of fat while animal No. 458 consumed 51.29 grams daily, making an average of 48.43
### TABLE 3. NUTRIENTS CONSUMED.

Results expressed in grams per day.

<table>
<thead>
<tr>
<th></th>
<th>LOW PROTEIN LOT</th>
<th>MEDIUM PROTEIN LOT</th>
<th>HIGH PROTEIN LOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Matter</td>
<td>976.31 1141.50 1058.90</td>
<td>1180.48 1405.19 1292.83</td>
<td>1253.01 1258.91 1255.96</td>
</tr>
<tr>
<td>Protein</td>
<td>112.46 140.39 126.42</td>
<td>174.94 202.25 188.59</td>
<td>225.30 230.95 228.12</td>
</tr>
<tr>
<td>Fat</td>
<td>34.771 36.933 35.852</td>
<td>45.566 51.291 48.428</td>
<td>50.193 51.237 50.715</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>763.51 872.90 818.20</td>
<td>869.87 1040.18 955.02</td>
<td>866.24 863.90 865.07</td>
</tr>
<tr>
<td>Ash</td>
<td>48.59 69.10 58.84</td>
<td>65.05 81.32 73.18</td>
<td>79.32 80.56 79.94</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>21.606 27.180 24.393</td>
<td>33.320 38.776 36.048</td>
<td>42.846 43.815 43.330</td>
</tr>
<tr>
<td>Water</td>
<td>5670. 5352. 5511.</td>
<td>5080. 4717. 4898.</td>
<td>4355. 4445. 4400.</td>
</tr>
<tr>
<td>Energy Value</td>
<td>3195.02 3357.60 3276.31</td>
<td>3713.26 4325.99 4019.62</td>
<td>3893.52 3908.98 3901.25</td>
</tr>
</tbody>
</table>
grams daily per individual. In the high-protein lot animals Nos. 453 and 451 consumed daily 50.19 and 51.24 grams of fat daily, respectively, which makes a lot average of 50.71 grams daily per lamb. The difference between the lot averages for the first two lots is considerable but that between the last two lots amounts to little. Expressing the average amounts of fat consumed daily by the lots, in per cent of the amount of the average consumption for the low-protein lot, the medium-protein lot consumed 135 per cent and the high-protein lot consumed 141 per cent as much as the low-protein lot.

The carbohydrates ingested daily by Lamb No. 468 amounted to 763.51 grams, and by Lamb No. 463 to 872.90 grams. The average for the low-protein lot was 818.26 grams per day per animal. In the medium-protein lot lambs Nos. 462 and 458 ingested daily 869.67 and 1040.18 grams, making a lot average of 955.02 grams daily per animal. In the high-protein lot the lambs Nos. 453 and 451 ingested 866.24 and 863.90 grams of carbohydrates per day, which made an average of 865.07 grams per day per animal. The variation among the lots in this respect was considerable but not regular in passing from the low-protein to the high-protein lot. Expressing the values of the amounts of ingested carbohydrates for the medium and high-protein lots, relative to the amount ingested by the low-protein lot, it is found that the medium-protein lot ingested 117 per cent and the high-protein lot 106 per cent as much as the low-protein lot.

The ash consumed by lambs Nos. 468 and 463 was 48.59 and 69.10 grams daily, which makes an average for the low-protein lot of 58.84 grams per day per animal. In the medium-protein lot, lambs No. 462 and 458 ingested daily 65.05 and 81.32 grams of ash, making an average of 73.18 grams daily per animal. The ash consumption in the high-protein lot amounted to 79.32 grams daily for animal No. 453 and 80.56 grams daily for animal No. 451, making an average of 79.94 grams daily per animal. Among the different lots, the variation in the amount of ash ingested was considerable. Relative to the amount ingested by the low-protein lot, the medium-protein lot ingested 124 per cent and the high-protein lot 136 per cent.

The phosphorus ingested by the lambs of the low-protein lot amounted to 2.94 grams daily for lamb No. 468, and 3.34 grams for lamb No. 463, making an average of 3.14 grams daily per individual. In the medium-protein lot, lambs Nos. 462 and 458 ingested daily 4.49 and 5.12 grams of phosphorus, which makes an average daily consumption for the lot of 4.81 grams. In the high-
protein lot, the lambs Nos. 453 and 451 ingested 5.70 and 5.81 grams of phosphorus daily, making an average of 5.75 grams. Here as in the case of the protein, there is a regular and relatively large variation among the lot averages. Relative to the amount of phosphorus ingested by the low-protein lot, the amounts ingested by the medium and high-protein lots were 153 per cent and 183 per cent of that for the low-protein lot respectively.

The total nitrogen consumed by the individuals of the low protein lot, amounted to 21.61 grams for lamb No. 468 and 27.18 grams for lamb No. 463, which makes an average of 24.39 grams daily per individual. In the medium protein lot, animal No. 462 ingested 33.32 grams and animal No. 458 ingested 38.78 grams of nitrogen daily, making an average for the lot of 36.05 grams daily. Animals Nos. 453 and 451 ingested daily 42.85 and 43.81 grams of nitrogen, making an average for the lot of 43.33 grams per day per animal. A variation similar to those for phosphorus and protein, is found here. Relative to the nitrogen consumption of the low-protein lot, the amount ingested by the medium and high-protein lots amount to 148 and 177 per cent of the amount for the low protein lot respectively.

The water consumed by the lambs Nos. 468 and 463 amounted to 5670 and 5352 grams daily, which makes an average of 5511 grams per day for the low-protein lot. In the medium-protein lot, animal No. 462 consumed 5030 grams and animal No. 458 consumed 4717 grams of water daily, making an average of 4898 grams per day per animal. Lambs No. 453 and 451 ingested daily 4355 and 4445 grams of water, which makes an average for the high-protein lot of 4400 grams daily. Among the lot averages in this case, there was a variation differing from any of the foregoing. The variation was consider-
able and regular but in the opposite direction to all of the others. Relative to the average daily consumption of water by the low protein lot, the amounts consumed by the medium and high-protein lots were 89 and 80 per cent of that consumed by the low-protein lot.

The energy value of the different rations ingested daily amounted to 3195.02 calories for animal No. 468 and 3357.60 calories for animal No. 463, which makes an average of 3276.31 calories per day per individual of the low-protein lot. In the medium-protein lot animals Nos. 462 and 458 ingested daily 3713.26 and 4325.99 calories of energy, making an average of 4019.62 calories daily per animal. Animals Nos. 453 and 451 ingested daily 3893.52 and 3908.98 calories of energy, which makes an average for the high-protein lot of 3901.25 calories daily per individual. In energy content of the rations ingested daily by the different lots, varied considerably but not consistently from the low-protein lot to the high-protein lot. Relative to the energy ingested daily by the low-protein lot the medium and high-protein lots ingested 123 and 119 per cent, as much as the low protein lot respectively.

In order to summarize the variations in the amounts of different nutrients consumed the following Table 4 is made up of the data stated above.
TABLE 4. NUTRIENTS CONSUMED.

Results expressed in per cent of the value for the low-protein lot.

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pro-</td>
<td>pro-</td>
<td>pro-</td>
</tr>
<tr>
<td>protein.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>100</td>
<td>122</td>
<td>119</td>
</tr>
<tr>
<td>Protein</td>
<td>100</td>
<td>149</td>
<td>180</td>
</tr>
<tr>
<td>Fat</td>
<td>100</td>
<td>133</td>
<td>141</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>100</td>
<td>117</td>
<td>106</td>
</tr>
<tr>
<td>Ash</td>
<td>100</td>
<td>124</td>
<td>136</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>100</td>
<td>153</td>
<td>183</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>100</td>
<td>148</td>
<td>177</td>
</tr>
<tr>
<td>Water</td>
<td>100</td>
<td>89</td>
<td>80</td>
</tr>
<tr>
<td>Energy</td>
<td>100</td>
<td>123</td>
<td>119</td>
</tr>
</tbody>
</table>

It is evident from the data of Table 4 that the greatest and most consistent variations were in the ingested protein, phosphorus, and nitrogen. Since the largest part and the most important part of the nitrogen, is accounted for in the protein, that factor may be eliminated as an independent important one. Therefore, this experiment will be considered, primarily, as one in which the protein and phosphorus of the rations were the main influencing factors.

1. Phosphorus of Rations. (a) General Statement of the Phosphorus Offered and Ingested. The amounts of the different forms of phosphorus found in the different feeds which made up the
rations, are stated in Table 5.

### TABLE 5. DIFFERENT FORMS OF PHOSPHORUS IN THE FEEDS.

Results expressed in per cent of the fresh substance.

<table>
<thead>
<tr>
<th>Kind of feed</th>
<th>Serial No.</th>
<th>Total phosphorus</th>
<th>Acid insoluble phosphorus</th>
<th>Acid Soluble Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>2844</td>
<td>0.2160</td>
<td>0.0580</td>
<td>0.1580</td>
</tr>
<tr>
<td>Corn</td>
<td>2831</td>
<td>0.2694</td>
<td>0.1334</td>
<td>0.1360</td>
</tr>
<tr>
<td>Oil meal</td>
<td>2832</td>
<td>0.8807</td>
<td>0.6514</td>
<td>0.2293</td>
</tr>
</tbody>
</table>

The total phosphorus content of the fresh alfalfa hay, corn, and oil meal was 0.2160, 0.2694, and 0.8807 per cent respectively. Compared with the amount of total phosphorus in alfalfa hay, the corn was 125 and the oilmeal 407 per cent as rich.

The three feeds, alfalfa hay, corn, and oilmeal contained 0.0580, 0.1334, 0.6514, per cent respectively of acid insoluble phosphorus. Expressing the richness, in acid-insoluble phosphorus, of the latter two feeds in per cent of that of the first, the corn and oilmeal contained 230 and 1123 per cent.

The alfalfa hay, corn, and oilmeal contained 0.1580, 0.1360, and 0.2293 per cent, respectively of acid-soluble phosphorus. The corn and alfalfa hay were 86 and 145 per cent, respectively, as rich in this form of phosphorus as the alfalfa hay.

The inorganic acid-soluble phosphorus content of the alfalfa hay, corn, and oilmeal was 0.1485, 0.0308, and 0.1267 per cent, respectively. The corn and oilmeal was 21 and 85 per cent.
respectively, as rich as alfalfa hay in inorganic acid-soluble phosphorus.

The organic acid-soluble phosphorus of the three feeds taken in the order above mentioned, amounted to 0.0095, 0.1052, and 0.1026 per cent respectively. Relative to the amount of this form of phosphorus in the alfalfa hay, the corn contained 111 per cent and the oilmeal 108 per cent as much.

For the purpose of comparison, in the following table the percentage content in phosphorus of the corn and oilmeal relative to that of alfalfa hay, will be restated.

**TABLE 6. DIFFERENT FORMS OF PHOSPHORUS IN FEEDS.**

Results expressed in per cents of the percentage content of the hay.

<table>
<thead>
<tr>
<th>Kind of Feed</th>
<th>Serial No.</th>
<th>Total Acid Phosphate</th>
<th>Acid Soluble Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay</td>
<td>2844</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Corn</td>
<td>2831</td>
<td>125</td>
<td>230</td>
</tr>
<tr>
<td>Oil meal</td>
<td>2832</td>
<td>407</td>
<td>1123</td>
</tr>
</tbody>
</table>

It will be recalled that the concentrates given to the low-protein lot consisted of 95 per cent of corn and 5 per cent of oilmeal, while that given the medium-protein lot was made up of 75 per cent of corn and 25 per cent of oilmeal, and that given the
high-protein lot consisted of equal parts of each. From the data of Table 5 it is clear that the oil meal was richer than the corn, in every form of phosphorus except organic acid-soluble phosphorus in which the two feeds were practically identical. Connecting the two facts, it is easy to predict that the high-protein lot received the ration which was richer in all forms of phosphorus, except the organic acid-soluble form, than the medium-protein lot and the low-protein lot. In the same way it may be concluded that the three lots received practically the same amounts of organic acid-soluble phosphorus. Table 7 indicates these facts to be true.

TABLE 7. PHOSPHORUS CONTENT OF CONCENTRATES OFFERED DIFFERENT LOTS.

<table>
<thead>
<tr>
<th>Concentrates received by different lots.</th>
<th>Total Acid insoluble phosphorus.</th>
<th>Acid Soluble Phosphorus.</th>
<th>Total. Inorganic. Organic.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-Protein Lot----</td>
<td>0.2999</td>
<td>0.1593</td>
<td>0.1407</td>
</tr>
<tr>
<td>Medium-Protein Lot--</td>
<td>0.4222</td>
<td>0.2628</td>
<td>0.1593</td>
</tr>
<tr>
<td>High-Protein Lot----</td>
<td>0.5750</td>
<td>0.3924</td>
<td>0.1826</td>
</tr>
</tbody>
</table>

In this connection it will also be of interest to have a statement of the distribution of the total phosphorus into the different forms of phosphorus in the different feeds. Therefore the following table is compiled.
DIFFERENT FORMS OF PHOSPHORUS IN THE FECES.

Results expressed in per cent of the total phosphorus.

<table>
<thead>
<tr>
<th>Kind of feed</th>
<th>Serial No.</th>
<th>Total phosphorus</th>
<th>Acid insoluble phosphorus</th>
<th>Acid Soluble Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa hay</td>
<td>2844</td>
<td>100</td>
<td>27</td>
<td>73</td>
</tr>
<tr>
<td>Corn</td>
<td>2831</td>
<td>100</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Oilmeal</td>
<td>2832</td>
<td>100</td>
<td>74</td>
<td>26</td>
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</tbody>
</table>

TABLE 8. DIFFERENT FORMS OF PHOSPHORUS INGESTED.

Results expressed in grams per day.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>LOW PROTEIN.</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>468</td>
<td>2.939</td>
<td>1.2406</td>
<td>1.6989</td>
<td>0.9944</td>
</tr>
<tr>
<td>463</td>
<td>3.387</td>
<td>1.3587</td>
<td>2.0284</td>
<td>1.3357</td>
</tr>
<tr>
<td>Average</td>
<td>3.163</td>
<td>1.2997</td>
<td>1.8636</td>
<td>1.1630</td>
</tr>
<tr>
<td>MEDIUM PROTEIN.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>462</td>
<td>4.486</td>
<td>2.2977</td>
<td>2.1887</td>
<td>1.3410</td>
</tr>
<tr>
<td>438</td>
<td>5.125</td>
<td>2.5917</td>
<td>2.5331</td>
<td>1.6100</td>
</tr>
<tr>
<td>Average</td>
<td>4.805</td>
<td>2.4447</td>
<td>2.3609</td>
<td>1.4755</td>
</tr>
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<td>HIGH PROTEIN.</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>453</td>
<td>5.700</td>
<td>3.2690</td>
<td>2.4313</td>
<td>1.6148</td>
</tr>
<tr>
<td>451</td>
<td>5.811</td>
<td>3.3326</td>
<td>2.4783</td>
<td>1.6438</td>
</tr>
<tr>
<td>Average</td>
<td>5.755</td>
<td>3.3008</td>
<td>2.4548</td>
<td>1.6293</td>
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</tbody>
</table>
b. Total Phosphorus Ingested. In the low-protein lot, lamb No. 468 ingested 2.939 grams and lamb No. 463 ingested 3.387 grams of phosphorus per day, which made an average of 3.163 grams of phosphorus per day per individual for the lot. The cause of this difference between the individuals of this lot is clear when it is noted from the data of Table 2, that lamb No. 468 ingested only 487.37 grams of hay and 628.72 grams of grain per day, while lamb No. 463 ingested 754.95 grams of hay and 556.45 grams of grain per day. Since the hay contained 0.216 per cent and the grain 0.2999 per cent of phosphorus, it is plain that the cause of lamb No. 463 ingesting more phosphorus than lamb No. 468 was due to its ingesting much more hay.

Animals Nos. 462 and 458 ingested 4.486 and 5.125 grams of phosphorus per day, respectively, which made an average for the lot of 4.805 grams of phosphorus per day per individual. The difference between the amounts of phosphorus ingested by the two lambs can be traced as before to the fact that lambs No. 458 ingested both more hay and grain than lamb No. 462. Lamb No. 462 ingested only 576.80 grams of hay and 758.60 grams of grain per day, while lamb No. 458 ingested 795.55 grams of hay and 820.00 grams of grain per day. The greatest difference was due to hay consumption.

In the high-protein lot, animals Nos. 453 and 451 ingested 5.700 and 5.811 grams of phosphorus per day, respectively, making an average daily consumption of 5.755 grams per day. The slight difference between the individuals in this regard may also be traced to the weights of the different parts of the ration. Lamb No. 453 ingested only 685.25 grams of hay and 734.80 grams of grain per day, while lamb No. 451 ingested slightly less hay, i.e.,
grams per day and relatively much more grain, i.e., 747.62 grams per day. Since the phosphorus content of the hay was 0.216 per cent while that for the grain fed this lot was 0.3924 per cent, it is evident that the difference between the amounts of phosphorus ingested by these animals was due mainly to the difference in the amount of grain consumed by the two animals.

The lot averages in the daily consumption of the total phosphorus were considerable. The low protein lot averages 3.163 grams, the medium protein lot 4.805 grams, and the high protein lot 5.755 grams per day. Expressing these values for the medium and high-protein lots in per cent of the value for the low-protein lot, the medium-protein lot ingested 153 per cent and the high-protein lot ingested 183 per cent. The cause of these differences was due to two main causes, first differences in the amounts of oilmeal in the rations, which was relatively very rich in phosphorus, and second to the fact that the low-protein lot did not ingest as much grain as the others, as is shown in the data of Table 2.

(c) Acid-Insoluble Phosphorus Ingested. The acid-insoluble phosphorus ingested by lamb No. 469 was 1.2406 grams per day while that of lamb No. 463 was 1.3587 grams per day, which makes an average for the low-protein lot of 1.29 grams per day per individual. The difference between the amounts of this form of phosphorus ingested by the two animals can be traced to the same cause as that which caused the similar difference for the total phosphorus.

In the medium-protein lot, animals Nos. 462 and 458 ingested 2.2977 and 2.5917 grams of phosphorus daily, respectively, making an average of 2.4447 grams of phosphorus per day per individual. The difference between the individuals in the amounts of
acid-insoluble phosphorus ingested, is due to a difference in the consumption of hay as was the case for the total phosphorus.

Animals Nos. 453 and 451 of the high-protein lot ingested 3.2690 and 3.3326 grams of acid-insoluble phosphorus, respectively, daily, making a lot average of 3.3008 grams per day per individual. The differences here was due to the same cause as in the case of the total phosphorus, i.e., in the difference in the amount of grain ingested.

The lot variation in the amount of acid-insoluble phosphorus ingested, was considerable. The amount of this form of phosphorus ingested by the medium-protein lot was 187 per cent and by the high-protein lot 254 per cent of that amount ingested by the low-protein lot. The cause of this large variation between the low and medium-protein lots was due mainly, first, to difference in the amount of grain consumed, and second, to the richness of the grain rations in this form of phosphorus. This is clear from the fact that, first, the average for the low-protein lot was 621.11 grams of hay and 591.08 grams of grain per day while the average for the medium-protein lot was 686.17 grams of hay and 789.30 grams of grain, and second that the acid-insoluble phosphorus content of the grain ration for the low and high-protein lots were 0.1593 and 0.2628 per cent respectively. The main cause for the differences between the amounts of acid-insoluble phosphorus ingested by the medium and high-protein lots was due to the fact that the acid-insoluble phosphorus content of the grain rations differed considerably, the first being 0.2628 per cent and the second 0.3924 per cent. The difference between the amounts of hay and grain consumed by the two lots amounted to little, that of the medium-protein lot being 686.17 grams of hay, and 789.30
grams of grain, and that of the high-protein lot amounting to 682.17 grams of hay and 741.21 grams of grain.

(d) Acid-Soluble Phosphorus Ingested. Animals Nos. 468 and 463 ingested 1.6989 and 2.0284 grams of acid-soluble phosphorus daily, making a lot average of 1.8636 grams per day per individual.

In the medium-protein lot, lamb No. 462 ingested 2.1887 grams per day and lamb No. 458 ingested 2.5331 grams per day, which made an average of 2.3609 grams per day per animal.

In the high protein lot, animals Nos. 453 and 451 ingested 2.4313 and 2.4783 grams per day respectively. From this the lot average was calculated to be 2.4548 grams per day per lamb.

The differences between the amounts of this form of phosphorus ingested by the individuals in the lots was due to the same causes as those which caused similar differences in the case of total phosphorus.

The differences between the lot averages in regard to the amount of this form of phosphorus ingested, were not quite as much as in the case of the other forms. The lot average for the medium-protein lot was 127 per cent and that for the high-protein lot was 132 per cent of that of the low-protein lot. Since the acid-soluble phosphorus content of the grain rations for the low, medium, and high-protein lots were 0.1407, 0.1593, and 0.1826 per cent respectively, the differences between the lots were due to the variation in the weights of hay and grain consumed, more than in any of the preceding cases.

(e) Inorganic Acid-Soluble Phosphorus Ingested. The inorganic acid-soluble phosphorus ingested by animal No. 468 was 0.9944 grams per day while the amount ingested by animal No. 463...
was 1.3357 grams per day. The average of these values made a lot average of 1.1650 grams per day per animal.

Animals Nos. 462 and 458 ingested 1.4310 and 1.6100 grams of inorganic acid-soluble phosphorus daily. The average for the medium-protein lot was then 1.4755 grams per day per period.

In the high-protein lot, animals Nos. 458 and 451 ingested 1.6148 and 1.6438 grams of inorganic acid-soluble phosphorus, respectively, daily. This made a lot average of 1.6293 grams per day per individual.

The lot average consumption of inorganic acid-soluble phosphorus varied about the same amount as for the other forms of phosphorus. The amount of this form of phosphorus ingested by the medium-protein lot was 127 per cent and the amount ingested by the high-protein lot was 140 per cent of the amount ingested by the low-protein lot. The cause of this variation was the same as that for the total phosphorus.

f. Organic Acid-Soluble Phosphorus Ingested. The organic acid-soluble phosphorus ingested by Lamb No. 463 amounted to 0.6927 grams per day. Therefore the lot average for the low-protein lot was 0.6986 grams daily per animal. Here as in the case of no other form of phosphorus, lamb No. 462 exceeded lamb No. 468 in the amount ingested. The cause for this lay in the fact that the alfalfa hay was only about one-tenth as rich in this form of phosphorus as the grain fed, the hay containing 0.0095 per cent while the grain contained 0.1051 per cent of organic soluble phosphorus. This is clear when it is noted that lamb No. 463 exceeded lamb No. 468 by 69.27 grams of grain but lamb No. 468 exceeded lamb No. 463 by only 267.48 grams of hay.

In the medium-protein lot, animal No. 468 ingested 0.8476
grams of organic acid-soluble phosphorus while animal No. 458 inges-
tested 0.9231 grams per day, making a lot average of 0.8853 grams
daily per animal. The difference between the individual consump-
tion of this form of phosphorus, was due to an all-around larger
consumption of both hay and grain by animal No. 458.

Animals Nos. 453 and 451 ingested daily 0.8165 and 0.8343
grams, respectively, of organic acid-soluble phosphorus, which made
a lot average for the high-protein lot of 0.8254 grams daily per
individual. The cause of the difference between the individuals
in this lot was due partially to a greater difference in the grain
than in the hay consumption and partially to the hay containing
only 0.0095 per cent while the grain contained 0.1039 per cent of
this form of phosphorus.

The lot averages for the low, medium, and high-protein
lots were 0.6986, 0.8853, and 0.8254 grams daily per animal. In
other words the lot average for the medium-protein lot was 127 per
cent and that of the high-protein lot was 118 per cent of the lot
average for the low-protein lot. The cause for the difference be-
tween the low and medium-protein lots in the amount of organic acid-
soluble phosphorus ingested is traceable to both the amount of the
feeds ingested and the richness of them in this form of phosphorus.
The lot average for the low-protein lot was 621.11 grams of hay
and 591.08 grams of grain, while the lot average for the medium-pro-
tein lot was 686.17 grams of hay and 789.30 grams of grain. The
medium-protein lot exceeded the low protein lot in the consumption
of both hay and grain but mainly grain. The phosphorus consumption
difference was also much affected by the hay containing only 0.0095
per cent of this phosphorus while the grain fed the low-protein lot
contained 0.1051 per cent and the grain fed the medium-protein lot contained 0.1045 per cent of organic acid-soluble phosphorus. The difference between the organic acid soluble phosphorus lot averages, for the medium and high-protein lots, was due chiefly to a difference in the amount of grain consumed. This is clear when it is noted that the lot average for the medium-protein lot was 686.17 grams of hay and 789.30 grams of grain, while that for the high-protein lot was 682.17 grams of hay, and 741.21 grams of grain. The medium-protein lot exceeded the high-protein lot in the consumption of both hay and grain. The organic acid-soluble phosphorus content of the grains fed the two lots also had a small influence, the grain fed the medium-protein lot containing 0.1045 per cent and the grain fed the high-protein lot containing 0.1039 per cent.

2. Protein of the Rations.

(a) Composition, in Protein, of the feeds of the Rations. In Table 9 the protein content of the three feeds used in this experiment, is stated in per cents of the feeds as fed.

<table>
<thead>
<tr>
<th></th>
<th>Hay</th>
<th>Corn</th>
<th>Oilmeal</th>
<th>Concentrates Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot</td>
<td></td>
<td></td>
<td></td>
<td>Low</td>
</tr>
</tbody>
</table>

TABLE 9. PROTEIN CONTENT OF FEEDS.

Results expressed in per cents of fresh samples.
Since the hay contained 12.50 per cent, the corn 6.89 per cent and the oilmeal 31.79 per cent of protein, it is clear that there was a possibility of making up rations of rather wide differences in their protein content. The corn contained only 55.1 per cent as much protein as the hay, while the oilmeal contained 254.3 per cent as much protein as the hay.

Since this is a phosphorus-protein problem it may be of value to note in this connection that the corn and oilmeal were 125 and 407 per cent, respectively, as rich in phosphorus as the hay. In comparing these figures with those of the paragraph above, it is evident that relative to the hay content, the corn and oil meal content in phosphorus and protein did not agree. However, if the protein and phosphorus content of the oilmeal is compared with that of the corn they are somewhat more concordant, the first being 327 per cent while the second is 461 per cent. In other words the ratio of the phosphorus content of protein to that of phosphorus in corn (25.6) was not entirely incomparable with the same ratio for oilmeal (36.1). These facts being true, any variation of the mixture of corn and oilmeal in making up a ration, could not vary the amount of protein in the ration without simultaneously varying the amount of phosphorus, approximately the same amount.

Since the concentrates fed the low-protein lot were made up of 95.0 per cent of corn and 5.0 per cent of oilmeal, those fed the medium-protein lot were made up of 75 per cent of corn and 25 per cent of oil meal, and those offered to the high protein lot contained equal parts of corn and oil meal, the percentage content in protein of the concentrates fed the different lots
can be calculated. Above in Table 9, these per cents are stated. The concentrates fed the low, medium, and high-protein lots contained 8.14, 13.12, and 19.34 per cent of protein, respectively. In other words, the medium and high-protein lots received concentrates 161 and 238 per cent as rich in protein as the concentrates received by the low-protein lot. In calculating from the data of Table 7, it is found that the concentrates offered the medium and high-protein lots were 141 and 192 per cent respectively, as rich in phosphorus as the concentrates offered the low-protein lot. Thus it is clear that richness in protein of the three rations offered, varied similarly to the richness of the rations in phosphorus. The closeness of the agreement in this variation in phosphorus and protein content is stated more exactly in the ratio of the protein content to the phosphorus content of the concentrates fed the low-protein lot was 27.2, for the medium-protein lot 31.1, and for the high-protein lot 33.7.

(b) Amounts of Protein Ingested. In Table 10 there is stated the amounts of protein ingested for the various periods, by the various animals and lots daily.

<table>
<thead>
<tr>
<th>Period No.</th>
<th>Low Protein Lot</th>
<th>Medium Protein Lot</th>
<th>High Protein Lot</th>
</tr>
</thead>
<tbody>
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<td>463</td>
<td>462</td>
</tr>
<tr>
<td>Weight I</td>
<td>113.2</td>
<td>140.3</td>
<td>178.5</td>
</tr>
<tr>
<td>Consumed II</td>
<td>115.0</td>
<td>139.8</td>
<td>173.4</td>
</tr>
<tr>
<td>III</td>
<td>109.2</td>
<td>141.0</td>
<td>172.9</td>
</tr>
<tr>
<td>Average</td>
<td>112.5</td>
<td>140.4</td>
<td>174.9</td>
</tr>
<tr>
<td>Lot Average</td>
<td>126.4</td>
<td>188.5</td>
<td>228.1</td>
</tr>
</tbody>
</table>

**TABLE 10. PROTEIN CONSUMED.**

Results expressed in grams per day.
In the low-protein lot animal No. 468 ingested 112.5 grams of protein, daily, and animal No. 463 ingested 140.4 grams of protein per day. Thus the lot average was 126.4 grams of protein per day per animal. The cause for the difference in the amount of protein ingested by the two animals of this group, was due, to animal 468 ingesting 487.37 grams of hay and 625.72 grams of grain while animal No. 463 ingested 754.85 grams of hay and 556.45 grams of grain. Since the hay contained 12.50 per cent of protein and the grain 8.13 per cent of protein, it is clear that the difference in the amounts of protein ingested was due mainly to the amount of protein of the hay.

Animals Nos. 462 and 458 ingested daily 174.9 and 202.2 grams, respectively, of protein, making an average of 188.5 grams of protein per day per animal. This difference between the protein consumption of the individuals of this lot was due to a greater consumption of both hay and grain by animal No. 462 than by animal No. 458. This is clear when it is noted that animal No. 462 ingested daily 576.80 grams of hay and 758.60 grams of grain, while animal No. 458 ingested daily 795.55 grams of hay and 820 grams of grain.

Lambs Nos. 453 and 451 of the high-protein lot ingested daily 225.3 and 230.9 grams respectively, of protein. This made an average of 228.1 grams of protein ingested daily per animal. The cause for the difference between the amounts of protein ingested by the two animals of this lot was due more to the difference between the amounts of grain consumed that the difference between the amounts of hay consumed. The difference was also due to the fact that the hay contained only 12.50 per cent of protein while the grain contained 19.34 per cent.
The lot averages in the amounts of protein consumed by the low, medium, and high-protein lots were 126.4, 188.5, and 228.1 grams per day per animal, respectively. In other words, the amount of protein consumed by the medium-protein lot was 149 per cent and the high-protein lot consumed 180 per cent of the amount of protein ingested by the low-protein lot. The cause for the differences between the lots was due mainly to the differences in the protein content of the grain fed the different lots and secondarily to the differences in the amounts of grain and hay consumed. This is plain when it is again stated that the grain offered to the low, medium, and high protein lots contained 8.14, 13.12, and 19.34 per cents, respectively, of protein. Also it is clearer when it is noted that the lot average feed consumption of the low-protein lot was 621.11 grams of hay and 591.08 grams of grain, of the medium-protein lot 686.17 grams of hay and 789.30 grams of protein, and of the high-protein lot 682.17 grams of hay and 741.21 grams of grain per day per animal. Between the low and medium-protein lots the differences in the grain and hay consumption were in the same direction and were consequently additive, and also additive to the composition influence. Between the medium and high-protein lots the differences in the grain and hay consumption were in the same direction and so were additive but since they were in the opposite direction to the protein consumption influence, the two influences tended to counterbalance one another.

It is well to note again the important fact that the relation of the protein and phosphorus ingested, was very close. The low, medium, and high-protein lots ingested amounts of protein, standing in the relation of 100, 149, and 180, while the amounts of phosphorus ingested by the lots respectively, was in
the same relation as 100, 153, and 183 respectively.
DIFFERENT FORMS OF PHOSPHORUS EXCRETED AND APPARENTLY DIGESTED.

A. DATA.

TABLE II. DIFFERENT FORMS OF PHOSPHORUS EXCRETED.

Results expressed in grams per day.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
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<tbody>
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<td></td>
<td>Feces</td>
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<tr>
<td>468</td>
<td>2.559</td>
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<td>2.1428</td>
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<td>0.3772</td>
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<tr>
<td>Average</td>
<td>2.677</td>
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<td>2.0977</td>
<td>0.1833</td>
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<tr>
<td>MEDIUM PROTEIN.</td>
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<tr>
<td>462</td>
<td>4.023</td>
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<tr>
<td>Average</td>
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<td>0.3436</td>
<td>0.0147</td>
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<tr>
<td>HIGH PROTEIN.</td>
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<tr>
<td>453</td>
<td>5.109</td>
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<td>0.7991</td>
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<td>4.1094</td>
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<tr>
<td>Average</td>
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<td>0.9337</td>
<td>4.2835</td>
<td>3.9731</td>
<td>0.3104</td>
<td>0.0159</td>
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</table>
TABLE 12. APPARENT DIGESTED PHOSPHORUS.

Results expressed in grams per day.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Total Acid insoluble phosphorus</th>
<th>Acid soluble phosphorus</th>
<th>Acid Soluble Phosphorus.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total inorganic, organic</td>
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</tbody>
</table>

**LOW PROTEIN.**

<table>
<thead>
<tr>
<th></th>
<th>468</th>
<th>0.380</th>
<th>0.8244</th>
<th>-0.4439</th>
<th>-1.0579</th>
<th>0.6141</th>
</tr>
</thead>
<tbody>
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<td>-0.8073</td>
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<td></td>
</tr>
<tr>
<td>Average</td>
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<td>0.9029</td>
<td>-0.4173</td>
<td>-0.9326</td>
<td>0.5153</td>
<td></td>
</tr>
</tbody>
</table>

**MEDIUM PROTEIN.**

<table>
<thead>
<tr>
<th></th>
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<th>0.464</th>
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<th>-1.4281</th>
<th>-1.9227</th>
<th>0.4944</th>
</tr>
</thead>
<tbody>
<tr>
<td>458</td>
<td>0.284</td>
<td>1.6823</td>
<td>-1.3979</td>
<td>-1.9669</td>
<td>0.5890</td>
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</tr>
<tr>
<td>Average</td>
<td>0.374</td>
<td>1.7871</td>
<td>-1.4130</td>
<td>-1.9548</td>
<td>0.5417</td>
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</tbody>
</table>

**HIGH PROTEIN.**

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<th>453</th>
<th>0.591</th>
<th>2.2006</th>
<th>-1.6091</th>
<th>-2.2220</th>
<th>0.6129</th>
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</thead>
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<td>-2.0483</td>
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<td>0.4172</td>
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</tr>
<tr>
<td>Average</td>
<td>0.538</td>
<td>2.3670</td>
<td>-1.8287</td>
<td>-2.3438</td>
<td>0.5150</td>
<td></td>
</tr>
</tbody>
</table>

1 Apparent digested phosphorus = Phosphorus ingested minus phosphorus excreted in feces.
B. DISCUSSION.

1. Weights of Total Phosphorus, Fecally excreted and Apparently Digested. Of total phosphorus, the low-protein lot excreted in the feces, 2.677 grams, the medium-protein lot 4.431 grams, and the high-protein lot 5.217 grams of phosphorus per day per individual. Then by subtracting these weights from the weights of the phosphorus ingested by the lots, it is found that, the low-protein lot assimilated 0.435 grams, the medium-protein lot 0.374 grams, and the high-protein lot 0.533 grams of phosphorus per day.

There are two questions to be answered, first, why was there such a variation in the amount of fecal phosphorus and second, why did the different lots assimilate different amounts of phosphorus? It may be said that the cause for both variations were dependent, first, on the amount of phosphorus ingested, and second, on the condition of the animals of the different lots. The condition of the animals of the different lots depended partially, first, on the individuality, second, on the body weight, third, on the age of the animal, and fourth on the normality of the food, and fifth on the contraction of ailments which were due to accident, as far as we can know. Of these factors which affect the condition of the animal, the third may be neglected, as the difference between the ages of the lambs was a matter of but a few days. The fourth factor may also be eliminated for the three rations used in the experiment are in common use for growing and fattening lambs. If these rations had, in common use produced abnormal animals, the practice of using them would have been discontinued and their harmful effects would be well known long ago.
By the statements above, it must be remembered that it is not meant that the different rations did not cause differences but those differences were such that the life processes as a whole were not widely altered.

Other things being equal and one factor acting at a time, one would naturally expect that within certain limits, first the amounts of phosphorus excreted in the feces and assimilated would be proportional to the amount of phosphorus excreted, and second, the body weights and the gains in body weights would be proportional to the amount of phosphorus assimilated and inversely proportional to the amount of phosphorus excreted in the feces.

Then the influence of, first, the amount of phosphorus ingested, and second, the kind of phosphorus ingested, third, the body weights of subjects, fourth, the gains in body weight, and fifth, the presence of abnormalities, will be discussed in relation to the amount of phosphorus excreted in the feces and the amount assimilated. But in the discussion we will be forced to assume that any one of the above named factors will cause the same effect while acting alone as when acting in combination with the other factors.

The difference between the amounts of phosphorus excreted fecally by the different lots, may have been due to a difference in the amounts of phosphorus ingested. If that were true, then one would expect that the number of grams of phosphorus fecally excreted per gram of phosphorus ingested, would be the same for all lots. The same would be the case for the phosphorus assimilated. In the case of the phosphorus excreted by the low, medium, and high-protein lots the values were 0.843, 0.920, and 0.906 grams
per gram of ingested phosphorus. In the case of the phosphorus assimilated by the lots in the same order, it is found that for every gram of phosphorus ingested 0.152, 0.080, and 0.094 grams of phosphorus are assimilated. In the case of the values given for the fecally excreted phosphorus, they are sufficiently constant to conclude that the amount of fecally excreted phosphorus was approximately proportionate to the amount of phosphorus ingested. The small deviation from the exact proportionality is left to be explained away by the other factors mentioned above. The series of values for the amount of phosphorus assimilated per gram of phosphorus ingested, were not so constant, the first being almost twice the size of the second, and the second differing from the third by about 16 per cent. As the weights of phosphorus assimilated per gram of phosphorus ingested by the three lots, is far from constant, showing that the amount of phosphorus assimilated was not proportional to the amount ingested, it cannot be said that the amount of phosphorus ingested affected to any appreciable extent the amount of phosphorus assimilated.

The differences in the amount of phosphorus in the feces may also have been affected by the body weights of the animals. To test this point the weight of phosphorus excreted in the feces per gram of phosphorus ingested per kilogram of body weight was calculated for each lot. The values for the low, medium, and high-protein lots were 0.0183, 0.0174, and 0.0179 grams respectively. The weights in this series were very constant, in fact more constant than the weights of phosphorus excreted per gram of phosphorus ingested. Thus it must be concluded that the body weights of the animals were influencing factors in determining the amount of phosphorus excreted in the feces. It is clear however, that the influ-
ence was relatively small compared with the influence of the amount of phosphorus consumed.

The body weights of the animals of the several lots seemed to have had some influence on the amount of phosphorus taken up by the circulation. It is found for the low, medium, and high-protein lots, that they assimilated 0.0105, 0.0071, and 0.0107 grams of phosphorus per kilogram of body weight, or 0.00327, 0.00151, and 0.00156 grams of phosphorus ingested, per grams of phosphorus ingested, per kilogram of body weight. It is clear that in the cases of the low and high protein-lots the body weight seems to have been the main, or only, governing factor in influencing the amount of phosphorus assimilated, but in the case of the medium protein lot the value is far removed from the others. It is also clear that relative to both the amount of phosphorus ingested and the body weight, the medium protein lot made the poorest use of its phosphorus.

As stated above, other things being equal, large gains in body weight should be followed by corresponding decreases in the amount of phosphorus in the feces. The weight of phosphorus in the feces of the low, medium, and high-protein lots were 0.0904, 0.0693, and 0.050 grams per gram of phosphorus ingested per kilogram of body weight. Since the amount of phosphorus excreted per gram of phosphorus ingested per kilogram of body weight, was constant, the above series of numbers should decrease regularly in order, if the gain in body weight were an influencing factor in determining the amount of phosphorus excreted. The low and medium-protein lots seem to show such a tendency but the high-protein lot does not fit with either of the others. Thus from this data, it cannot be concluded that the amount of phosphorus fecally excreted is governed by the
in any definite way by the gain in body weight, especially in such short periods.

It seems that there must have been some relation between the gains in body weight and the amounts of phosphorus taken up by the blood. That there was a relation between the amount of phosphorus assimilated and the amount of gain in body weight, is apparent from the fact that the low, medium, and high-protein lots assimilated 0.435, 0.374, and 0.533 grams of phosphorus while they gained 0.2025, 0.2511, and 0.1701 kilograms per day per animal. That these values are inversely proportional is evident from the constancy of their products, 98.21, 93.91, and 91.51 for the lots in order. That the assimilation of a food like phosphorus should vary inversely to the amount of gain in body weight is unreasonable. On closer examination of the data, it is surprising to find that this relation was probably due to a mathematical accident. In the first place, both series of values were not determined directly, but by differences between values, relatively very much larger than themselves. This is a condition that admits of the multiplication of errors. In the second place, if the values, both for the amount of assimilated phosphorus and the amount of gain in body weight for the individuals are examined, it is found that the variation within lots was larger than between lots and the range of variation for one lot covered the range for the other lots almost completely. Therefore no weight can be put on the fact that the amount of phosphorus assimilated by these lots of animals was inversely proportional to their gains in body weight.

The influence of a simultaneous variation in the amounts of the different kinds of phosphorus with the variation in the to-
otal amount of phosphorus ingested, probably had some influence on the amount of total phosphorus in the feces, and the amount of phosphorus assimilated. Yet, it is evident from slight relative variations in the different kinds of phosphorus ingested, such an influence would be small. The brief discussions of this point will come under the discussion for the different forms of phosphorus individually.

The only evidences of abnormality in the case of any of the lambs was that Lamb No. 463 which ingested a ration relatively very high in the roughage-concentrate ratio, and lamb No. 458 which ingested much the largest weight of total feed and excreted very soft feces throughout the experiment. These evidences of abnormality were not evidenced in either the phosphorus fecally excreted or assimilated.

2. Weights of Acid-Soluble Phosphorus, Fecally Excreted and Apparently Digested. The amount of acid-soluble phosphorus excreted daily in the feces of the low, medium, and high-protein lots was 2.2810, 3.7440, and 4.2835 grams per day per animal. For the lots in order, the amounts of acid-soluble phosphorus in the feces made up 85.1, 85.5, and 82.0 per cent of the total phosphorus in the feces.

The cause of the variation in the amount of this form of phosphorus in the feces, may have been due to the amount of acid-soluble phosphorus ingested, the amount of total phosphorus ingested, the body weight, or the gain in body weight.

It is found that the low, medium, and high-protein lots excreted in the feces 122.7, 160.2, and 174.4 per cent of the ingested acid-soluble phosphorus or 72.1, 78.6, and 74.4 per cent of the ingested total phosphorus. The size of the first series of per
cents indicates clearly that there was some addition to that form of phosphorus while the food passed through the body. The variability of that series of per cents indicates, also, that the amount of this form of phosphorus in the feces was not proportional to the amount ingested. The second series of per cents were quite constant indicating that the amount of acid-soluble phosphorus in the feces was practically proportional to the amount of total phosphorus ingested.

The influence of the body weight on the amount of acid-soluble phosphorus in the feces is indicated by the constancy of the amount of acid soluble phosphorus in the feces per gram of total phosphorus ingested per kilogram of body weight. These values for the low, medium, and high protein lot were 0.0156, 0.0148, and 0.0147, respectively. The constancy of this series of values is slightly greater than that for the amount of acid-soluble phosphorus in the feces, expressed in per cents of the total phosphorus ingested. This shows that the amount of acid soluble phosphorus in the feces was influenced to some extent by the weight of the animals.

The gain in body weight, as in the case of the total phosphorus, did not seem to influence the fecal output of acid-soluble phosphorus. It is found that the amount of this form of phosphorus excreted fecally by the low, medium, and high-protein lots was 0.007, 0.0058, and 0.0086 grams of acid-soluble phosphorus per gram of total phosphorus ingested, per kilogram of body weight per kilogram of gain in body weight. The irregularity of these values, while the amount of acid-soluble phosphorus in the feces per gram of total phosphorus ingested per kilogram of body weight was so constant, is a certain indication of the lack of influence of the gain in body weight.
weight on the fecal output of acid-soluble phosphorus.

That quantity of acid-soluble phosphorus, which was calculated in the same way as the amount of total phosphorus assimilated, will be discussed, but not as the amount of this form of phosphorus taken into the blood system. The values of these quantities are \(-0.4173\), \(-1.4130\), and \(-1.8287\) grams of acid-soluble phosphorus for the low, medium, and high-protein lots, respectively. In other words, these lots of lambs daily, per individual, excreted fecally \(0.4173\), \(1.4130\), and \(1.8287\) grams of acid-soluble phosphorus more than was ingested. This can mean only, that these amounts of acid-insoluble phosphorus were transformed into the acid-soluble form. But in connection it must be remembered that the low, medium, and high-protein lots assimilated \(0.485\), \(0.372\), and \(0.538\) grams of total phosphorus daily, which could have been derived only from the acid-soluble form. Thus it was not the "apparent digested" acid-soluble phosphorus, alone, which was derived from the acid-insoluble phosphorus ingested, but the sum of the "apparent digested" acid-soluble phosphorus and the total phosphorus assimilated. Then this means that the lots of animals in order, transformed \(0.9023\), \(1.7870\), and \(2.3667\) grams of acid-insoluble phosphorus into acid-soluble phosphorus, daily, per individual.

The factors which seemed related to the variation in the amount of this form of phosphorus in the feces, seem likely to be related to the degree of transformation of acid-insoluble phosphorus to the acid-soluble form.

The constancy in the amount of acid-insoluble phosphorus transformed per gram of acid-insoluble phosphorus ingested, shows that the amount of ingested acid-insoluble phosphorus governed
very largely the amount of acid-soluble phosphorus transformed to
the acid-soluble form. The low, medium, and high-protein lots trans-
formed daily to the acid-soluble form 0.694, 0.731, and 0.717
grams of acid-insoluble phosphorus, per individual.

From the fact that 0.0149, 0.0139, and 0.0142 grams of
acid-insoluble phosphorus per gram of acid-insoluble phosphorus
ingested, per kilogram of body weight, were transformed by each
individual daily to the acid-soluble form by the low, medium, and
high-protein lots respectively, is evidence that the body weight
had practically no influence on the amount of transformation of the
one form of phosphorus to the other. The series of values just
stated were slightly less constant than the series representing the
amounts transformed daily per individual per gram of acid-insoluble
phosphorus ingested.

It is not necessary to do more than state that the low,
medium, and high-protein lots gained 0.2025, 0.2511, and 0.1701
to kilograms per day per animal, for it be plain that the gains in
body weight were not at all related with the amount of acid-soluble
phosphorus gained in the process of digestion.

3. Weights of Inorganic Acid-Soluble Phosphorus, Fecal-
ly Excreted and Apparently Digested. The inorganic acid-soluble
phosphorus daily excreted by the low, medium, and high-protein lots
was 2.0977, 3.4303, and 3.9731 grams respectively, per animal. This
form of phosphorus made up 78.4, 77.7, and 76.1 per cent of the to-
tal phosphorus in the feces, 66.5, 71.5, and 69.0 per cent of the
the total phosphorus ingested, and 183.4, 233.4, and 243.8 per cent
of the inorganic acid-soluble phosphorus ingested.

The relation of the factors, amount of inorganic acid-sol-
uble phosphorus ingested, amount of total phosphorus ingested, body weights, and gains in body weight, to the amount of inorganic acid-soluble phosphorus in the feces will be discussed.

As stated above, the inorganic acid-soluble phosphorus in the feces was represented by 183.4, 233.4, and 243.6 per cent of the amount of this form of phosphorus ingested. It is clear from the size of these per cents that it was impossible for all of the inorganic acid-soluble phosphorus to have been derived from the inorganic acid-soluble phosphorus ingested. From the relatively large and constant differences between the per cents for the different lots it is evident that the amount of inorganic acid-soluble phosphorus ingested was not a large factor, if any at all, in influencing the fecal output of this form of phosphorus.

In restating the percents, 66.5, 71.5, and 69.0 for the low, medium, and high-protein lots, representing the amounts of inorganic acid-soluble phosphorus, fecally excreted in reference to the amount of total phosphorus ingested, quite a different situation presents itself. It is notable, that the major part of the total phosphorus ingested, was excreted in the feces in all cases. From the relatively small and irregular differences between the per cents for the three lots, it is plain that the amount of total phosphorus ingested was a powerful influence in determining the amount of inorganic acid-soluble phosphorus in the feces.

The influence of body weight is well shown by the amounts of acid-soluble phosphorus in the feces per gram of total phosphorus ingested per kilogram of body weight. The low, medium, and high-protein lots excreted fecally, 0.0144, 0.0135, and 0.0136 grams of inorganic acid-soluble phosphorus per gram of total phosphorus ingested per kilogram of body weight, daily. The fact that these
values were practically as constant as the values for the amount of inorganic acid-soluble phosphorus excreted in the feces per gram of total phosphorus ingested, shows that the body weight did not exert any marked influence at all upon the fecal output of this form of phosphorus.

The lots taken in order excreted daily in the feces, 3.29, 2.85, and 4.05 grams of inorganic acid-soluble phosphorus per gram of total phosphorus ingested per kilogram of gain in body weight. The large differences in these values indicate that the gain in body weight as obtained for this period was not an influential factor in reference to the amount of inorganic acid-soluble phosphorus excreted in the feces.

The "apparent digested" inorganic acid-soluble phosphorus was determined in this case, as in the others by taking the difference between the ingested and fecal inorganic acid-soluble phosphorus. In the case of the lots taken in the regular order, the "apparent digested" inorganic acid-soluble phosphorus was -0.9326, -1.9548, and -2.3438 grams per day per individual. This means that at least 0.9326, 1.9548, and 2.3438 grams of some other forms of phosphorus had been changed to the inorganic acid-soluble form. Of course, all of the phosphorus which was assimilated had to come from either the inorganic or organic acid-soluble form or both. Therefore the low, medium, and high-protein lots may have transformed to the inorganic acid-soluble form, as much as the sum of the "apparent digested" total phosphorus and "apparent digested" inorganic acid-soluble phosphorus, or 1.4176, 2.3288, and 2.8818 grams daily per animal. So the question is, did the animals assimilate inorganic acid-soluble phosphorus alone or organic acid-soluble phosphorus alone, or some of both. In other words, did
the lots of animals in this experiment taken in order, transform to the inorganic acid-soluble form 0.9326, 1.9548, or 2.3438 grams of phosphorus or 1.4176, 2.3288, and 2.8818 grams of phosphorus or some quantities between the limits just stated?

The acid-insoluble and the organic acid-soluble phosphorus ingested were the only possible courses of additional inorganic acid-soluble phosphorus. Then, other things being equal, the amount of inorganic acid-soluble phosphorus gained by the transformation of the other forms, should be proportioned to the amount of the other forms ingested. Through calculations assuming that all of the assimilated phosphorus was inorganic, it is found that for the low, medium, and high-protein lots there was a transformation to the inorganic acid-soluble form of 0.709, 0.699, and 0.698 grams per gram of acid-insoluble and organic acid-soluble phosphorus ingested. Assuming that only the organic acid-soluble phosphorus was, as far as possible, assimilated, it is found that in the case of the lots in order, there was a transformation of 0.467, 0.587, and 0.562 grams of other forms of phosphorus to the inorganic acid-soluble form per gram of acid-insoluble and organic acid-soluble phosphorus ingested. The remarkable constancy of the first set of values obtained when it was assumed that only the inorganic was assimilated, and the inconstancy of the last series, obtained when it was assumed that only the organic was assimilated, indicates that in all probability it was the inorganic acid-soluble phosphorus which was assimilated.

4. Weights of Total Phosphorus in Urines. The total phosphorus in the urines was obviously soluble, and was considered as inorganic. The average excretion of phosphorus for the low protein lot was 0.0134 grams, that for the medium-protein lot was
0.0147 grams, and that for the high-protein lot was 0.0159 grams per day per animal. The lots in order excreted in their urines 0.4, 0.3, and 0.2 per cent of the total phosphorus ingested or 0.5, 0.3, and 0.3 per cent of the total phosphorus excreted.

Through an examination of the absolute weights, given above, of the total phosphorus in the urines, it is evident that there was a gradual increase as we pass from the first to the third lot of animals. That this regular increase should not be considered significant is made clear by two facts. First, the individuals differed within the lots more than the lots differed within themselves and the range of variation of any one lot overlaps the range of variation of the other two lots, as for example, the animals of the medium-protein lot varied over the entire range covered by both of the other two lots. Second, the differences between the lots were too small to be considered as they might readily be due to analytical errors only. Therefore no further discussion of the amount of phosphorus in the urines is necessary.
### PHOSPHORUS BALANCE.

**A. DATA.**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Total Acid in-soluble phosphorus</th>
<th>Acid Soluble Phosphorus</th>
<th>Total Inorganic Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LOW PROTEIN.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>468</td>
<td>0.3643</td>
<td>0.8244</td>
<td>-0.4596 -1.0737 0.6141</td>
</tr>
<tr>
<td>463</td>
<td>0.5794</td>
<td>0.9815</td>
<td>-0.4020 -0.8185 0.4165</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>0.4721</td>
<td>0.9029</td>
<td>-0.4308 -0.9461 0.5153</td>
</tr>
<tr>
<td><strong>MEDIUM PROTEIN.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>462</td>
<td>0.4522</td>
<td>1.8920</td>
<td>-1.4398 -1.9344 0.4944</td>
</tr>
<tr>
<td>458</td>
<td>0.2666</td>
<td>1.6823</td>
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<tr>
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<td>0.3594</td>
<td>1.7871</td>
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<td><strong>HIGH PROTEIN.</strong></td>
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<td></td>
<td></td>
</tr>
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<td>0.5224</td>
<td>2.3670</td>
<td>-1.8446 -2.3597 0.5150</td>
</tr>
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</table>
TABLE 14. DISTRIBUTION OF THE EXCRETION OF THE TOTAL PHOSPHORUS INGESTED.

Results expressed in per cent of total phosphorus ingested.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Total Phosphorus</th>
<th>Acid insoluble phosphorus</th>
<th>Acid soluble phosphorus</th>
<th>Total Inorganic Phosphorus</th>
<th>Total Organic Phosphorus</th>
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</thead>
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<tr>
<td></td>
<td>Dietary Total</td>
<td>Feces</td>
<td>Urine</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Ingested</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW PROTEIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>468</td>
<td>87.1</td>
<td>14.2</td>
<td>72.9</td>
<td>69.8</td>
<td>2.1</td>
</tr>
<tr>
<td>463</td>
<td>82.6</td>
<td>11.1</td>
<td>71.4</td>
<td>63.3</td>
<td>8.2</td>
</tr>
<tr>
<td>Average</td>
<td>84.8</td>
<td>12.6</td>
<td>72.1</td>
<td>66.5</td>
<td>5.6</td>
</tr>
<tr>
<td>MEDIUM PROTEIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>462</td>
<td>89.7</td>
<td>9.0</td>
<td>80.6</td>
<td>72.8</td>
<td>7.9</td>
</tr>
<tr>
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<td>94.4</td>
<td>17.7</td>
<td>76.7</td>
<td>70.2</td>
<td>6.5</td>
</tr>
<tr>
<td>Average</td>
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<td>13.3</td>
<td>78.6</td>
<td>71.5</td>
<td>7.2</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>453</td>
<td>89.6</td>
<td>18.7</td>
<td>70.9</td>
<td>67.3</td>
<td>3.6</td>
</tr>
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<td>91.7</td>
<td>13.8</td>
<td>77.9</td>
<td>70.7</td>
<td>7.2</td>
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<td>Average</td>
<td>90.6</td>
<td>16.2</td>
<td>74.4</td>
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</table>
### Table 15. Distribution in the Feces of the Different Forms of Phosphorus

Results expressed in per cent of the ingested forms.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Total Phosphorus</th>
<th>Acid Insoluble Phosphorus</th>
<th>Acid Soluble Phosphorus</th>
<th>Total Inorganic Phosphorus</th>
<th>Total Organic Phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOW PROTEIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>468</td>
<td>87.1</td>
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<td>126.1</td>
<td>206.4</td>
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<td>463</td>
<td>82.6</td>
<td>27.3</td>
<td>119.3</td>
<td>160.4</td>
<td>39.9</td>
</tr>
<tr>
<td>Average</td>
<td>84.3</td>
<td>30.6</td>
<td>122.7</td>
<td>183.4</td>
<td>26.3</td>
</tr>
<tr>
<td>MEDIUM PROTEIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>462</td>
<td>89.7</td>
<td>17.7</td>
<td>165.3</td>
<td>243.4</td>
<td>41.7</td>
</tr>
<tr>
<td>458</td>
<td>94.4</td>
<td>35.1</td>
<td>155.2</td>
<td>223.4</td>
<td>36.2</td>
</tr>
<tr>
<td>Average</td>
<td>92.0</td>
<td>26.4</td>
<td>160.2</td>
<td>233.4</td>
<td>38.9</td>
</tr>
<tr>
<td>HIGH PROTEIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>453</td>
<td>89.6</td>
<td>32.7</td>
<td>166.2</td>
<td>237.6</td>
<td>24.9</td>
</tr>
<tr>
<td>451</td>
<td>91.7</td>
<td>24.0</td>
<td>182.6</td>
<td>250.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Average</td>
<td>90.6</td>
<td>28.3</td>
<td>174.4</td>
<td>243.8</td>
<td>37.4</td>
</tr>
</tbody>
</table>
### TABLE 16. DISTRIBUTION OF DIFFERENT FORMS OF PHOSPHORUS IN FECES.
Results expressed in per cent of the total.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Total Acid in—</th>
<th>Acid Soluble Phosphorus—</th>
<th>——Acid Soluble Phosphorus—</th>
<th>Total</th>
<th>Inorganic</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>phos—soluble</td>
<td>phos—soluble</td>
<td>phos—soluble</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>468</td>
<td>100.0</td>
<td>16.3</td>
<td>83.7</td>
<td>80.2</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>463</td>
<td>100.0</td>
<td>13.5</td>
<td>86.5</td>
<td>76.6</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>100.0</td>
<td>14.9</td>
<td>85.1</td>
<td>78.4</td>
<td>6.7</td>
<td></td>
</tr>
</tbody>
</table>

**LOW PROTEIN.**

| 462        | 100.0          | 10.1                       | 89.9                        | 81.1  | 8.8       |         |
| 458        | 100.0          | 18.8                       | 81.2                        | 74.3  | 6.9       |         |
| Average    | 100.0          | 14.4                       | 85.5                        | 77.7  | 7.3       |         |

**MEDIUM PROTEIN.**

| 453        | 100.0          | 20.9                       | 79.1                        | 75.1  | 4.0       |         |
| 451        | 100.0          | 15.0                       | 85.0                        | 77.2  | 7.3       |         |
| Average    | 100.0          | 17.9                       | 82.0                        | 76.1  | 5.9       |         |

**HIGH PROTEIN.**

### TABLE 17. BODY WEIGHTS AND GAINS IN BODY WEIGHTS.
Results expressed in kilograms and kilograms per day.

<table>
<thead>
<tr>
<th>Low Protein Lot.</th>
<th>Medium Protein Lot.</th>
<th>Low Protein Lot.</th>
</tr>
</thead>
<tbody>
<tr>
<td>468</td>
<td>463</td>
<td>462</td>
</tr>
<tr>
<td>458</td>
<td>451</td>
<td>453</td>
</tr>
<tr>
<td>Body weight,</td>
<td>47.4012</td>
<td>45.1332</td>
</tr>
<tr>
<td>&quot; average,&quot;</td>
<td>46.2672</td>
<td>52.9578</td>
</tr>
<tr>
<td>&quot; gains,&quot;</td>
<td>0.1782</td>
<td>0.2268</td>
</tr>
<tr>
<td>&quot; &quot; aver.&quot;</td>
<td>0.2025</td>
<td>0.2511</td>
</tr>
</tbody>
</table>
| Note: Gains = One-fourteenth of difference between the weights for the 19th and 21st weeks of experiment.
B. DISCUSSION OF PHOSPHORUS BALANCES. The balances for the different forms of phosphorus were calculated from the amounts of "apparent digested" forms of phosphorus and the amounts of total phosphorus in the urines. In the cases of the acid-insoluble and organic acid-soluble phosphorus there is no difference between the "apparent digested" and the "balance". In the cases of the other three forms of phosphorus, the "balances" were obtained by subtracting from the "apparent digested" phosphorus the total phosphorus of the urines. From such methods of calculation the values for the phosphorus balances, given in Table 13 were obtained.

Since for the acid-soluble and organic acid-soluble forms of phosphorus, the "apparent digested" and the "balance" are identical, a repetition of the discussion of "apparent digested" acid-soluble and organic acid-soluble phosphorus under the head of phosphorus balance, is unnecessary.

In reality, the difference between the amounts of the "apparent digested" phosphorus and the phosphorus "balance" for the total, and soluble and inorganic acid-soluble phosphorus is negligible. This is clear when it is calculated that the limit of error in the analytical work was larger than the differences between the "apparent digested" and the "balance" for the three forms of phosphorus. Since the total phosphorus of the urines of the low, medium, and high-protein lots amounted to only 0.4, 0.3, and 0.2 percent of the total phosphorus ingested or 0.4, 0.3, and 0.13 percent of the total phosphorus in the feces, an error of from 0.2 to 0.4 percent in the analysis of the feces ingested or an error of 0.3 to 0.4 percent in the analyses of the feces would cause as great a difference in the amount of "apparent digested" phosphorus as the
subtraction of the total phosphorus in the urine. Similarly an error of 0.6 to 0.7 per cent in the analysis of acid-soluble phosphorus in the feeds, or an error of 0.4 to 0.5 per cent in the analysis for the acid-soluble phosphorus in the feces, would have caused as great a difference in the amount of "apparent digested" phosphorus as the subtraction of the total phosphorus in the urines. Similarly, an error of 0.9 to 1.0 per cent in the analysis of the feeds for inorganic acid-soluble phosphorus or an error of 0.4 to 0.6 per cent in the analysis of the feces for inorganic acid-soluble phosphorus would account for as much difference in the "apparent digested" inorganic acid-soluble phosphorus as the subtraction of the total phosphorus of the urines. Therefore numerically all the phosphorus "balances" were practically equal to the "apparent digested" phosphorus, and their relations to the other factors influencing the life of the animal, were the same as that of the "apparent digested" phosphorus. Excepting in the case of total phosphorus balance, no additional significance can be placed on the "balances" than was placed on the "apparent digested" forms of phosphorus.

By the term "apparent digested" total phosphorus, is meant that amount of total phosphorus which was taken up by the blood. By the term "total phosphorus balance" the amount of total phosphorus stored or retained in the body tissues is designated. As in the case of the other forms of phosphorus there was freedom of transformation from one form to another, and as the degree and direction of this transformation was not directly measureable, no such exact interpretation can be placed on the terms, "apparent digested", or "balance" for the other forms of phosphorus. Under the head of "apparent digested" total phosphorus above, it is stated that the "apparent di-
gested total phosphorus was influenced, practically not at all by the amount of phosphorus ingested, some by the body weight, and not at all by the gain in body weight. Such a condition is evidently for the phosphorus balance as is shown by the following table.

**Phosphorus Balance Relative to other Factors.**

<table>
<thead>
<tr>
<th></th>
<th>Low Protein lot.</th>
<th>Medium Protein lot.</th>
<th>High Protein lot.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus stored per gram of phosphorus ingested,</td>
<td>0.1492</td>
<td>0.0748</td>
<td>0.0908</td>
</tr>
<tr>
<td>Phosphorus stored per kilogram of body weight,</td>
<td>0.0102</td>
<td>0.0068</td>
<td>0.0103</td>
</tr>
<tr>
<td>Phosphorus stored per kilogram of body weight, per kilogram gain body weight,</td>
<td>2.3310</td>
<td>1.4313</td>
<td>3.0710</td>
</tr>
</tbody>
</table>

Therefore the amount of total phosphorus stored was little related to either the amount of phosphorus stored or the body weight and not at all by the gain in body weight.
SUMMARY.

Twenty-one lambs were fed in three lots, on three different rations, from about the time of weaning, (July 2nd, 1910) till the time of the slaughter test, about six months later. On December 19, two of the most representative lambs from each lot were chosen and put into metabolism stalls. A metabolism experiment of 12 days in duration was run.

During the metabolism experiment the rations of the lambs were the same as that received by them during the previous feeding. The rations were practically the same in all the nutrients and heat values, except in the case of the phosphorus and protein. The greatest variation in absolute weights was in protein but the ratio of protein to phosphorus in all three rations was constant. Since such was the case the problem was a protein-phosphorus problem and not a purely phosphorus or purely protein problem. Thus in the discussion of this experiment the protein, in name, has been left out till at this point, because the relation of factors to the total phosphorus ingested, would be the same for the protein ingested. So any conclusions made above, in reference to the influence of the total phosphorus ingested, must be interpreted as the influence of one or both protein and total phosphorus ingested.

The data obtained by direct methods was first, the protein offered and refused, second, the total, acid-soluble and inorganic acid-soluble phosphorus offered, rejected, and excreted in the feces, third, the total urinary phosphorus and fourth, the body weights before and after the experiment. In addition to this data, other
data was obtained by calculation from it, such as the protein ingested, the total, acid-soluble, acid-insoluble, inorganic acid-soluble, and organic acid-soluble phosphorus in the feces, and the gains in body weight. The comparative results, given in Tables 14, 15, and 16 probably state general conditions better than any brief exposition of the same.
CONCLUSION.

When, in this experiment, protein and phosphorus were made equally and practically the only variable nutrients in rations made up of alfalfa hay, corn, and oilmeal, the effect on the phosphorus metabolism of lambs was as follows:

First. The three lots of lambs excreted daily in the feces from 2.677 to 5.217 grams of total phosphorus per animal or from 84.8 to 92.0 per cent of the total phosphorus ingested. The lots excreted daily in their urines from 0.0314 to 0.0159 grams of total phosphorus per animal, or from 0.2 to 0.4 per cent of the total phosphorus ingested.

Second. The lots of animals took into their blood circulation from 0.485 to 0.538 grams of total phosphorus daily per animal or from 8.0 to 15.2 per cent of the total phosphorus ingested. The animals of the three lots stored daily in the body of each animal from 0.4721 to 0.5224 grams of total phosphorus or from 7.7 to 14.8 per cent of the total phosphorus ingested.

Third. The amount of total phosphorus in the feces was largely influenced by the amount of total phosphorus and protein ingested and to a considerable extent by the body weights of the animals.

Fourth. The amount of total phosphorus taken up by the blood and the amount of total phosphorus stored seemed to have been to some extent influenced by the amount of total phosphorus and protein ingested and the body weights of the animals. That a close mathematical agreement between the amount of ingested phosphorus or the body weight with the amounts of assimilated and stored phos-
phorus, could not be expected. The reason is clear when it is noted that an error of one per cent in the analysis of either the rations offered, the rations refused, or the feces excreted would amount to about ten per cent in the amount of phosphorus assimilated.

Fifth. From 73.6 to 69.4 per cent of the acid-insoluble phosphorus ingested was transformed to the acid-soluble form, and directly or indirectly to the inorganic acid-soluble form, except in the case of one animal (No. 451) in which a very small amount of acid-insoluble phosphorus was transformed into the organic acid-soluble form.

Sixth. Inorganic acid-soluble phosphorus was apparently the main form of phosphorus which was assimilated.

Note of Appreciation. The work just described was undertaken at the suggestion of Professor H. S. Grindley. Valuable assistance and suggestions have been given by Professor H. S. Grindley and Mr. A. D. Emmett. To these men, the author wishes to express his most sincere gratitude.

The hearty cooperation of Mr. W. E. Carroll, who had charge of another phase of this experiment, has been most helpful in the successful carrying out of the work described.

Thanks are due Mr. P. A. Hoffman, for laboratory assistance in many ways, during the entire experiment.
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38. Carriere. Ibid. 1901, 133:314.