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THE EFFECTS OF DIETARY PHOSPHORUS ON THE ABSORPTION, DEPOSITION AND EXCRETION OF COPPER IN CALVES SUPPLEMENTED WITH MOLYBDENUM OR MOLYBDENUM AND SULFATE

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THE EFFECTS OF DIETARY PHOSPHORUS ON THE ABSORPTION, DEPOSITION AND EXCRETION OF COPPER IN CALVES SUPPLEMENTED WITH MOLYBDENUM OR MOLYBDENUM AND SULFATE

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INTRODUCTION

It has been known for many years that copper is present in biological materials. Elvehjem (49) in 1935 presented a rather comprehensive review of the early research on copper metabolism. Although most work before the 1900's was concerned with levels of copper found in biological materials, two copper-containing proteins had been isolated and studied. One was the respiratory enzyme, hemocyanin, isolated from some marine animals, and the other was a protein, turacin, found in the feathers of a South American bird.

Bodansky (14) in 1921 was one of the first investigators to prove conclusively that copper was naturally present in tissue of higher animals and not just an accidental contamination of tissues. McHargue (102, 103) in 1925 reported levels of copper in many materials and concluded that high copper values of some tissues were probably biologically significant. McHargue (101) in 1926 showed evidence that copper as well as manganese and zinc were factors in metabolism of animals. He was one of the earliest investigators to use a purified diet, but his experimental results were not conclusive because of the inability of the supplemented purified diet to maintain normal growth and health of his experimental animals.

Hart et al. (76) showed that ash of certain plant

and animal tissues could correct the condition of milk anemia in rats, but he could not cure the condition with iron alone. In a later study, Hart et al. (77) found that the other factor involved was copper. He showed conclusively that copper as well as iron was necessary for hemoglobin production in rats.

Since Hart demonstrated the need for copper in hemoglobin production, many metabolic activities of copper have been discovered. However, very little is known of the mechanism of copper metabolism in the body, or of the activity of most of the copper enzymes which have been isolated.

Copper deficiencies have been reported from many areas of the world. Some of the symptoms reported for this disease were anemia, depressed growth, bone disorders, depigmentation of hair or wool, abnormal wool growth, neonatal ataxia, impaired reproductive performance, heart failure, and gastrointestinal disturbances.

Later it was shown that copper deficiency could occur in areas when copper was present in the feed at relatively high levels. Ferguson et al. (58) in 1938 showed that molybdenum was a significant factor in a local disease in British cattle known as "teart". Interrelationships of molybdenum and copper have been studied extensively for many years, but there appear to be many discrepancies in the manifestation of copper deficiency when molybdenum is present in feed.

In 1953, Dick et al. (41) showed that sulfate as well as molybdenum had a very marked effect on the metabolism of copper and that sulfate as well as molybdenum was necessary to produce copper deficiency in sheep. With this discovery, many of the discrepancies in the earlier work could be explained.

The effects of other inorganic compounds have been studied on the premise that there may be interrelationships between copper metabolism and other substances. Zinc, phosphorus, and iron have been shown to have some effect on the copper status of animals.

Our interest in the interrelationship of copper, molybdenum and sulfate arose from an earlier investigation by Vanderveen (132) at this station in which the effects of molybdenum, sulfate and certain other ions, and effects of stress imposed by outdoor housing, were studied. The influence of molybdenum and sulfate on the absorption of radioactive copper was also investigated. He reported that the effects of the molybdenum, and molybdenum plus sulfate treatments were not so great as expected from a survey of the literature. The results of absorption studies, using Cu^{64}, as well as certain questions arising from other aspects of their studies, prompted the series of experiments reported in this manuscript. With the addition of new laboratory facilities for determination of minor elements, it was believed a more extensive study of the effects of molybdenum, sulfate and phosphorus on the

absorption, excretion, and deposition of copper might be fruitful. During the course of this investigation, various components of blood were also studied. This dissertation reports the results of the study.

REVIEW OF LITERATURE

COPPER METABOLISM

The absorption, transport, storage, and excretion of copper has not been completely elucidated. Studies on absorption of copper are limited. Gitlin et al. (64), in studies with mice, considered the absorption of copper an enzymic and a first order process. Sacks et al. (121) demonstrated in dogs that copper was absorbed in the more distal intestinal loops. Tompsett's results (130) indicated that most of the copper is absorbed in the upper tract where intestinal contents are acidic. Bowland et al. (15) in a study with swine showed that the absorption of copper was mainly in the colon and small intestine.

Dietary factors, including the form in which copper is consumed, affect the absorption of copper. Lassiter and Bell (91) fed several types of copper compounds and found in the first experiment that the absorption of cupric chloride was greater than that of either cupric oxide needles or cupric sulfate. In another experiment, cupric carbonate gave higher blood copper levels than cupric or cuprous oxide powders or cupric oxide needles.

In an experiment carried out by Buescher et al. (20), two millicuries of Cu^{64} were fed to pigs as a sulfate, carbonate, or an oxide. Each sample contained 10 mg of copper. Their studies revealed that the type of copper

salts fed did not affect the absorption of copper. However, excretion of copper in the urine from $CuCO_3$-fed animals was much higher. Tompsett (130) showed that high levels of calcium in the diet of mice could reduce total body copper by as much as 50 per cent.

In a study by Mills (107) on the availability of copper in different freeze-dried plants fed to rats, herbage supplements gave a greater response as measured by growth, blood formation, melanin pigment regeneration, and hepatic copper values than did copper sulfate. He suggested that this effect was caused by the greater availability of the copper complexes for absorption as compared to the free copper ions. In other studies (106, 108) Mills showed that very little of the plant copper was in the form of soluble extracts, but these extracts gave a much greater physiological response than did copper sulfate. He showed that there was not much difference in the amount of soluble copper in plants from either normal pastures or those producing sway-back in sheep. Both were equal in value when fed to experimental animals.

McCall et al. (99) showed that high levels of protein in the diet of rats decreased both the amount of copper absorbed and total body copper. Zinc had the same effect but to a lesser degree. Cox and Harris (30) found that supplementary dietary zinc resulted in a loss of iron in rats and suggested that this caused the anemic condition observed. The copper stores did not decrease until the

iron reserves in the body became very low, and anemia commenced. Iron supplementation on high zinc rations prevented a reduction of copper in the body.

It was demonstrated by Gubler et al. (74) that manganese poisoning had an effect on body copper levels of rats. Large amounts of manganese in the feed increased plasma and brain copper and decreased urinary copper. Microcytic hypochromic anemia developed even when copper was added to high-manganese diets. Total body copper was twice as great in the animals supplemented with manganese and copper as in the animals supplemented with copper alone.

Kulwich et al. (88) found an increased retention of copper in rats and swine when molybdenum was fed at high levels. When molybdenum was fed at the level of 1000 ppm of the diet, there was no apparent interference with copper balance or metabolism.

Dick (43) studied the accumulation of copper in the liver of pen-fed sheep and found the amount to be proportional to the copper intake. During a six-month period, accumulation was between 4.5 and 5 per cent of the intake. The addition of ferrous sulfite to the ration reduced the accumulation of copper in the liver by 50 per cent. It was suggested that sulfite from ferrous sulfite formed an insoluble salt with copper making it unavailable. No effects on copper retention were observed when the diet contained thiosulfate or the elements, sulfur, zinc or nickel. Molybdenum with sulfate had a severe limiting effect on

copper retention. Calcium carbonate depressed copper retention. Schultze et al. (123) showed that even severely anemic rats could not absorb over 5 per cent of the copper fed to them.

It was reported by Ferguson (57) that lead acetate in the ration could cause a drop in blood copper in ewes. When a mixture of salts of fluorine, barium, silver, and tin were added to the ration, an even greater decrease of blood copper was noted. Lambs born to the ewes fed lead acetate were either weak or stillborn. No signs of sway-back or pathological lesions were noted in the lambs.

Scheinberg et al. (122) described the pathway taken by radioactive copper after its absorption as follows: Copper first appears in the blood plasma as a cupric ion, loosely bound to an albumen fraction. It then is transported throughout the body and accumulates in the liver and certain other tissues. Later, there appears in the blood stream a copper-protein complex called ceruloplasmin containing the radioactive copper. The liver seems to be necessary for the incorporation of copper into ceruloplasmin, but this fact has not been positively proven.

It has been shown by Gubler et al. (72) that copper appears in two forms in the blood serum. One is a stable copper-protein called ceruloplasmin which will not release its copper when treated with sodium diethyldithiocarbonate. This portion of serum copper is not immediately affected by either dietary or injected copper. The second form of

copper is associated with a serum albumin to which the copper is more loosely bound. The concentration of this fraction is readily influenced by dietary copper. When copper is absorbed, it becomes loosely bound to the serum albumin and is transported to other parts of the body where it is removed by the liver and other tissues.

Bush et al. (21) showed that red blood cells take up injected radioactive copper quite rapidly and release it slowly to the plasma fraction. These studies showed that red blood cells released copper rapidly at first and then more slowly as equilibrium was approached. Gubler (70) indicated that there was both a stable and a labile fraction of copper in the blood cells, the latter being in equilibrium with albumin copper.

The distribution of absorbed copper has been studied by Comar et al. (26, 27) using radioactive copper. Cattle were either fed 250 mg or injected with 100 mg of radioactive copper. Seventy-five per cent of the radioactive copper that was given orally was recovered in the feces within five days. Only 3 per cent was recovered from the urine during this period. When injected, 3 per cent of the radioactive dose was recovered in the feces and 3 per cent in the urine. The injected copper was removed rapidly from the blood plasma. The liver, kidney, intestinal tract, adrenals, thymus, gall bladder, and bile had the highest concentrations of radioactivity. The tissues having a very low radioactivity were white bone marrow, muscle, hide,

urinary bladder, ligaments, cartilage, bones, and nerves. The mucosal layer of the intestine contained several times more radioactivity than did the muscle layers. The liver showed the highest concentration of radioactive copper and served as the chief storage organ. The kidney contained the next highest amount of radioactive copper. In rats, it was shown that the kidney had a higher level of labelled copper than the liver, thus furnishing evidence of a species difference.

The excretion of copper has been found to be mainly via the intestinal tract with very little appearing in the urine. Mahoney et al. (96) injected Cu^{64} intravenously into normal dogs and into dogs with ligated bile ducts in which the flow of bile was diverted to the urinary bladder. The excretion of copper in the urine accounted for only 0.6 per cent of the administered copper, 7 to 10 per cent being recovered in the bile, and 1.5 per cent being excreted through the intestinal wall. Untagged copper injected into dogs having obstructed bile ducts increased the excretion of copper in the urine, while in the normal dogs it did not. This finding indicated that under certain conditions, urinary excretion could be increased. In dogs and pigs given several large doses of copper intravenously, about 70 per cent of the copper was retained in the liver, thus demonstrating the high capacity of the liver for copper storage.

Gitlin (64) observed an increase of Cu^{64} in the

liver of mice up to seven hours after Cu^{64} injection. After seven hours there was a decrease of Cu^{64} in the liver and an increase in the bile. Later there was an increase in radioactive copper in the feces.

Sheep seem to be more sensitive than other animals to copper toxicity. Beck (5) showed that reduction of the concentration of copper in the liver of sheep after a large oral dose of copper is slower than that in other species.

Several copper-containing enzymes have been isolated in higher animals. In a review by Scheinberg and Sternlieb (122), information on several mammalian copper proteins has been summarized. Ceruloplasmin, cerebrocupein, cytochrome oxidase, uricase, hepatocuprein and tyrosinase were listed. Tyrosinase and cytochrome oxidase are the only two which are relatively well understood. Tyrosinase has an oxidase activity and is concerned with biological oxidation in tissues. Ceruloplasmin is a copper-protein found in the serum of blood and probably is concerned with the normal transportation and metabolism of copper. It has a weak oxidase activity but no logical function for it can be found. When there is a deficiency of ceruloplasmin, there also is a very large increase in liver and tissue copper. In humans, this condition is known as "Wilson Uzmans disease". Copper concentration will build up in the liver until it becomes toxic to the tissue. This disease usually is fatal. The functions of the other copper proteins have not been reported.

Copper toxicity has been reported by many investigators. Underwood (131) has described the general symptoms of copper toxicity. Initially, there is a very large increase in liver copper to an upper limit characteristic of each species. Then there is a catastrophic liberation of the liver copper into the blood stream. Hemolysis of the red blood cells and jaundice follow. Copper toxicity often is fatal to the animal. Symptoms of copper poisoning have been described in cattle and sheep but not in many other species.

Sheep are very susceptible to copper toxicity. Pierson et al. (117) and Ryff et al. (120) studied copper toxicity in this species. They showed a rapid uptake of copper by the liver up to levels exceeding 500 ppm. A rapid increase of ionic copper in the blood stream was noted. Hemolysis of blood cells and jaundice developed. Many of the animals died. It was suggested by Pierson that death resulted from kidney impairment associated with accumulation of the products of hemolysis and not because of the high level of copper or the lack of red blood cells.

Cunningham (32) studied the effects of various levels of copper supplementation in the bovine and showed that single doses of up to 80 grams of copper sulfate were not poisonous to yearling heifers or mature cows. A single dose of 400 grams was fatal to cattle of both age groups. Kidder (86) reported jaundice in cattle when large doses of copper were consumed over a long period of time.

Scheinberg et al. (122) has indicated that copper toxicity is not very common in humans even though much of the food consumed is processed in copper utensils. When humans consume a large quantity of copper, it usually causes nausea, vomiting and sometimes diarrhea and cramps. This generally results in a rapid passage of copper through the digestive tract and prevents the absorption of any substantial quantity of the element. It was concluded that copper is less toxic to humans than to most other species of animals.

Other investigators (16, 65, 115, 138) have reported copper toxicity in rats, pigs, and other non-ruminant animals. These species usually are less sensitive to copper toxicity. The symptoms are generally depressed growth, lack of appetite and even death, if the levels of copper are too high. There is no degeneration of the liver or release of copper, such as is the case in cattle and sheep.

COPPER DEFICIENCY

The earliest work with copper deficiency was concerned with the interrelationships of copper and iron and their ability to cure certain types of anemia. Early work by Hart et al. (76) showed that the ash of some plant and animal tissues could correct the condition of milk anemia in rats, but iron alone was not effective. In a later study Hart et al. (77) found the limiting factor to be copper. They showed conclusively that copper as well as iron was necessary for hemoglobin production in rats.

The interrelationship of copper and iron has been studied extensively in rats and in many other animals since that time. Scheinberg (122) showed some evidence that iron absorption was affected by the copper status of the animal. Chase et al. (25) reported that there was an increase in the uptake of iron from the digestive tract when copper was fed to copper-deficient rats. Wintrobe et al. (139) showed that it was necessary to supplement the diet with copper as well as with iron to cure certain types of anemia in swine. Lahey et al. (90) found that copper-deficient swine had an impaired ability to absorb iron from the gastrointestinal tract, an inability to mobilize much of the tissue iron, and also an inability to utilize injected iron for hemoglobin synthesis. It was shown by Brookbank (19) that copper-deficient swine were unable to utilize iron. Only copper

supplementation could correct this condition. Houk et al. (80) have reported that animals on a low-copper diet retained less iron than those on a high-copper diet, and that stores of copper were lost more readily than were iron stores.

Elvehjem and coworkers (50, 51, 52) showed in a series of studies that there was no increase in iron absorption when copper was fed to rats. There was evidence, however, that copper did affect the mobility of body iron. Morgan (110) presented evidence that normal rats, made anemic by bleeding, had a reduction of plasma iron, an increased total iron-binding capacity, and reduced stores of iron in the liver and spleen. Rats that were copper-deficient and made anemic by bleeding developed anemia more quickly and recovered more slowly than the normal controls.

Gubler et al. (73) demonstrated a reduction of iron absorption from the gastrointestinal tract of swine as a result of copper deficiency. It was necessary to supplement the diet with iron as well as with copper for complete recovery. In a later study Gubler et al. (71) measured the enzyme activities of several tissues. They found a decrease of cytochrome oxidase in the heart and liver but not in the kidney of copper-deficient pigs. Catalase and hemoglobin concentrations were reduced during both copper and iron deficiencies. Cytochrome c levels were high in copper-deficient animals and low in iron-deficient animals. Tissue iron was reduced markedly during copper deficiency.

Josephs (83) showed that iron in rats on low copper diets was utilized by the tissues to maintain a minimum concentration of iron. When copper was added to their diets, more iron was used in the production of hemoglobin and less was stored in the tissues.

Lahey et al. (89) studied the relationship of iron and copper deficiencies in swine. Microcytic hypochromic anemia developed when iron, copper, or both were deficient in the diet. The similarity of the deficiency symptoms of both metals indicated that copper was concerned with the normal metabolism of iron. There was an increase in the iron-binding capacity of the blood plasma.

In another study Cartwright et al. (24) showed that swine had only a single type of anemia whether it resulted from iron or from copper deficiency. Their findings differed from those of other investigations using different species of animals.

Van Wyk et al. (137) produced both iron and copper deficiency in dogs. There was, however, a difference in the resulting anemias. In iron deficiency, there was no change in the number of red blood cells, but cell size was smaller, and the concentration of hemoglobin was much lower. In copper deficiency, there was a reduction in the number of red cells but no reduction in hemoglobin. There was evidence of defective maturation of erythrocytes and defective development of the cells in the erythrocytic series. Maass et al. (95) found that both iron and copper supplements

in the diet were needed to cure milk anemia in dogs.

Enzyme studies by Gallager et al. (62) in rats demonstrated that liver slices from copper-deficient rats were normal in oxidative phosphorylation and anaerobic glycolysis ability and in catalase, diphosphopyridine nucleotide (DPN), isocitric dehydrogenase, malic dehydrogenase and glutamic dehydrogenase activity. Cytochrome oxidase and succinic oxidase levels were severely reduced.

Keil et al. (84) showed that contrary to an earlier report (85) by the same authors, iron added to milk diets was incapable of regenerating the hemoglobin of the blood in rats. The rats showed a change in hair color and had difficulty in rearing young. Kletzien et al. (87) found a small increase of iron in rat brain, heart, kidney, lung, muscle, spleen, testicle and bone tissue, and a large increase of iron in the liver when diets were supplemented with copper, thus suggesting an increase in the absorption of iron.

Muntwyler and Hanza (112) reported that copper supplementation in the rat increased the mobilization of available tissue iron for hemoglobin production and also increased the number of red blood cells. Schultze and Simmons (124) showed an increase in the cytochrome c oxidase level in bone marrow, rapid reticulocytosis, and a gradual increase in erythrocyte count and hemoglobin content in copper-deficient rats, when their diets were supplemented with copper. The absorption and retention of

copper appeared to be less in iron-deficient rats. Of the tissues sampled, the liver showed the greatest absolute retention of radioactive copper. Bing et al. (13) reported that rats injected with iron were able to retain more copper than those dosed orally.

There have been many other diseases and symptoms appearing in copper-deficient animals in addition to anemia and impairment of iron metabolism. Becker et al. (6) showed that the copper content of the livers of "salt sick" animals in Florida was very low. Supplementation with copper alleviated the condition.

Bennetts et al. (10, 12), in a series of studies, found the "falling disease" of cattle to be caused by the lack of copper in the diet. This disease was described as the terminal phase of a copper deficiency and could be controlled by the addition of a copper supplement to the ration. The animals usually exhibited a mild to severe anemia and much of the heart tissue had been replaced with fibrous or collagenous tissue. It was suggested that the failure of the heart might be due to the high degree of fibrous tissue, but there was great variability in the amount of connective tissue in the hearts of animals which had died from this disease. Gubler et al. (71) showed a marked decrease of cytochrome oxidase in the hearts of copper-deficient swine and also cardiac failure. Swine hearts exhibited cardiac hypertrophy as a prominent symptom while, in cattle, the heart was in a state of atrophy

with tissue being replaced by fibrous tissue.

Shearer et al. (126) reported cases of swayback in England and compared them with a similar disease in Australia. He showed that swayback was not an uncomplicated copper deficiency as was assumed by the Australians. Both the soil and feed were found to have a relatively high copper content. Lead was thought to be implicated but such was not borne out by Ferguson's investigations (57). Sellers (125) and Butler et al. (22) found that the copper content was low in the feed consumed by sheep in the swayback areas. In lambs, blood copper was low, and postmortem examinations revealed extensive demyelination of the cerebrum. Ewes showed a large drop in whole blood copper during the winter. This was reportedly caused by the low copper content of winter feed as compared with the summer pasture. There was a decreased incidence of the disease during copper supplementation. Butler et al. (22) attempted, unsuccessfully, to produce swayback in newborn lambs by feeding molybdenum with sulfate.

In 1944 Ferguson (55) in England was the first to show that copper supplementation improved the health of the cattle on "teart" pastures. Later, Ferguson et al. (56, 59) discovered that molybdenum levels were higher in "teart" than in "non-teart" pastures, and evidence indicated that molybdenum was implicated in the high incidence of "teart" in cattle on these pastures. The symptoms were diarrhea and unthriftiness, but no other symptoms of copper

deficiency were apparent. The administration of copper sulfate prevented diarrhea in the animals.

Enzootic ataxia is a disease in Australia, similar to swayback in England. Enzootic ataxia was described by Bennetts and Beck (9) as a disease in unweaned lambs caused by copper deficiency. In all cases a low copper status was demonstrated in ewes, lambs, and pastures.

Bennetts and Chapman (11) in 1937 found that copper deficiency existed in lambs exhibiting enzootic ataxia. The disease was prevented by feeding copper supplements to the ewes. Adequate copper appeared to be essential for the proper development of the central nervous system in lambs and normal erythropoiesis in the adults. The liver of the affected animals was very deficient in copper. There was evidence that low copper intakes prevented the animals from utilizing the iron stored in their bodies.

Pathological changes, described by Underwood (131), for enzootic ataxia in sheep are cerebral demyelination, degeneration of the motor tracts in the spinal cord and changes in the neurons of the central nervous system. Animals have an incoordinated movement of the hind limbs, a stiff and staggering gait and a swaying of the hind quarters. The lesions are irreversible and apparently develop before the lamb is born. This disease has been reported in species other than sheep.

Marston (97) found that it was possible for enzootic ataxia to develop in lambs when the copper content of the

liver of the ewe was below 20 μg/g dry weight, and that it was the invariable result when the copper concentration in the ewe's liver fell below 10 μg/g dry weight. In lambs examined pathologically, there were characteristic plaques of demyelination in the ventrolateral columns of the spinal cord.

Marston and Lee (98) described the beneficial effects of copper supplementation in preventing enzootic ataxia in sheep. Copper supplementation increased wool quality but when too much copper was fed, the sheep experienced a crisis and soon died.

Jensen and Flint (81) reported cases of enzootic ataxia in Colorado. Both the brain and central nervous system were found to be damaged and the copper content of the liver was quite low. The symptoms of the disease were partial to complete paralysis, retarded prenatal growth, and posterior muscular incoordination. Demyelination of the cerebrum and spinal cord, and fragility of the bones were common.

Davies and Farmer (36) showed evidence that "ryegrass staggers" in lambs was due to the low copper status of the animals. They showed that pastures being used were normal in copper and sulfate content, but relatively high in molybdenum.

Bennetts (7, 8) showed that "stringy" wool appeared to be the earliest and frequently the most obvious sign of copper deficiency in sheep, even when the deficiency was

not severe enough to cause ataxia in lambs. In lambs the deficiency caused decreased growth and development.

COPPER, MOLYBDENUM, AND SULFATE INTERRELATIONSHIPS

In 1938 Ferguson et al. (58) showed that high molybdenum levels in herbage caused cows to be unthrifty, to drop to a varying extent in production, and in some cases even to die. In a later study, Ferguson et al. (59) were among the earliest workers to report the effect of molybdenum on the copper status of animals. In their study it was shown that there was a relatively high molybdenum content in the forages of "teart" areas.

Dick and Bull (46) reported the effects of molybdenum on copper retention in herbivorous animals. An increase of molybdenum in the ration decreased the amount of copper in the liver. On high levels of molybdenum, the amount of liver copper was reduced even when the ration was supplemented with copper. In another study, Dick (39) showed that the type of ration also could have an effect on the level of liver copper when the ration was supplemented with molybdenum. The decrease in liver copper was much more rapid when sheep were fed chaffed lucerne hay, rather than chaffed oat hay.

Lesperance and Bohman (93, 94) found that more molybdenum was retained on grass than on lucerne hay when the rations were supplemented with 100 ppm molybdenum. In all cases, heifers receiving molybdenum showed typical signs of molybdenosis, although susceptibility of individual

cattle to this disease was variable. The animals showed achromotrichia after sixty days and there was some lameness. Plasma molybdenum increased 100-fold during the first thirty days. Animals receiving alfalfa and molybdenum had an increase of plasma copper and retained a high liver tissue copper concentration. Molybdenum decreased the energy balance but had no effect on digestibility of the ration and on copper, phosphorus, sulfur, and nitrogen balance. Plasma molybdenum was thought to be the best immediate criterion for excessive molybdenum intake, but liver molybdenum concentration was found to be more indicative of molybdenum toxicity.

Cunningham (33) reported on both copper deficiency and the disease called "peat scours" in New Zealand. The diseases were both considered copper deficiencies, with peat scours being much more severe. The symptoms were similar, both diseases being characterized by unthriftiness, brittleness of bones, changes in hair color, rough and staring hair and anemia. The animals with peat scours were even more unthrifty, had mild to severe cases of diarrhea, and dropped dramatically in milk production. Adult sheep were usually not affected as markedly as were cattle. There was sometimes a decrease in the quality of wool, but other symptoms seldom appeared. The lambs showed very severe effects of the low copper status, and these were similar in both types of copper deficiency. The lambs were born with ataxia and were afflicted with acute

osteoporosis and a tendency for bone fracture.

Investigations have indicated that molybdenum is the probable cause of peat scours. This condition develops in areas where the copper content of forages is relatively high. It was shown (33) that molybdenum at the level of 30 ppm of the ration was sufficient to reduce the copper reserve in the liver, but not to produce diarrhea. When the molybdenum level was doubled, severe diarrhea appeared. In these studies several of the copper-deficient animals developed fractures. The fractured bones showed a mild degree of osteoporosis but were virtually normal in appearance. Blood phosphorus and calcium levels were normal.

Marston (97) showed that nervous disorders sometimes developed in lambs born to ewes having liver copper levels below 20 ppm dry weight. When the levels dropped below 10 ppm dry weight, the lambs were invariably afflicted with nervous disorders. It was shown that molybdenum could decrease liver copper levels in sheep, but its ability to reduce copper levels in the liver changed with the seasonal variation of pastures. In these experiments, 50 ppm molybdenum in the feed did not reduce blood or liver copper levels below that needed for normal physiological activity. These findings were different from those with sheep in other areas where the addition of molybdenum at a 50 ppm level caused the more extensive symptoms of copper deficiency.

Molybdenum toxicity was reported in California by

Britton and Goss (18). The symptoms were diarrhea, pigmentary changes of the coat, hypochromic microcytic anemia and emaciation. This condition was reproduced experimentally in a Holstein calf. When molybdenum was withdrawn, the animal quickly recovered. Bardshad (2) studied the molybdenum levels of plants and soil in California. The level of molybdenum in some plants was as high as 220 ppm. Legumes generally had higher levels of molybdenum than did grasses. Succulent pastures caused more severe digestive disturbances in cattle than did non-succulent pastures. As little as 15 ppm molybdenum caused severe digestive disturbances.

Thomas and Moss (129) showed that molybdenosis caused lack of sexual interest in Holstein bulls. Postmortem examinations of the testes showed marked damage of the interstitial cells and germinal epithelium, with little evidence of spermatogeneses. Gleming et al. (60) studied the effects of molybdenosis on reproduction in a heifer. Although the growth rate of the heifer with molybdenosis was reduced, she recovered very rapidly after treatment with copper, and was able to produce an apparently normal calf.

Early studies on molybdenum toxicity are misleading and confusing because the effect of sulfate on molybdenum absorption and excretion was not recognized. It was not until Dick (40, 45) in 1935 showed that sulfate with molybdenum had an important effect on copper metabolism, that

many of the conflicting results of investigations in different areas could be reconciled. Dick (42, 44, 45) found that sulfate affected both the absorption and excretion of molybdenum in sheep. On a constant intake of molybdenum, excretion of this element was greater, and blood and tissue accumulation smaller when sulfate was included in the ration. When dietary sulfate was increased, there was also an increase in fecal molybdenum indicating a reduction in the absorption of molybdenum from the digestive tract. Although sulfate reduced the level of molybdenum in the blood and tissues, it increased the toxicity of molybdenum in these animals.

Dick (41, 43) showed that the amount of copper retained by sheep was affected by the ingestion of both molybdenum and sulfate, but neither separately had much effect on copper storage. When copper content of the ration was very low, it took only a small amount of molybdenum on high sulfate intakes to affect copper retention.

Studies by Wynne et al. (141, 142) indicated that sheep in South Wales had a copper deficiency resulting from the interaction of molybdenum, copper, and sulfate, thus confirming the work of Dick (40, 45) on the interrelationship of these elements. Harvey et al. (78) studied the interrelationship of molybdenum, copper and sulfate in Australia. Cattle on pasture lost their copper reserves more quickly than did animals fed a similar type of dry ration containing similar copper, molybdenum and sulfate

levels. The conclusion was drawn that some substance other than those mentioned were affecting the copper status.

Mills et al. (109) showed that ewes on a high intake of molybdenum and sulfate would give birth to ataxic lambs. Degenerative lesions, similar to those in swayback animals, were found in the nervous system. Very low liver copper levels were found in both the ewes and the lambs.

Butler and Barlow (23) attempted to produce swayback in lambs. Although blood and liver copper values were similar to those found in sheep with this disease, typical symptoms did not appear. It was not known whether the nature of their diet or the breed of sheep prevented the occurrence of the disease.

Cunningham et al. (34) studied the effect of molybdenum intake in young sheep. Sheep appeared to be less sensitive to the effects of molybdenum and sulfate than were cattle. In another experiment using cattle on a low-copper ration, Cunningham et al. (35) found that molybdenum or molybdenum and sulfate caused a reduction of both liver and blood copper. The addition of sulfate to a ration containing molybdenum reduced both blood and tissue molybdenum. These animals did not develop diarrhea when fed molybdenum at the level of 4 or 9 ppm.

Monogastric animals are generally not as sensitive to molybdenum or molybdenum with sulfate as are ruminants. Neilands et al. (113) showed that molybdenum was toxic to rats when fed at levels of 50 and 100 mg per 100 g of diet.

Supplementation with small amounts of copper to bring the copper content of the ration up to 9.7 mg per 100 g helped relieve molybdenum toxicity. Tracer studies with Mo^{99} showed that copper did not alter molybdenum distribution. Gray and Ellis (69) reported that molybdenum in the diet of the rat would retard growth, and high zinc levels would cause anemia. Copper supplementation helped alleviate the symptoms caused by both diets. The addition of both zinc and molybdenum to the diet caused an even more drastic reduction of growth.

Comar et al. (28) studied the effects of high molybdenum intake in rats. When rats were given 1200 to 1600 mg of molybdenum per kilogram of body weight, it was always fatal. Growth rate was reduced when rats received 120 to 600 mg of molybdenum per kilogram of body weight. Neither molybdenum nor phosphorus decreased liver copper in the animals. When molybdenum was fed to cattle, it accumulated in the bone at about the same rate as did phosphorus. Blood alkaline phosphatase levels increased when there was a copper-molybdenum imbalance.

It was shown by Gray and Daniel (67) that molybdenum depressed growth in rats when fed at a level of 0.08 per cent of their diet. Methionine and copper supplements in their diets, and weekly injections of vitamin B_{12} improved the rate of growth in these animals.

Kulwich et al. (88) found an increase in tissue copper concentration in both rats and pigs when fed 1000

ppm molybdenum in their diets. Twenty ppm of copper did not counteract the effects of molybdenum. Increase in tissue copper was greatest in the liver and kidneys. The addition of 1000 ppm zinc in the diets had no effect on copper metabolism. Swine appeared to be more resistant to molybdenum toxicity than were rats.

Jeter and Davis (82) showed that diets containing 80 ppm molybdenum and 20 ppm copper had no effect on the growth of weanling rats. When the level of copper was 5 ppm, molybdenum reduced the growth of rats. There was no evidence of anemia at any of the molybdenum levels. Achromotrichia and alopecia appeared when molybdenum levels were 80 and 140 ppm. Reproduction was hampered at these levels, and there was some infertility in the males.

Miller et al. (105) reported that 2200 ppm inorganic sulfate added to rations containing molybdenum at levels of 75, 100, and 300 ppm, increased the rate of growth in rats. At the lower molybdenum levels, sulfate restored the growth rate to that of the controls. Sulfate also reduced the levels of copper and molybdenum in the blood and liver.

Van Reen and Williams (136) have reported the effects of different sulfur compounds on a diet containing 1200 ppm molybdenum. Methionine, sodium sulfate and sodium thiosulfate relieved some of the adverse effects of molybdenum on growth. Cysteine was less effective in this respect.

It was shown by Williams et al. (140) that molybdenum fed to rats depressed their appetite and retarded their growth. Some rats had diarrhea, but none died. Alkaline phosphatase activity was depressed in both the intestines and kidney. Van Reen (135) fed 0.8 mM of molybdenum to rats and supplemented the diets with sodium sulfate, citrate, tartrate, acetate, bromide, chloride, or nitrate. Only sodium sulfate helped alleviate molybdenum toxicity.

Halverson et al. (75) showed that rats on a diet low in both copper and sulfate were affected by the addition of dietary molybdenum. They developed a slight anemia, retardation of growth, and diarrhea. The addition of cysteine along with molybdenum to the diet caused severe anemia but increased the rate of growth. The effects of molybdenum were reversed by the addition of copper.

Brinkman and Miller (17) reported that zinc had no effect on rats whose diets contained 200 ppm molybdenum when zinc oxide levels were 0.05, 0.10, and 0.15 per cent. There was almost a fifty per cent reduction of growth when 200 ppm molybdenum were fed.

It was found by Gray and Daniel (68) that the copper status of rats had an effect on their reaction to molybdenum and sulfate supplementation, and when body copper was low enough, they showed basically the same symptoms as did ruminants. Molybdenum had two different actions in this study. In copper-depleted rats consuming a copper deficient

diet, dietary molybdenum and sulfate aggravated the copper deficiency while copper administration lessened it. In rats receiving normal amounts of copper and having normal copper stores, molybdenum did not induce copper deficiency but caused some other disfunction in metabolism, which was partially corrected by methionine and completely corrected by sulfate supplementation. It was suggested that the difference in the response of ruminant and non-ruminant animals to molybdenum and sulfate might be explained on the basis of the copper status of the animals and their dietary intake.

Feaster and Davis (54) gave S^{35} orally to rabbits receiving either 0.15 per cent molybdenum or no supplemental molybdenum in their diets. There was no difference in total S^{35} in the liver, muscle, or hair of the two groups. Less S^{35} was taken up by the liver and slightly more S^{35} was excreted in the molybdenum-fed animals. The bones of the animals given molybdenum were richer in sulfur than those of the controls.

McCarter et al. (100) produced molybdenum toxicity in rabbits and described the symptoms of the toxicity. These symptoms appeared very quickly and included bone abnormalities. Fractures of the epithysis of the long bones were commonly noted. Many rabbits died during the treatment. Arrington and Davis (1) observed molybdenum toxicity in rabbits when more than one per cent of their diets consisted of molybdates. The rabbits exhibited

anorexia, loss of weight, alopecia, slight dermatosis and anemia. In young rabbits, abnormalities of the front legs developed. When 0.02 per cent copper was included in the molybdenum diets, there was no sign of the toxicity.

Chickens seem to have a relatively high tolerance for molybdenum. Davies et al. (37) reported low mortality rates in chickens fed up to 4000 ppm molybdenum. Hemoglobin concentration, packed cell volume and red blood cell count were increased when up to 2000 ppm molybdenum was fed, but with 4000 ppm and above, anemia appeared in the animals. There was no sign of diarrhea, and supplementation with sulfate did not reduce molybdenum deposition in the tibia. Sulfate in sufficient quantities alleviated the physical symptoms of molybdenosis. Miller and Denton (104) fed 2200 ppm of sulfate as sodium and potassium salts to growing chicks. Sulfates had no effect on their growth over a three-week period. When molybdenum was fed at the level of 200 ppm, there was no adverse effect on growth. Growth was retarded slightly at the level of 500 ppm, and levels of 1500 to 2500 ppm gave a considerable reduction in growth. Sulfate was more effective than taurine, bisulfite, sulfite or sulfides in this respect. Thiosulfate did not counteract the effect of molybdenum but further increased depigmentation of the animals. Sulfate reduced liver storage of molybdenum, and copper reduced it even more. Copper did not improve the growth rate.

There has been some evidence presented which

indicates that phosphorus metabolism may be affected by copper deficiency. Davis (38) reported that molybdenum supplements in low-copper rations caused poor bone formation in calves. In sixty days on low copper rations, calves developed swelling of the joints, beading of the ribs, and typical rachitic appearance. One calf suffered a fractured bone. Molybdenum appeared to follow the route of phosphorus in the body. Blood phosphorus and blood phosphatase were nearly normal. In extreme cases, the blood phosphorus and blood alkaline phosphatase increased when molybdenum was at toxic or slightly below toxic levels, and molybdenum did not lower liver copper levels. When dietary copper was low, the addition of molybdenum decreased the copper levels even further. Copper did not interfere with molybdenum accumulation. Phosphorus excretion in cattle was affected by molybdenum, and when molybdenum was fed a larger amount of phosphorus was excreted in the feces and a smaller amount in the urine. An increase of phosphorus in the ration had no effect on bone deformities caused by molybdenum.

Baxter et al. (3, 4) described bone disorders resulting from copper deficiency in dogs. There was a severe reabsorption of bone along the medullary surface of the shafts, a reduced amount of cancellous bone and a wider epithysis. There was no evidence of any gross disturbance of calcification. The bone abnormalities showed no resemblance to rickets and copper supplementation relieved the condition.

A report by O'Dell et al. (114) showed that copper deficiency in chicks caused bone deformities. Bones were more easily fractured, and there was evidence of internal hemorrhages. Histological studies of the aorta suggested a derangement of connective tissue metabolism with a major defect in the elastic tissue.

Follis et al. (61) studied bone deformities in pigs receiving copper-deficient diets. These pigs developed gross deformities of the legs. Histological examination of the bones in copper-deficient animals showed little osteoblastic activity and poor development of the bone cortex. The activity of the chondroblasts appeared to be normal.

In studies with cattle, Shirley et al. (127) found that molybdenum in the ration changed the pathway of excretion of phosphorus. More of the phosphorus was excreted in the feces than in the urine. Molybdenum plus copper had less effect on the pathway of phosphorus excretion than did molybdenum alone.

Feaster and Davis (53) showed that molybdenum did not affect the absorption of calcium and phosphorus. Rabbits fed molybdenum had nearly as much calcium and phosphorus in their bones as did the controls even though the bones of the former were smaller. Radioactive calcium and phosphorus were higher in the bones of the molybdenum-fed animals than of the controls. There was a decrease of organic matter in the bones of the former group.

Several investigators have shown that zinc has an effect on the copper status of the animal. Cox and Harris (30) found that neither copper nor iron would improve the growth of rats when they were fed 0.4 to 0.6 per cent zinc as the oxide. The rats developed anemia and achromotrichia, and these symptoms were prevented by copper and iron supplementation. Zinc caused reduction of liver iron and in some cases also of liver copper. Neither iron nor copper prevented deposition of zinc in the liver.

Smith and Larson (128) showed that the addition of excess zinc to the diets of rats caused microcytic hypochromic anemia. Cobalt and iron had no effect but copper partially cured this condition. Mixtures of iron, cobalt, and copper were more effective in this respect than copper alone. Van Reen (134) fed excess zinc to rats and found that it reduced cytochrome oxidase, catalase, and hemoglobin levels in the liver and increased alkaline phosphatase. Copper supplementation increased hemoglobin, catalase and cytochrome oxidase, but it did not increase the rate of body growth.

In swine, Ritchie (118) found that zinc could reduce copper toxicity. The addition of zinc to high-copper diets prevented such symptoms as severe anemia, hemolytic jaundice, internal hemorrhage, loss of weight, and death of the animals.

Grant-Frost and Underwood (66) studied the interrelationship of dietary copper and zinc in rats. Zinc reduced growth, food consumption, hemoglobin level and copper

retention. It was concluded that the main depressing effect of zinc on growth was the reduction of food consumption. Anemia was caused by a zinc-induced copper deficiency.

Cox et al. (29) reported that excess dietary zinc could reduce liver iron but not liver copper in swine. With 0.4 per cent zinc diet, hemoglobin, hemosiderin, and ferritin levels in the liver were decreased. In another experiment (31) rats, particularly male rats, fed 0.4 per cent zinc in the diet exhibited decreased liver xanthine oxidase in a few days. Liver molybdenum was not affected.

McCall et al. (99) demonstrated that high protein diets had a greater effect on decreasing liver copper levels in rats than did zinc supplements. Soybean protein proved to be more beneficial to the animals than was casein protein when high levels of zinc were fed. The animals fed soybean protein were significantly heavier, showed higher hemoglobin levels, and accumulated less total zinc. Liver iron concentrations decreased in all groups concurrently with the increase of zinc levels in the diet. Animals consuming soybean protein and showing no signs of zinc toxicity had the same liver zinc levels and the same decrease in liver iron as those consuming casein protein. Either iron or copper relieved the symptoms of zinc toxicity. There were signs of copper deficiency when high levels of zinc were fed. The deficiency symptoms were alopecia, loss of coat color, poor growth, and anemia. Zinc had no effect on the percentage of doses of radioactive copper, zinc, or total copper which accumulated in the liver.

EXPERIMENTAL PROCEDURE

The purpose of this experiment was to study the effect of a high or low dietary phosphorus intake on the absorption, deposition, and excretion of copper in animals being supplemented with molybdenum, molybdenum and sulfate, or neither.

Twelve Holstein bull calves were randomly divided into four groups. Two groups received a high and two groups a low phosphorus ration. Each group then was subdivided with one animal receiving 50 to 75 ppm molybdenum, another 50 to 75 ppm molybdenum plus 0.3% sulfur as a sulfate, and the third neither (Control). Table 1 shows the plan by which individual animals were assigned to treatment groups.

Treatment animals were given either molybdenum (Mo) or molybdenum and sulfate (Mo + SO_4) in their milk soon after they were born in an attempt to maximize the length of time they received these supplements. At about two months of age, the calves were taken off milk and given the appropriate concentrate mixture. It was assumed that at this age the calves could subsist on forage and a concentrate mixture containing a high level of urea. The rations with their ingredients and the nutrient composition are described in Tables 2 and 3.

Hay intake was adjusted weekly in an attempt to keep the ratio of hay to concentrate at about 2:1 by weight.

Table 1. Pattern by which animals were assigned to treatments

Group number	Animal number	Ration[a]	Phosphorus level	Supplemented with	
				Molybdenum	Sulfate
I	609	Hay + Grain mixture (No. 3)	Low	Yes	Yes
I	610	Hay + Grain mixture (No. 1)	Low	No	No
I	611	Hay + Grain mixture (No. 2)	Low	Yes	No
II	612	Hay + Grain mixture (No. 6)	High	Yes	Yes
II	613	Hay + Grain mixture (No. 4)	High	No	No
II	614	Hay + Grain mixture (No. 5)	High	Yes	No
III	615	Hay + Grain mixture (No. 5)	High	Yes	No
III	616	Hay + Grain mixture (No. 6)	High	Yes	Yes
III	617	Hay + Grain mixture (No. 4)	High	No	No
IV	618	Hay + Grain mixture (No. 1)	Low	No	No
IV	619	Hay + Grain mixture (No. 2)	Low	Yes	No
IV	620	Hay + Grain mixture (No. 3)	Low	Yes	Yes

[a] See Table 2 for detailed description of concentrate mixtures.

Table 2. Description and ingredient composition of concentrate mixtures

Low phosphorus concentrate mixtures

Mixture no. 1 (Control)			Mixture no. 2 (Mo)			Mixture no. 3 (Mo + SO_4)		
Corn	200	lb	Corn	200	lb	Corn	200	lb
Molasses	15	lb	Molasses	15	lb	Molasses	15	lb
Urea	8	lb	Urea	8	lb	Urea	8	lb
Plain salt	0.5	lb	Plain salt	0.5	lb	Plain salt	0.5	lb
$NaHCO_3$	10.5	lb	$NaHCO_3$	10.5	lb	$NaHCO_3$	10.5	lb
$CaCO_3$	22.6	lb	$CaCO_3$	2.6	lb	$CaCO_3$	2.6	lb
Vitamins A & D[a]			Vitamins A & D[a]			Vitamins A & D[a]		
			Na molybdate	59.7	g	Na molybdate	59.7	g
						Sodium sulfate	9.44	g

High phosphorus concentrate mixtures

Mixture no. 4 (Control)			Mixture no. 5 (Mo)			Mixture no. 6 (Mo + SO_4)		
Corn	200	lb	Corn	200	lb	Corn	200	lb
Molasses	15	lb	Molasses	15	lb	Molasses	15	lb
Urea	8	lb	Urea	8	lb	Urea	8	lb
Plain salt	0.5	lb	Plain salt	0.5	lb	Plain salt	0.5	lb
Na_2HPO_4	10.0	lb	Na_2HPO_4	10.0	lb	Na_2HPO_4	10.0	lb
Dicalcium phosphate	3.5	lb	Dicalcium phosphate	3.5	lb	Dicalcium phosphate	3.5	lb
Vitamins A & D[a]			Vitamins A & D[a]			Vitamins A & D[a]		
			Na molybdate	59.7	g	Na molybdate	59.7	g
						Sodium sulfate	9.44	g

[a] Eight grams vitamin A supplement (5000 IU/g), 4 g irradiated yeast.

Table 3. Nutrient composition[a] of rations

	TDN	Crude protein equivalent	Phosphorus	Calcium
	(%)			
Timothy hay	51.7	7.6	0.21	0.40
Mixture no. 1 (Control)	46.6	16.4	0.23	0.45
Mixture no. 2 (Mo)	46.6	16.4	0.23	0.45
Mixture no. 3 (Mo + SO_4)	44.8	15.8	0.22	0.43
Mixture no. 4 (Control)	46.6	16.4	1.42	0.38
Mixture no. 5 (Mo)	46.6	16.4	1.42	0.38
Mixture no. 6 (Mo + SO_4)	44.8	15.8	1.36	0.37

[a] Calculated from tables in Feeds and Feeding, by F. B. Morrison, 22 ed. The Morrison Publishing Company. Ithaca, N. Y. 1956.

With this ration, certain animals received supplementary Mo at a level of 75 ppm and supplementary sulfur, as SO_4, at a level of 0.3% of the total ration. In Table 4, the calculated amounts of Mo and SO_4 consumed by the various animals are shown.

Phosphorus levels were approximately 1.42% in the high-phosphorus and 0.23% in the low-phosphorus concentrate mixtures. The timothy hay was estimated, using Morrison's tables (111), to contain about 0.21% phosphorus. The low phosphorus (LP) ration contained near or below the amount necessary for the normal maintenance and growth of young dairy animals according to Morrison's standards. On the high phosphorus (HP) rations, animals received about three times the amount of phosphorus provided by the rations.

Blood samples were taken every two weeks for the determination of calcium, inorganic phosphorus, alkaline phosphatase, plasma protein, and hemoglobin. Monthly blood samples were taken for the determination of copper, zinc, and molybdenum.

Heparinized blood was poured into two 50-ml centrifuge tubes and centrifuged at 4000 rpm for two hours. Two 20-ml aliquots of the resulting plasma were pipetted into platinum dishes for the determination of zinc and copper. The remaining plasma was frozen and held for subsequent determination of molybdenum. One 20-ml sample of whole blood was pipetted into a platinum dish for the determination of zinc and copper. An estimation of blood cell volume

Table 4. Calculated levels of supplemental molybdenum and sulfur consumed by the animals by four-week periods

Animal no.	609	611	612	614	615	616	619	620
Days on experiment	Molybdenum (ppm)							
57 to 84	82	120	99	100	144	104	109	66
85 to 112	61	100	68	85	97	86	80	68
113 to 140	68	79	67	77	181	72	71	68
141 to 168	69	71	73	68	68	68	74	62
169 to 196	69	73	61	72	63	75	65	58
197 to 224	65	94	50	87	80	61	82	59
225 to 252	76	63	53	68	68	40	77	27
253 to 282	--	--	12	80	68	57	--	--

Animal no.	609	612	616	620
Days on experiment	Sulfur (%)			
57 to 84	0.329	0.396	0.415	0.265
85 to 112	0.245	0.274	0.342	0.273
113 to 140	0.270	0.286	0.288	0.273
141 to 168	0.275	0.292	0.271	0.248
169 to 196	0.277	0.245	0.300	0.232
197 to 224	0.258	0.201	0.244	0.234
225 to 252	0.304	0.212	0.160	0.109
253 to 282	---	0.047	0.230	---

was made from centrifuged samples in order to calculate blood cell zinc, copper and molybdenum values. Another sample of whole blood was centrifuged and the resulting plasma was used for the determination of inorganic phosphorus, protein, blood alkaline phosphatase, and calcium. The remaining whole blood was frozen for subsequent determinations of molybdenum and hemoglobin.

Blood in platinum dishes was dried in a convection oven at 60 C for 8 to 12 hours, then in a vacuum oven over night at the same temperature. Samples were transferred to a muffle furnace and ashed for eight hours at 500 C. After samples had cooled, they were dampened with redistilled water. Four milliliters of perchloric acid were added and the mixture was brought to dryness on a gas plate. Samples were re-ashed in a muffle furnace for an additional four hours. Ashed samples were dissolved in 20 ml of 2N HCl and transferred to separatory funnels. Zinc and copper determinations were made by methods described in a mimeographed report by Lazer (92).

Plasma calcium was determined by the Clark-Collip modification of the Kramer-Tisdall method described in a textbook by Hawk et al. (79). Inorganic phosphorus and alkaline phosphatase were determined by a modification of the Bodansky method using the principles of Fish and Subbarow for the determination of phosphorus. This method is described by Hawk et al. (79).

Blood plasma protein was estimated using a protein

refractor. Hemoglobin was determined by a cyanohemoglobin method similar to that of Evelyn and Mallow as described by Hawk et al. (79). A purchased standard hemoglobin solution was used as a reference for the determination of hemoglobin.

Radioactive copper studies were started when animals reached the age of eight months. Three animals were fed and three animals were injected with Cu^{64} at two-week intervals. The Cu^{64} was purchased from ISO/Serve Inc. in Cambridge, Mass. and transported to the laboratory on the day it was used. It was diluted, and three equal portions were removed as oral use. The remaining solution was diluted further and equal samples were used for injection into the jugular vein. The radioactivity of the copper ranged from 5 to 8 millicuries for the oral dose and 0.5 to 1.5 millicuries for the injected dose, depending upon the radioactivity of the material received. A lead shield was used to protect the persons diluting and dividing the material. The samples to be fed were placed in lead-shielded bottles, and the solution to be injected was placed in the original bottle enclosed in a lead container. The samples then were transported to the barn for feeding or injection.

The animals were placed in collection stalls before receiving the radioactive copper. The Agricultural Experiment Station Veterinarian injected the radioactive copper intravenously into the jugular vein. Orally administered radioactive copper was given by means of a stomach tube.

Urine and fecal samples were collected at four- to six-hour intervals during the first three days and at longer intervals thereafter.

Blood samples were taken from the jugular vein at four-hour intervals except for the animals injected with Cu^{64}. Samples were taken from these animals every 1 to 2 hours for the first few hours after injection and at four-hour intervals thereafter. After 3 days, blood samples were taken at longer intervals.

Two weeks after the animals had received both an injected and oral dose of Cu^{64}, they were given a third dose, either oral or injected, and sacrificed 32 hours later. Various tissues were sampled and the radioactivity determined. At that time, additional samples of tissue were collected for determination of total tissue copper, zinc and molybdenum.

Radioactivity in the tissue samples was determined by the use of a Model DS5-5 gamma well scintillation counter, manufactured by Nuclear of Chicago Corporation. A 5-milliliter sample of blood was counted for 1 to 15 minutes, depending on the level of radioactivity present. Urine was mixed and 75 to 200 ml portions were taken. Ten milliliters of nitric acid were added to the urine to destroy organic matter and the solution was brought to dryness on an electrically heated sandbox under a hood. Samples were dissolved and transferred into test tubes, each containing 5 ml of solution, for counting. Feces were wrapped in wax paper, weighed on an analytical balance, and placed in test

tubes to be counted. Samples were counted for various lengths of time depending on the radioactivity.

Tissue samples from sacrificed animals were placed in previously weighed test tubes and counted immediately. The tubes containing the tissues were weighed later, and weights of tissues were calculated. Tissue samples from the digestive tract were rinsed in three different beakers of distilled water and blotted dry with paper towels. "Clean" tissues, such as liver and muscle, were placed in previously weighed tubes without first being rinsed.

Tissue samples used in the determination of total copper or zinc were placed in previously weighed plastic bottles. Then the bottles were weighed again and held in a convection oven for at least 24 hours and until the tissues appeared dry. The tissues then were stored at room temperature until they were analyzed. Before analysis, the tissues were re-dried in a vacuum oven for twelve hours and then re-weighed. These weights constituted the dried tissue weights used in the calculations. For determination of zinc and copper, samples of tissues were removed from the plastic bottle, weighed in a platinum dish and ashed in a muffle furnace for eight hours at 500 C. The method from this point on was identical to that described for blood.

For determination of molybdenum in tissue and blood, a wet ashing method was employed. Tissue samples were weighed, and blood samples were pipetted into 100-ml beakers. Samples were digested with a mixture of nitric

and perchloric acids on a steam bath until the tissue dissolved. The beakers then were placed on a gas hot plate and heated just to dryness using a low flame. Samples were removed from the hot plate, dissolved in 0.02N HCl and made up to 50 ml with water in a volumetric flask. An appropriate portion of the resulting solution was used for the determination of molybdenum as described below.

A portion of the sample was pipetted into a 50-ml glass-stoppered centrifuge tube and diluted to 10 ml with water. The sample was neutralized with a 1:1 aqueous dilution of distilled NH_4OH, using one drop of phenophthalein as an indicator, and was made just acid with a 1:1 aqueous dilution of distilled HCl. Four milliliters of HCl, 1 ml of $Fe(NH_4)_2SO_4$ (approximately 50 ppm), 1 ml of 5% NaF, 2 ml of KSCN solution (50 g KSCN in 50 ml of H_2O), 5 ml of 5% ascorbic acid, and 10 ml of isoamyl alcohol were added to the centrifuge tube. The mixture was shaken for 3 minutes, and the aqueous layer was removed. Another 5 ml of 5% ascorbic acid was added, and the sample was shaken for 15 minutes. The aqueous layer again was removed, and the remaining isoamyl alcohol was poured into 15 ml centrifuge tubes and spun at 2500 rpm for 10 minutes. Samples then were read in a Model 2400 Beckman D U Spectrophotometer at 465 mμ for determination of molybdenum. A known molybdenum standard was read along with the samples.

RESULTS AND DISCUSSION

The effect of treatments on the physical condition of the animals varied among groups. The animals in Groups I and IV, which were on the low phosphorus (LP) rations, generally were in good health. Only one animal showed any signs of copper deficiency. Animal 609, which was fed the LP, molybdenum-plus-sulfate (Mo + SO_4)-supplemented ration showed signs of achromotrichia. The hair around the eyes turned gray, and there were some patches of reddish brown hair on the face and body. The molybdenum (Mo) animals of both groups did not show any signs of copper deficiency. Animals 609 and 611 in Group I had rough hair coats and a less thrifty appearance than the corresponding control animal, 610. None of the animals in Group IV showed any obvious physical treatment effects.

Two animals on the high phosphorus (HP) rations in Groups II and III showed rather striking treatment effects. Animal 612 in Group II, which received the HP, Mo + SO_4-supplemented ration, showed about as much hair discoloration as Animal 609 on the corresponding LP ration. In addition, the front legs of Animal 612 were bowed and misshapen, one knee was enlarged, and lameness was pronounced. The legs of this animal resembled those of an animal having rickets. Animal 612 (Mo + SO_4) and animal 614 (Mo) both had rough hair coats and appeared less thrifty than the corresponding

control animal, 613.

The HP, Mo + SO_4-supplemented animal (616) in Group III showed the most extreme effects of treatment. This animal lost weight during the last few months of the experiment and exhibited severe achromotrichia. The hair on the face was very light in color, and there was a great deal of brown hair on the head and body of the animal. Toward the end of the experiment, this animal exhibited diarrhea and became very weak. During the second treatment with radioactive copper, the animal stopped eating, and the condition became grave. The animal refused to lie down in the collection stall for over 48 hours and had to be carried to a stall at the end of the collection period. To prevent the animal dying, milk was fed in place of the concentrate mixture for three days. The animal started to eat some concentrate the second day and was placed on the regular feed the third day. Eleven days later, the animal was given the final Cu^{64} treatment and sacrificed 32 hours later. In this group, the control animal (617) died from pneumonia during the sixth month of the experiment. The animal did not appear normal during the third month of the experiment and would not eat concentrate and hay readily. At this time his condition did not appear to be serious, but several weeks later, the animal showed a tendency to bloat and at times appeared to vomit. Later he became very ill and finally succumbed. An autopsy was performed by the Agricultural Experiment Station veteranian, and the report

indicated that the animal died from lung congestion, apparently caused by foreign material that gained access to the lungs. This caused a purulent type of pneumonia with pus pockets over a large area of both lungs. The pericardial sac of the heart was thick and edematous, and the abomasal pylorus appeared to be constricted, although apparently not blocked. Since this experiment had been in progress for several months, another animal was not substituted. Animal 615 of this group, which received the HP, Mo-supplemented ration, showed no treatment effects except for a rough hair coat and a less thrifty appearance than the control. In Tables 5 and 6 the amount of feed consumed by the animals and changes in their body weights are recorded.

It appeared desirable in this experiment to have the LP concentrate mixtures as low in phosphorus as practically possible. Corn was used as the main ingredient of the grain mixture, because of its relatively low phosphorus content, and urea was used in place of a plant protein supplement for the same reason. A ration lower in phosphorus would have been desirable, but there was no practical way to decrease the phosphorus level further without making the ration even less palatable. Sodium carbonate was used in rations to balance the large amount of sodium from the sodium phosphate and sodium sulfate supplements added to the experimental grain mixtures. Molasses was used to improve the palatability of all the mixtures.

When the radioactive copper work was begun, some of

Table 5. Feed consumption and changes in body weight of animals on low-phosphorus rations

Days on experiment	Concentrate	Hay	Wt gain	Concentrate	Hay	Wt gain
	(lb)			(lb)		
Treatment	Control					
Animal number	610			618		
29 to 56	73.7	43.8	8	87.4	44.8	39
57 to 84	107.1	93.2	45	107.3	116.0	50
85 to 112	108.0	133.3	35	81.0	164.3	41
113 to 140	79.3	146.5	29	80.8	171.7	41
141 to 168	68.6	149.6	34	81.4	171.6	25
169 to 196	77.6	160.1	-20	84.0	192.9	41
197 to 224	76.5	106.8	27	93.9	226.8	33
225 to end	58.8	150.8	28	35.7	102.0	8
Totals	649.6	984.1	186	651.5	1190.1	278
Means	2.94	4.45	0.84	3.19	5.83	1.36
Treatment	Molybdenum					
Animal number	611			619		
29 to 56	92.9	50.0	35	34.1	47.3	32
57 to 84	111.0	109.2	44	107.5	114.2	40
85 to 112	103.7	158.5	54	66.1	118.8	22
113 to 140	95.0	178.0	39	66.0	141.7	18
141 to 168	86.4	183.0	45	68.4	128.7	11
169 to 196	105.8	193.8	17	58.3	142.0	26
197 to 224	108.0	181.9	31	82.4	144.5	3
225 to end	88.2	167.6	32	29.4	56.0	24
Totals	791.0	1222.0	297	512.2	893.2	176
Means	3.58	5.53	1.34	2.51	4.38	0.86
Treatment	Molybdenum + Sulfate					
Animal number	609			620		
29 to 56	57.6	84.6	31	55.4	58.0	33
57 to 84	73.3	126.8	42	62.5	150.1	46
85 to 112	64.3	171.8	50	67.3	155.4	41
113 to 140	81.1	188.8	41	68.0	156.5	38
141 to 168	77.3	175.5	38	69.7	183.3	17
169 to 196	78.4	176.3	-10	62.5	179.5	36
197 to 224	67.9	168.6	30	74.4	211.7	26
225 to end	61.8	121.1	8	10.7	77.3	6
Totals	561.7	1213.5	230	470.5	1171.8	243
Means	2.54	5.49	1.04	2.17	5.40	1.12

Table 6. Feed consumption and changes in body weight of animals on high-phosphorus rations

Days on experiment	Concentrate	Hay	Wt gain	Concentrate	Hay	Wt gain
	(lb)					
Treatment	Control					
Animal number	613			617		
29 to 56	47.5	38.2	18	a	a	a
57 to 84	111.2	108.1	57	a	a	a
85 to 112	106.9	176.7	71	79.2	37.8	19
113 to 140	100.0	207.2	52	59.5	60.1	18
141 to 168	101.5	221.9	53	57.2	49.9	- 5
169 to 196	121.5	231.6	33	56.0	25.5	-14
197 to 224	102.0	182.2	19	25.6	9.3	-21
225 to 252	84.0	249.6	43		died	
253 to end	10.5	35.6	27			
Totals	785.1	1451.1	373			
Means	3.66	6.78	1.74			
Treatment	Molybdenum					
Animal number	614			615		
29 to 56	43.3	19.1	14	10.6	5.2	39
57 to 84	108.1	100.0	60	85.8	48.3	59
85 to 112	100.1	165.2	60	96.6	127.1	46
113 to 140	89.3	173.2	40	75.8	133.2	23
141 to 168	83.9	193.5	50	79.0	183.5	49
169 to 196	100.6	213.5	8	78.2	202.0	25
197 to 224	101.3	161.0	22	99.9	180.4	31
225 to 252	95.2	217.2	40	95.1	217.3	22
253 to end	13.6	24.2	2	35.7	99.0	30
Totals	735.4	1267.2	296	656.7	1196.0	324
Means	3.49	5.92	1.38	2.97	5.41	1.47
Treatment	Molybdenum + Sulfate					
Animal number	612			616		
29 to 56	35.8	28.6	4	6.2	3.3	18
57 to 84	90.0	114.2	56	27.6	32.2	35
85 to 112	76.3	174.7	48	62.6	101.9	35
113 to 140	78.6	184.9	35	63.1	134.2	21
141 to 168	74.6	155.3	- 3	64.9	151.3	30
169 to 196	74.8	199.6	43	59.4	117.8	-10
197 to 224	62.5	216.4	18	33.9	91.1	-22
225 to 252	75.3	243.4	20	23.4	106.4	- 1
253 to end	1.8	32.5	14	9.4	27.4	6
Totals	569.7	1349.6	235	350.5	765.6	112
Means	2.66	6.30	1.09	1.59	3.46	0.56

[a] Animal 617 would not consume the experimental concentrate.

the blood studies were discontinued because of lack of time. Analysis of blood for zinc, copper and molybdenum was continued until the end of the experiment, however.

Blood inorganic phosphorus values are recorded in Table 7. The blood inorganic phosphorus levels were higher for the animals on the HP rations than for those on the LP rations and remained so throughout the experiment. The mean values for inorganic phosphorus were 8.4 to 10.4 mg/100 ml of blood plasma for the animals fed high phosphorus levels, and 7.6 to 8.4 mg/.00 ml for the animals fed low phosphorus levels.

It was expected that the LP rations would be slightly deficient in phosphorus. The blood inorganic phosphorus levels of the LP-fed animals indicated that the dietary phosphorus intake apparently was nearly adequate, however. In studies by Palmer et al. (116), blood inorganic phosphorus levels were usually about 8 mg/100 ml of plasma for calves and about 6 mg/100 ml of plasma for mature cattle. The blood inorganic phosphorus levels decreased in most of the animals toward the end of the experiment, indicating an inability of the animals to maintain a high blood inorganic phosphorus level as they became older. Blood inorganic phosphorus in the LP-fed animals appeared to decrease at a more rapid rate than that in the HP-fed animals. Animals 609 and 610 showed relatively low blood inorganic phosphorus levels during the last sampling period. The Mo-supplemented animals of all groups maintained higher blood inorganic

Table 7. Concentration of inorganic phosphorus in the blood

Low dietary phosphorus

Treatments	Control		Mo		Mo + SO_4	
Animal no.	610	618	611	619	609	620
Days on experiment	(mg/100 ml)					
0	8.0	9.8	10.8	9.6	9.1	9.4
14	9.9	8.0	10.5	9.6	9.5	8.6
28	10.2	8.2	9.2	9.8	8.6	9.8
42	7.7	10.2	8.2	11.2	6.9	9.8
56	10.2	8.7	9.2	10.4	9.2	8.2
70	9.6	8.0	9.8	8.0	8.0	6.8
84	8.6	7.1	10.8	6.9	8.6	6.4
98	5.8	5.9	7.3	6.5	5.8	6.5
112	9.1	6.9	8.8	6.1	6.5	6.4
126	8.0	6.3	7.2	7.2	6.7	6.8
140	7.5	5.1	6.0	6.4	6.5	6.8
154	6.5	7.9	7.5	5.9	8.3	6.7
168	4.3	6.4	6.1	6.9	4.9	7.2
Means	8.1	7.6	8.6	8.0	7.6	7.6

High dietary phosphorus

Treatments	Control		Mo		Mo + SO_4	
Animal no.	613	617	614	615	612	616
Days on experiment	(mg/100 ml)					
0	9.1	9.0	10.4	9.1	8.9	8.8
14	8.5	9.4	9.7	11.4	8.8	10.2
28	10.4	6.4	9.6	9.2	9.4	9.4
42	9.0	8.2	9.4	12.2	8.0	11.8
56	9.8	8.6	10.2	10.6	9.6	8.2
70	10.2	8.0	11.8	9.4	8.8	9.0
84	11.2	9.9	11.2	9.9	11.0	9.5
98	9.8	7.9	11.8	7.5	8.6	7.9
112	10.2	---	11.2	8.1	9.9	9.3
126	8.0	9.3	9.9	8.8	7.8	6.7
140	7.2	8.9	9.3	8.9	7.7	8.8
154	8.8	9.1	12.1	10.7	7.5	7.1
168	6.9	9.2	8.7	10.3	7.7	8.0
Means	8.7	8.8	10.4	9.7	9.2	8.7

phosphorus levels than did either the control or the Mo + SO_4-supplemented animals. The calculated percentage of phosphorus consumed by each animal during 28-day periods is recorded in Table 8. The HP rations were approximately three times as high in phosphorus as were the LP rations.

Blood alkaline phosphatase values were quite variable among animals as may be seen in Table 9. Only Animal 617 showed a consistently low level of blood alkaline phosphatase. This effect may have been the result of this animal's having been ill much of the time before its death. Comar (28) indicated that normal blood alkaline phosphatase values range from 6 to 9 Bodansky Units. The values in this experiment were generally lower than 6 Bodansky Units except for a few exceptionally high results early in the experiment. These high values were not consistent and did not appear to be related to treatments. There were no apparent treatment effects on blood alkaline phosphatase.

Plasma calcium and protein levels are listed in Tables 10 and 11 respectively. Neither plasma calcium nor protein levels showed any treatment effect and were apparently in the normal range for cattle.

Hemoglobin levels are recorded in Table 12. There appears to be no treatment effect on hemoglobin levels, but such levels were generally lower than those published by Dukes (47), who reported that average hemoglobin levels for cattle should be about 12 g per 100 ml of blood. The mean hemoglobin values ranged from 8.1 to 10.5 g per 100 ml of

Table 8. Calculated percentage of phosphorus in the total ration consumed

Treatments	Low dietary phosphorus					
	Control		Mo		Mo + SO_4	
Animal no.	610	618	611	619	609	620
Days on experiment	(%)					
57 to 84	0.017	0.018	0.016	0.017	0.016	0.016
85 to 112	0.020	0.019	0.019	0.014	0.018	0.017
113 to 140	0.021	0.020	0.018	0.016	0.020	0.017
141 to 168	0.021	0.020	0.017	0.016	0.020	0.019
169 to 196	0.023	0.021	0.018	0.015	0.020	0.018
197 to 224	0.021	0.025	0.014	0.018	0.018	0.022
225 to 252	0.022	0.027	0.018	0.017	0.016	0.017

Treatments	High dietary phosphorus					
	Control		Mo		Mo + SO_4	
Animal no.	613		614	615	612	616
Days on experiment	(%)					
57 to 84	0.064		0.062	0.047	0.054	0.016
85 to 112	0.067		0.063	0.059	0.052	0.042
113 to 140	0.066		0.058	0.048	0.054	0.042
141 to 168	0.068		0.057	0.054	0.049	0.044
169 to 196	0.079		0.067	0.055	0.053	0.039
197 to 224	0.065		0.067	0.064	0.048	0.024
225 to 252	0.061		0.065	0.065	0.060	0.020
253 to	---		---	0.065	---	0.017

Table 9. Concentration of alkaline phosphatase in blood plasma

Treatments	Low dietary phosphorus: Control		Mo		Mo + SO_4	
Animal no.	610	618	611	619	609	620
Days on experiment	(Bodansky units)[a]					
0	---	9.1	---	7.2	---	9.0
14	---	8.4	---	9.6	---	8.2
28	2.4	8.3	4.2	12.3	4.4	15.5
42	5.7	12.8	3.8	7.2	4.5	13.4
56	6.6	5.6	2.6	5.6	5.2	8.0
70	4.2	4.4	4.0	5.0	3.2	4.8
84	7.6	4.9	5.6	5.3	5.5	7.7
98	5.6	3.7	3.5	3.1	6.9	5.5
112	4.0	4.8	3.5	4.4	3.5	5.6
126	5.5	4.5	4.0	3.5	4.8	4.4
140	4.5	5.6	4.4	4.7	5.7	5.3
154	4.4	4.4	4.5	3.7	4.5	4.5
168	5.9	6.0	4.1	4.5	5.5	5.1
Means	5.1	6.3	4.0	5.9	4.9	7.5

Treatments	High dietary phosphorus: Control		Mo		Mo + SO_4	
Animal no.	613	617	614	615	612	616
Days on experiment	(Bodansky units)[a]					
0	---	3.2	---	4.3	---	8.6
14	---	3.0	---	9.0	---	11.5
28	9.2	4.2	11.4	9.2	6.4	10.9
42	6.9	2.2	2.8	9.9	9.0	11.2
56	6.7	2.6	5.6	6.2	4.0	6.7
70	5.0	2.2	4.8	6.6	4.2	4.6
84	5.8	2.1	5.8	5.1	5.5	6.6
98	7.1	2.1	5.6	2.6	6.4	3.9
112	4.5	---	4.5	2.7	4.5	4.3
126	6.1	1.3	5.1	3.7	5.8	5.2
140	6.4	1.6	5.6	3.3	5.3	4.3
154	5.3	1.2	3.4	2.7	4.3	4.9
168	5.6	1.7	6.6	4.0	4.1	4.5
Means	6.2	2.3	5.6	5.3	5.4	6.7

[a] One Bodansky unit corresponds to the liberation of one mg of inorganic phosphate per 100 ml of serum during a one-hour period of incubation.

Table 10. Concentration of calcium in blood plasma

Treatments	Low dietary phosphorus					
	Control		Mo		Mo + SO_4	
Animal no.	610	618	611	619	609	620
Days on experiment	(mg/100 ml)					
0	----	10.6	----	13.2	----	12.0
14	----	11.1	----	11.0	----	11.1
28	10.8	----	11.0	----	11.6	----
42	11.3	----	11.5	11.1	11.1	----
56	----	12.0	----	12.4	----	12.0
70	11.9	12.6	11.2	11.9	11.2	12.8
84	12.3	13.3	12.3	13.3	12.3	13.7
98	12.5	11.9	10.8	11.0	11.6	11.6
112	11.4	12.6	11.1	11.9	12.4	12.9
126	12.3	12.2	11.9	11.9	12.0	12.8
140	12.4	13.2	12.7	12.3	12.2	12.5
154	11.5	----	11.2	----	12.4	----

Treatments	High dietary phosphorus					
	Control		Mo		Mo + SO_4	
Animal no.	613	617	614	615	612	616
Days on experiment	(mg/100 ml)					
0	----	12.2	----	11.8	----	13.7
14	----	11.3	----	11.8	----	10.9
28	12.2	----	11.8	----	11.0	----
42	10.9	13.0	10.2	11.8	10.6	12.1
56	----	11.5	----	12.3	----	12.2
70	12.4	11.2	10.8	11.9	11.6	11.7
84	11.7	11.2	11.5	13.0	13.0	12.6
98	12.0	11.0	11.5	11.0	11.7	11.2
112	12.0	----	11.2	11.6	11.1	11.5
126	12.4	10.9	12.6	11.0	11.7	11.7
140	12.3	11.1	11.6	11.2	11.7	12.1
154	11.9	----	11.0	----	11.0	----

Table 11. Concentration of protein in blood plasma

	Low dietary phosphorus					
Treatments	Control		Mo		Mo + SO_4	
Animal no.	610	618	611	619	609	620
Days on experiment	(%)					
0	6.2	7.8	6.1	6.8	7.8	6.5
14	---	7.4	---	6.6	---	6.8
28	6.7	7.3	6.6	5.9	7.1	6.8
42	7.1	7.4	5.8	6.2	6.8	7.1
56	6.9	7.1	5.9	6.8	6.8	7.0
70	7.2	6.7	6.6	6.5	7.3	7.8
84	6.9	7.2	6.9	6.9	7.8	7.5
98	7.1	7.1	7.2	7.8	7.0	7.2
112	8.0	7.2	7.2	7.2	7.4	7.6
126	7.6	7.8	6.8	7.3	7.2	8.0
140	---	7.8	---	7.2	---	7.9
154	7.4	6.8	6.9	6.8	7.8	7.1
168	7.7	7.0	7.2	7.2	7.6	7.3
Means	7.2	7.3	6.7	6.9	7.3	7.3

	High dietary phosphorus					
Treatments	Control		Mo		Mo + SO_4	
Animal no.	613	617	614	615	612	616
Days on experiment	(%)					
0	---	6.2	---	6.8	---	7.0
14	8.2	6.8	7.8	7.1	6.8	7.6
28	7.1	6.4	6.9	6.8	6.7	6.9
42	7.3	7.1	7.0	7.2	6.7	7.6
56	6.8	6.8	6.9	6.7	6.7	7.1
70	6.6	6.4	6.9	6.8	7.2	6.9
84	6.8	6.7	6.6	7.2	6.8	7.3
98	7.2	6.8	7.0	7.4	7.3	7.7
112	7.4	---	7.6	7.8	7.1	7.4
126	7.0	6.8	6.9	7.8	7.0	7.6
140	---	6.8	---	7.8	---	7.3
154	7.4	7.6	7.1	7.4	7.8	7.7
168	7.8	6.8	7.3	7.6	7.6	7.1
Means	7.2	6.8	7.1	7.3	7.1	7.3

Table 12. Blood hemoglobin values

Treatments	Low dietary phosphorus						High dietary phosphorus					
	Control		Mo		Mo + SO_4		Control		Mo		Mo + SO_4	
Animal no.	610	618	611	619	619	620	613	617	614	615	612	616
Days on experiment	(mg/100 ml)											
0	--	9.2	---	6.5	---	10.1	---	12.4	---	10.0	---	9.0
14	--	8.1	---	6.8	---	8.8	10.3	---	10.6	---	12.1	---
28	10.7	9.3	11.5	6.8	11.5	9.3	10.2	8.7	10.1	8.0	9.7	7.3
42	10.0	9.6	10.0	7.5	10.5	9.3	9.6	9.1	10.3	8.5	10.7	8.0
56	9.6	8.5	9.7	8.0	10.2	9.3	---	9.0	---	9.1	---	8.7
70	9.8	8.6	10.2	8.8	9.1	8.6	10.0	9.2	8.2	10.3	10.2	9.5
84	9.1	8.8	9.2	9.7	9.5	8.3	9.6	9.1	8.8	10.6	10.0	9.3
98	9.5	8.7	10.8	7.8	10.0	7.5	10.0	8.2	8.7	8.1	9.4	9.3
112	9.4	8.8	---	8.0	10.0	8.7	11.3	8.7	9.4	9.8	10.9	9.1
124	9.1	9.1	12.7	9.1	9.9	9.3	10.1	---	9.3	9.3	10.1	8.2
140	9.4	8.7	9.1	9.2	10.1	9.8	11.5	8.2	10.0	9.3	9.8	8.7
154	10.9	---	10.1	---	11.6	---	10.8	---	8.6	---	10.1	---
168	10.1	9.3	9.8	8.2	10.5	8.4	---	7.8	---	9.2	---	9.3
182	10.6	9.1	9.1	8.1	11.2	8.4	10.9	a	8.7	---	10.1	---
196	10.2	8.5	8.8	8.3	10.9	8.7	---		---	9.2	---	8.6
210	10.2	---	9.8	---	10.2	---	---		---	8.8	---	7.7
224	9.5	8.7	10.3	8.2	10.1	8.7	10.8		8.1	---	8.8	---
238	10.5	9.6	10.3	9.2	10.9	9.6	---		---	9.3	---	9.1
252	9.2	---	10.1	---	10.1	---	9.6		8.0	---	10.2	---
Means	9.8	8.9	10.1	8.1	10.5	8.9	10.3	9.0	9.1	9.3	10.2	8.7

[a] Died

blood and these values were within the range reported in mature cows by Vanderveen (132) at this station.

Blood copper values are recorded in Tables 13 and 14. Throughout this experiment there was a large variation in blood copper values, most of the high ones being found when the calves were young. Whole blood copper values ranged from 68 to 162 µg per 100 ml of blood with the majority of values ranging from 75 to 120 µg. These values compare favorably with those reported for cattle by Underwood (131). The normal range given by this worker was 0.5 to 1.5 mg of copper per liter of blood with most of the values being between 0.8 to 1.2 mg. It was shown by Eden (48) that copper concentrations of whole blood and plasma may be expected to be approximately the same.

Red blood cell copper levels appeared to be quite low toward the end of the experiment in the animals supplemented with Mo + SO_4, even though plasma copper was high. This indicates that there may have been an interference with the transport of copper from the blood plasma to the red blood cells. This finding is in contrast with that in control animals, which maintained high red blood cell copper levels even though the plasma copper levels were very low at the end of the experiment.

Whole blood and plasma copper levels were higher in the animals supplemented with Mo + SO_4 than in the animals supplemented with Mo or in the controls, thus indicating a treatment effect. Plasma copper values in the animals

Table 13. Concentration of copper in the blood of animals on low phosphorus rations

Days on experiment	Whole blood	Blood plasma	Blood cells	Whole blood	Blood plasma	Blood cells
	μg/100 ml					
	Control					
Animal no.	610			618		
0	109.4	88.3	108.0	87.4	41.0	186.0
14	76.7	64.2	100.0	---	---	---
28	85.6	82.1	93.0	87.5	78.5	106.0
56	73.4	62.7	97.0	79.7	63.5	75.0
84	75.2	67.1	92.0	69.8	69.8	70.0
112	90.2	91.4	87.1	71.1	54.8	111.2
140	83.9	76.8	98.5	114.3	68.3	220.5
168	74.1	69.2	85.0	70.5	55.3	106.4
196	70.2	69.1	72.4	71.4	58.9	103.2
224	76.8	66.1	100.0	68.7	50.0	113.6
252	75.0	69.6	95.7			
Means	81.0	73.3	93.6	80.0	55.0	121.3
	Molybdenum					
Animal no.	611			619		
0	88.4	74.0	106.0	69.6	58.9	85.1
14	78.5	62.4	102.0	---	---	---
28	75.0	91.0	45.0	82.1	89.3	65.0
56	93.1	106.5	63.0	83.2	83.2	83.2
84	87.7	89.5	88.0	66.5	61.5	76.8
112	80.9	84.3	75.1	78.1	72.3	119.5
140	86.6	85.7	88.4	67.9	72.3	58.0
168	77.7	70.9	100.0	67.8	62.5	81.5
196	85.7	101.8	51.4	71.4	67.7	105.9
224	78.6	75.5	80.8	69.6	67.8	74.0
252	82.1	84.5	76.4			
Means	84.5	84.1	79.6	72.9	63.5	83.1
	Molybdenum + Sulfate					
Animal no.	609			620		
0	162.5	76.6	250.0	82.1	58.9	123.0
14	132.0	103.5	131.9	---	---	---
28	123.1	100.0	163.0	82.2	76.2	95.0
56	110.0	103.8	127.0	125.3	148.6	76.0
84	136.0	127.1	155.0	93.0	105.7	62.8
112	89.7	89.7	89.9	94.2	108.9	60.6
140	103.6	114.3	80.0	104.5	128.6	54.6
168	104.5	115.6	81.5	88.0	100.9	53.3
196	101.8	116.5	69.6	80.4	96.4	73.2
224	104.5	131.2	46.8	84.8	96.4	58.0
Means	115.6	107.9	114.2	92.7	102.3	72.9

Table 14. Concentration of copper in the blood of animals on high phosphorus rations

Days on experiment	Whole blood	Blood plasma	Blood cells	Whole blood	Blood plasma	Blood cells
	μg/100 ml					
	Control					
Animal no.	613			617		
0	72.0	74.0	70.0	108.8	94.6	127.0
28	a	a	a	105.3	107.1	102.0
56	80.3	71.4	99.0	103.8	116.4	75.6
84	82.0	74.3	100.0	84.3	78.9	97.0
112	69.0	63.5	81.2	93.1	98.1	81.0
140	73.1	66.5	73.1	107.2	109.8	102.3
168	83.0	70.1	111.1	110.7	104.5	129.2
196	73.2	68.7	84.7			
224	75.0	71.4	82.2		died	
252	71.4	63.4	90.7			
Means	75.4	69.3	88.0	101.9	101.3	102.0
	Molybdenum					
Animal no.	614			615		
0	97.7	65.1	133.1	121.3	94.6	167.0
28	a	a	a	90.7	81.2	113.0
56	107.0	105.3	111.0	96.7	74.3	143.0
84	80.6	80.6	80.6	81.4	70.6	107.1
112	74.8	76.0	73.2	86.4	88.9	80.7
140	74.8	77.7	67.8	98.2	92.0	112.1
168	107.1	88.4	162.5	86.6	75.0	115.8
196	74.1	82.1	56.3	99.0	92.9	113.5
224	79.5	90.2	51.4	90.2	69.2	140.2
252	76.8	71.4	92.8	87.1	89.3	81.8
Means	85.8	81.9	92.1	93.8	82.8	117.4
	Molybdenum + Sulfate					
Animal no.	612			616		
0	104.1	78.1	141.0	108.8	98.1	133.0
28	---	---	---	103.5	94.6	103.0
56	110.6	107.1	118.0	102.3	86.0	102.3
84	109.2	118.1	89.8	98.0	100.6	92.8
112	103.0	118.8	69.6	95.6	107.2	71.6
140	104.0	120.0	69.3	95.2	114.3	84.9
168	111.8	128.5	74.4	89.3	113.4	32.1
196	96.4	106.7	76.7	67.1	89.3	40.7
224	83.0	87.5	71.6	67.0	73.8	47.1
252	80.4	92.0	53.2	51.8	48.2	61.5
Means	100.3	106.3	84.8	87.9	92.5	76.9

[a] blood analysis not completed.

supplemented with Mo + SO_4 on either high or low levels of phosphorus averaged about 75% higher than those of the molybdenum-supplemented animals and about 65% higher than those of the controls. The whole blood copper values averaged nearly 60% higher in the Mo + SO_4-supplemented animals than in the Mo-supplemented animals and 30% higher than the controls. Dick (39, 40, 42, 44) showed that Mo + SO_4-supplementation increased both blood and plasma levels in sheep although this effect was not demonstrated by Vanderveen et al. (133) in mature milking cows at this station.

One Mo + SO_4-supplemented animal, 616, showed a rapid decrease in whole blood and plasma copper during the last three months of the experiment, which was indicative of copper deficiency. This probably was the result of the HP, Mo + SO_4 treatment coupled with a sharp reduction in feed consumption. Copper levels in both hay and concentrates were between 3 and 4 ppm. With such low levels of copper in the feed, any large decrease in feed consumption probably would reduce dietary copper intake to a dangerously low level. Blood and plasma copper values in the controls were quite low toward the end of the experiment, indicating that dietary copper may not have been sufficient for the needs of these animals as they became more mature.

Blood zinc values recorded in Tables 15 and 16 show a treatment effect. In all groups except Group III, the levels of whole blood and red blood cell zinc were much

Table 15. Concentration of zinc in the blood of animals on low phosphorus rations

Days on experiment	Whole blood	Blood plasma	Blood cells	Whole blood	Blood plasma	Blood cells
	μg/100 ml					
	Control					
Animal no.	610			618		
0	500	210	1016	260	80	644
14	300	150	596	---	---	---
28	260	85	610	303	104	728
56	261	125	554	317	124	777
84	301	135	680	259	90	666
112	250	92	643	261	96	655
140	292	134	617	258	97	630
168	283	116	650	247	110	570
196	297	115	662	216	88	540
224	192	115	359	275	87	726
252	269	89	690			
Means	291	124	643	266	97	660
	Molybdenum					
Animal no.	611			619		
0	345	180	546	240	140	668
14	300	145	427	---	---	---
28	240	100	524	283	114	694
56	205	69	482	342	140	816
84	283	133	613	284	105	651
112	229	95	463	250	97	642
140	272	132	547	235	85	577
168	208	107	483	247	119	574
196	225	92	509	216	142	469
224	217	92	479	275	116	674
252	209	80	516			
Means	248	111	508	264	118	641
	Molybdenum + Sulfate					
Animal no.	609			620		
0	450	185	773	370	95	858
14	440	130	947	---	---	---
28	375	120	814	400	120	1036
56	303	102	831	422	145	1032
84	401	133	997	334	90	916
112	334	78	942	367	98	980
140	408	117	999	372	118	881
168	372	116	906	386	138	1049
196	357	134	840	365	106	1093
224	365	126	882	389	111	1061
252	355	92	1002			
Means	379	121	903	377	113	990

Table 16. Concentration of zinc in the blood of animals on high phosphorus rations

Days on experiment	Whole blood	Blood plasma	Blood cells	Whole blood	Blood plasma	Blood cells
	μg/100 ml					
	Control					
Animal no.	613			617		
0	345	175	544	320	100	589
28	a	a	a	253	106	566
56	283	132	601	232	90	558
84	234	124	485	225	50	633
112	234	110	511	242	75	641
140	242	113	516	207	58	628
168	250	125	518	222	53	729
196	289	142	665			
224	254	124	510		died	
252	214	104	608			
Means	261	127	551	243	76	621
	Molybdenum					
Animal no.	614			615		
0	330	180	522	360	130	485
28	a	a	a	325	104	855
56	330	83	826	409	143	959
84	242	130	538	334	90	916
112	217	110	496	312	75	784
140	205	108	424	333	121	811
168	233	121	564	320	93	892
196	235	138	452	345	113	897
224	200	91	514	350	93	961
252	195	91	505	300	104	775
Means	243	117	537	339	107	834
	Molybdenum + Sulfate					
Animal no.	612			616		
0	365	135	694	310	125	771
28	a	a	a	297	125	724
56	408	126	972	329	137	753
84	401	103	1047	267	92	663
112	400	95	948	292	130	637
140	359	115	1016	262	128	580
168	408	98	1101	272	121	631
196	450	137	1047	282	138	716
224	387	117	1074	205	109	485
252	327	115	824	262	108	668
Means	389	116	969	278	121	663

[a] Blood analysis not completed.

higher in the Mo + SO_4-supplemented animals than in the Mo-supplemented and control animals, regardless of dietary phosphorus levels. This difference was sometimes as much as 60% higher (average, 40%) than in the Mo-supplemented and control animals. There was no substantial increase of plasma zinc in the Mo + SO_4-supplemented animals.

In Group III, the reverse was true with the Mo-supplemented animal having a higher blood zinc value than the Mo + SO_4-supplemented animal. No explanation is apparent for this reversal. In some preliminary work, two Mo + SO_4-supplemented animals showed the same increase in blood zinc levels. The blood zinc values ranged from 192 to 500 μg/100 ml of blood with the majority of values being between 250 and 375 μg. In only the Mo + SO_4-supplemented animals and one Mo-supplemented animal (616) were blood zinc values consistently higher than 300 μg/100 ml of blood. There is not much information in the literature on the normal values for blood zinc in cattle. Gessert et al. (63) reported that whole blood zinc levels in mature cows range from 237 to 375 μg zinc/100 ml of blood. The blood zinc levels in the animals of this experiment ranged from 208 to 500 μg/ml of blood. Some of the young calves showed very high blood zinc values, thus suggesting that calves may have higher levels than mature animals. The blood zinc values for the older age periods were usually within the range reported by Gessert et al. (63) for mature cows.

Tissue zinc concentrations are recorded in Table 17. There was no apparent treatment effect on tissue zinc concentrations. These values varied depending upon the type of tissue assayed but were generally similar for the same type of tissue from different animals. It is interesting to note the relatively large differences in zinc content between shoulder, thigh and loin muscle. The mean zinc content of the dried thigh, loin and shoulder muscle of all animals was 80, 86, and 159 ppm, respectively. There have been very few data reported in the literature on zinc concentrations in different tissues in cattle. Liver zinc concentrations in this experiment averaged 112 ppm for all the animals with a range of 81 to 152 ppm zinc on the dry tissue basis. This compares favorably with Gessert's report (63) on liver zinc concentrations for thirty cows. The mean reported by Gessert was 125 ppm with a range of 106 to 170.

The concentration of molybdenum in samples of blood drawn during the time each animal received its respective experimental concentrate mixture is recorded in Table 18. The Mo-supplemented animals showed high levels of blood molybdenum, these levels being several times higher than those of the Mo + SO_4-supplemented or control animals. In this study, the range of blood molybdenum concentrations for animals receiving about 60 ppm molybdenum or more in their rations was 4.3 to 16.5 µg/ml, with a mean of 9.0 µg/ml. The levels of molybdenum in the blood of the

Table 17. Concentration of zinc in various selected dried tissues

Treatments	Low dietary phosphorus						High dietary phosphorus				
	Control		Mo		Mo + SO_4		Control	Mo		Mo + SO_4	
Animal no.	610	618	611	619	619	620	613	614	615	612	616
Tissues	(ppm)										
Thigh	106.3	73.1	78.7	80.2	74.1	68.6	75.1	72.9	76.6	91.9	81.9
Shoulder	240.3	171.6	166.2	187.6	127.9	167.1	120.4	120.0	133.2	198.8	118.7
Loin	97.8	82.6	84.8	93.2	76.8	73.7	75.3	71.0	91.0	80.7	102.7
Spleen	86.5	85.7	105.7	95.2	96.2	100.3	95.7	95.0	97.7	80.1	93.0
Pancreas	115.3	95.8	139.1	115.3	177.6	160.2	72.1	107.4	157.4	120.2	217.5
Kidney	76.1	75.6	70.0	72.1	70.7	75.7	69.5	70.0	71.2	69.5	88.0
Heart	60.8	71.8	64.2	62.8	76.4	64.0	75.1	72.3	77.0	63.5	72.7
Lung	69.2	80.2	53.2	87.9	70.2	82.0	71.5	80.2	70.3	68.4	72.9
Liver	141.9	126.0	114.1	110.6	109.1	151.7	112.1	99.8	116.3	150.0	81.0
Reticulum	120.8	117.4	121.0	116.9	112.2	125.8	115.2	129.0	115.3	109.9	126.2
Rumen	106.6	113.5	116.8	98.5	127.7	104.1	102.6	107.4	115.1	102.1	129.6
Abomasum	100.2	80.1	91.2	85.2	61.3	75.5	38,2	100.3	130.6	71.5	75.3
Duodenum	67.6	72.8	86.6	73.6	62.8	70.4	57.4	64.6	75.0	60.5	82.2
Jejunum	65.7	71.3	87.6	69.8	79.0	84.0	70.1	75.3	77.9	65.4	76.5
Ileum	65.1	67.5	74.9	85.1	69.3	79.1	70.2	71.0	77.9	73.7	71.2
Colon	91.6	84.8	122.7	50.0	69.5	86.5	53.8	59.9	66.4	67.8	81.8
Cecum	101.4	100.7	91.2	80.5	98.2	98.8	96.4	84.9	101.1	102.1	111.6
Omasum	161.5	147.6	90.8[a]	158.2	140.3	156.3	152.4	145.7	158.3	157.9	150.2
Brain	47.3	49.1	39.6	35.3	44.5	48.7	55.1	37.5	52.5	41.5	43.6
Fat	2.3	4.7	4.9	4.1	13.3	9.9	3.3	2.1	9.6	9.6	---

[a] Sample contained a large proportion of fatty tissue and was not considered representative.

Table 18. Molybdenum content of blood

Days on experiment	Low dietary phosphorus				High dietary phosphorus			
	Whole blood	Blood plasma	Whole blood	Blood plasma	Whole blood	Blood plasma	Whole blood	Blood plasma
	(μg/ml)							
Treatment	Control							
Animal no.	610		618		613		617	
84	0.07	0.15	0.04	0.06	0.30	0.04	0.10	0.02
112	0.15	0.15	0.08	0.13	0.20	0.14	0.25	0.05
140	0.03	0.20	0.08	0.04	0.10	0.07	0.17	0.08
168	0.07	0.20	0.20	0.08	0.10	0.07		
196	0.20	0.40	0.20	0.20	0.30	0.04	died	
224	0.30	0.60	0.30	0.08	0.35	0.04		
252	0.30	0.60	0.25	0.23	0.10	0.14		
Treatment	Molybdenum							
Animal no.	611		619		614		615	
84	1.40	1.50	1.60	1.80	2.10	2.40	3.30	3.60
112	4.30	6.40	13.70	16.50	5.20	5.80	6.60	7.24
140	8.90	10.30	12.40	14.00	10.20	11.40	5.50	6.70
168	8.90	10.10	12.50	15.60	4.20	4.60	5.20	6.30
196	10.80	11.70	15.20	16.20	10.00	11.80	5.00	7.00
224	10.40	10.60	16.50	20.80	7.10	9.80	6.00	7.40
252	10.20	12.60	12.60	16.00	7.60	8.60	12.40	12.40
Treatment	Molybdenum + Sulfate							
Animal no.	609		620		612		616	
84	6.00	6.50	1.15	1.35	1.05	1.17	0.95	1.05
112	0.85	1.15	1.40	1.65	1.10	1.20	1.30	1.60
140	1.40	1.00	1.85	2.15	1.25	1.46	1.45	1.75
168	1.60	2.00	1.45	1.75	1.00	1.20	1.05	1.27
196	1.30	1.70	1.65	2.10	1.20	1.20	0.53	0.80
224	1.20	1.30	1.25	1.30	1.10	1.37	0.70	0.75
252	1.35	1.40	1.20	1.40	1.60	1.46	0.60	0.50

Mo + SO_4-supplemented animals ranged from 0.50 to 6.0 ppm with a mean of 1.35 µg/ml. The depressing effect of sulfate on the concentration of blood molybdenum has been reported by several other investigators (35, 45, 105, 133). It was shown by Dick (45) that sulfate not only prevented the absorption of molybdenum from the gut but also increased the excretion of molybdenum in the urine, thereby reducing both blood and tissue molybdenum concentrations. Molybdenum levels in the blood of both the high and low phosphorus, Mo-supplemented animals were higher than those reported by several investigators, (33, 45, 132). Vanderveen (132) reported molybdenum levels of 1.5 to 5.6 µg/ml of blood in mature cows receiving 50 ppm molybdenum in their ration. This was much lower than the blood values of the animals in this experiment which were being fed about 60 ppm molybdenum.

Molybdenum levels in the blood of the control animals ranged from 0.07 to 0.35 µg/ml with a mean of 0.17 µg/ml. The molybdenum content of the blood in the control animals increased, and the blood copper levels decreased as the animals matured. The decrease in blood copper as the animals matured may have influenced the molybdenum content of the blood. The molybdenum content of the feed of the controls was less than one ppm. These blood molybdenum levels were higher than those reported by other investigators (32, 45, 132). Cunningham (33) showed mean molybdenum levels in two groups of cattle of 0.13 to 0.16 µg/ml of

blood. Vanderveen (132) at this station reported blood molybdenum levels of 0.05 ug/ml or less in mature cows not receiving any supplemental molybdenum.

Tissue molybdenum values are recorded in Table 19. These studies show the effect of dietary sulfate on the deposition of molybdenum in the tissue, presumably because of the effect of sulfate on the absorption and excretion of molybdenum. There apparently was no difference in effect between HP and LP treatment. The addition of 0.3% sulfur as a sulfate in the ration reduced tissue molybdenum levels so that some of the tissue molybdenum concentrations approached those recorded for the control animals. Some of the tissues of the Mo + SO_4-supplemented animals did not show this decrease, particularly certain tissues of the digestive tract. Tissues from the kidney, rumen, reticulum and omasum showed high molybdenum concentrations compared with other tissues despite the addition of sulfate to the ration. This high molybdenum concentration of the tissues of the rumen, reticulum and omasum could be the result of the high molybdenum content of the ingesta or possibly a blocking of the transfer of the molybdenum across the epithelium of the forestomach by the sulfate, thereby trapping molybdenum in the tissue. The tissues of the small and large intestines do not show such high molybdenum concentrations. It is possible that much of the molybdenum may be absorbed by the rumen, reticulum, and omasum. If such were true and these organs were

Table 19. Concentration of molybdenum in various selected dried tissues

Treatments	Low dietary phosphorus						High dietary phosphorus				
	Control		Mo		Mo + SO_4		Control	Mo		Mo + SO_4	
Animal no.	610	618	611	619	609	620	613	614	615	612	616
Tissues	(ppm)										
Thigh	0.36	0.56	4.91	7.69	0.68	0.05	1.18	4.55	4.57	0.98	0.64
Shoulder	0.00	0.00	7.05	1.12	0.75	1.57	2.55	5.65	7.50	0.70	0.92
Loin	0.00	0.17	3.64	3.47	0.10	1.32	0.27	3.55	4.62	0.17	0.30
Spleen	2.25	2.69	26.49	27.10	4.28	6.54	1.25	29.10	27.30	5.63	5.48
Pancreas	2.29	1.06	18.00	26.00	2.44	3.08	0.33	17.60	21.10	1.80	1.67
Kidney	12.40	4.64	63.90	19.30	28.60	24.00	3.20	63.50	73.50	26.30	13.10
Heart	1.24	0.70	15.90	20.00	1.93	3.15	0.00	12.20	20.12	1.51	1.50
Lung	2.10	2.00	66.60	41.40	3.20	5.94	1.00	30.00	38.00	2.83	3.76
Liver	7.06	5.00	31.00	36.00	14.70	17.40	5.20	39.20	36.70	13.60	15.00
Reticulum	2.11	0.80	39.10	33.40	31.20	22.60	1.70	48.40	49.80	36.30	10.02
Rumen	1.20	1.58	41.90	32.10	36.30	13.80	0.15	36.20	31.30	10.00	11.20
Abomasum	1.93	0.77	27.70	33.50	3.20	5.34	0.35	27.10	21.10	3.19	2.20
Duodenum	1.80	1.41	32.70	35.50	5.80	5.45	1.30	35.00	40.30	2.63	3.75
Jejunum	2.06	0.68	40.20	34.50	7.70	5.48	0.52	29.50	38.70	4.28	6.82
Ileum	2.70	1.79	45.90	45.30	8.46	6.42	1.48	34.70	48.70	3.21	3.96
Colon	2.10	0.78	28.00	61.50	5.08	5.19	1.71	16.40	31.90	3.82	4.06
Cecum	2.76	0.81	29.20	38.50	7.70	6.25	2.65	49.10	43.20	5.16	3.30
Omasum	2.81	1.22	19.00[a]	70.90	39.10	45.20	2.65	41.60	33.20	45.30	20.70
Brain	0.91	0.29	3.46	5.43	1.01	0.28	0.87	1.43	5.30	0.75	0.29

[a] Sample contained a large proportion of fatty tissue and was not considered representative.

responsible for absorption of a large proportion of the dietary molybdenum, then one might explain why ruminants are much more susceptible to molybdenum toxicity than are monogastric animals. The tissues of the small intestine contained a substantial amount of molybdenum as compared with most of the other tissues investigated, but these levels were not nearly as high as those recorded for the rumen, reticulum, and omasum. Muscle, pancreas, heart, lung, abomasum and brain tissues from the Mo + SO_4-supplemented animals did not show any substantial increase in molybdenum when compared with those of the controls. Tissue molybdenum in the control animals usually ranged from 0 to 3 ppm with the liver and kidney tissues being somewhat higher.

On a dry matter basis, liver tissue molybdenum in animals that received no molybdenum supplements in their feed was similar to that reported by Cunningham et al. (33) and Vanderveen et al. (133) in cattle. Liver molybdenum concentrations were 4.9, 5.7 and 7.1 ppm for the three control animals. When Mo and Mo + SO_4 supplements were consumed by the animals, the liver molybdenum concentrations increased. Liver molybdenum levels in the Mo + SO_4-supplemented animals on both high and low phosphorus rations ranged from 13.6 to 17.4 ppm and in the Mo-supplemented animals from 31.0 to 39.2 ppm on a dried weight basis. These values are comparable to those found in mature cows at this station by Vanderveen et al. (133).

Tissue copper concentrations are presented in Table 20. These values show large variations, but at least part of these variations appear to be due to animal rather than treatment effects. Tissues such as liver, kidney, rumen, reticulum and omasum apparently show some treatment effect. The liver, which is generally considered the most sensitive tissue for assessing the copper status of the animals, showed the greatest variability in copper content. The values recorded for tissue copper concentrations, especially for the control animals, appear to be in agreement with the tissue copper concentrations reviewed and tabulated by Rusoff (119).

Molybdenum supplementation did not appear to affect the liver copper concentrations in the LP animals. The two animals on this treatment (612 and 619) had liver copper concentrations of 89.0 and 132.8 ppm, which was in the same general range as the controls (610 and 618) with liver copper concentrations of 113.8 and 109.1 ppm. Molybdenum appeared to lower liver copper concentrations when it was fed with rations high in phosphorus. Animals 614 and 615, which were on the HP, Mo-supplemented ration, showed a devrease in liver copper concentrations, the decrease being very pronounced in Animal 614. This effect of molybdenum supplementation was not found in the corresponding animals on the LP ration.

The Mo + SO_4-supplemented animals on both LP and HP rations showed a decrease in liver tissue copper as

Table 20. Concentration of copper in various selected dried tissues

Treatments	Low dietary phosphorus						High dietary phosphorus				
	Control		Mo		Mo + SO_4		Control	Mo		Mo + SO_4	
Animal no.	610	618	611	619	609	620	613	614	615	612	616
Tissues	(ppm)										
Thigh	3.4	1.5	2.5	3.6	1.8	1.8	2.0	1.7	3.1	2.3	2.2
Shoulder	4.0	2.7	3.1	2.6	2.6	2.0	2.4	1.8	4.0	1.9	2.1
Loin	3.5	2.3	3.3	3.3	2.7	1.7	2.7	1.9	3.5	2.2	2.5
Spleen	8.1	4.0	5.4	4.7	8.0	4.5	3.4	3.7	3.9	4.4	4.6
Pancreas	4.6	4.4	5.6	5.4	7.4	4.3	4.1	4.2	6.2	3.7	3.6
Kidney	12.1	11.5	17.1	11.5	32.5	18.7	11.6	16.8	13.1	24.7	17.6
Heart	14.8	15.6	14.8	15.5	17.2	15.6	13.1	14.7	16.6	12.1	13.7
Lung	6.3	5.7	6.8	6.9	9.4	6.1	6.0	6.2	7.2	5.3	8.1
Liver	113.8	109.1	89.0	132.8	20.7	30.9	87.9	10.2	63.2	15.8	18.8
Reticulum	5.1	5.1	4.3	4.1	10.3	10.5	4.9	8.7	5.1	11.7	6.8
Rumen	6.2	3.8	8.6	4.2	12.9	10.1	3.3	9.2	4.2	7.4	4.2
Abomasum	8.1	9.8	7.6	11.3	4.8	12.8	1.0	7.3	6.6	2.9	9.5
Duodenum	6.7	7.1	6.9	5.0	6.9	5.2	4.8	3.9	6.8	4.6	5.2
Jejunum	4.5	4.3	5.4	3.6	5.2	5.0	3.6	3.8	5.0	3.3	3.2
Ileum	3.8	3.6	3.2	5.3	5.0	3.7	3.7	3.5	3.9	3.7	3.2
Colon	5.5	3.3	7.3	2.8	4.7	3.4	2.6	3.2	3.5	2.8	4.0
Cecum	6.3	4.1	5.8	5.0	5.8	4.0	3.3	4.6	4.6	2.9	3.7
Omasum	10.2	6.8	1.9[a]	6.2	10.0	11.3	6.7	9.3	6.6	8.2	6.5
Brain	13.5	6.3	6.5	5.8	15.1	7.0	10.5	5.3	8.2	4.4	5.6
Fat	1.9	0.9	0.9	1.3	1.3	1.8	0.6	0.5	0.6	1.8	---

[a] Sample contained a large proportion of fatty tissue and was not considered representative.

as compared with their controls. The LP, Mo + SO_4-supplemented animals did not show as great a decrease in liver copper concentration as was found in the HP, Mo + SO_4-supplemented animals. The LP, Mo + SO_4 supplemented animals had liver copper concentrations of 20.7 and 30.9 ppm on a dry weight basis as compared with 113.8 and 109.1 ppm for their corresponding controls, and the HP, Mo + SO_4-supplemented animals had liver copper concentrations of 15.8 and 18.8 ppm on a dry weight basis as compared with 87.9 ppm for their corresponding control.

In this study, molybdenum appeared to exert little effect in reducing liver copper concentrations in young bulls on the LP ration but apparently did have an effect when molybdenum was added to the HP ration. The physical condition and the reduced liver copper concentration of the HP, Mo + SO_4-supplemented animals as compared with the LP, Mo + SO_4-supplemented animals indicates that phosphorus, as well as sulfate, may affect copper metabolism.

It is well established that sulfate has the ability to reduce tissue molybdenum and to decrease the absorption of molybdenum from the digestive tract. Dick (42) was the first to show that a sulfate supplement not only reduced the absorption and deposition of molybdenum but also reduced the liver copper concentration in sheep. He also demonstrated that molybdenum did not reduce liver copper concentrations in sheep when there was a low concentration of sulfate present in the ration. Cunningham et al (34)

and Wynne et al. (141, 142) also made the same conclusion in their studies in which it was shown that the sulfate content of the ration had to exceed 0.2% before molybdenum could depress liver copper concentrations. Cunningham et al. (35) showed that cattle were more sensitive than sheep to dietary molybdenum and that 0.1% sulfate in the ration was sufficient to allow molybdenum to decrease liver copper concentrations in cattle. Vanderveen et al. (133) showed that molybdenum, in a ration low in total sulfur, could reduce the concentration of copper in the liver of lactating cows. In this study, molybdenum exerted little effect in reducing liver copper concentration in young bulls on the LP ration but did have an effect when molybdenum was supplemented in the HP ration.

The copper content of the kidney was highest in the Mo + SO_4-supplemented animals and second highest in the Mo-supplemented animals. Tissues from the rumen, reticulum and omasum of the Mo + SO_4-supplemented animals generally contained higher concentrations of copper than did the corresponding tissues of the Mo-supplemented or control animals. An exception was the HP, Mo + SO_4 animal (614) which also had high copper levels in these tissues. These specific tissues are the same ones that were high in molybdenum in the Mo + SO_4-supplemented animals even though sulfate supplementation reduced the molybdenum concentrations markedly in most of the other tissues. These same animals exhibited reduced liver copper concentrations and

were not able to utilize copper as readily as did the other animals. This suggests a possible blockage of the transport system thus causing high concentrations of copper as well as of molybdenum to be deposited in these tissues.

Studies on the absorption and excretion of copper using Cu^{64} were not conclusive, but some treatment effects were evident. The amount of Cu^{64} injected into each animal and the amount recovered in the feces and urine are recorded in Table 22. The phosphorus in the HP rations apparently decreased the fecal excretion of injected Cu^{64}. The HP-supplemented animals excreted 0.64 to 1.87% of the injected Cu^{64} in the feces while the LP-supplemented animals excreted from 2.27 to 4.68% of the injected copper by the same route.

Urinary excretion of Cu^{64} was very low in all animals except one which excreted over 7% of the dose in the urine. This animal (618) excreted over 90% of the recovered urinary Cu^{64} during a 10-hour period on the second day after injection. With the exception of this one period the excretion of Cu^{64} by this animal was comparable to that of the other animals. The Lp control animals excreted more Cu^{64} than did the Mo- and Mo + SO_4-supplemented animals, but such was not generally the case in the HP animals. The excretion of Cu^{64} in the urine was less than one per cent of the injected dose, with most of the animals excreting less than 0.5%. Comar (27) indicated that three per cent of an injected dose of copper was excreted in the urine and three per cent in the feces in

normal animals. In these studies the excretion of Cu^{64} in the urine of cattle was less than one per cent of the injected dose in most cases, although it is possible for an animal to excrete a much larger percentage of the injected dose as was demonstrated by Animal 618.

When Cu^{64} was administered orally to the animals, there appeared to be no effect of treatment on the excretion of Cu^{64} in the feces. In Table 21, the amount of Cu^{64} fed and percentage recovered in the excreta are recorded. In five days, about 90% of the Cu^{64} was recovered in the feces. The amount of Cu^{64} excreted in the urine was very small, however these figures were not considered reliable because of the possibility that the urine was contaminated by the highly radioactive feces. Orally-administered copper was compared with intravenously injected copper with respect to the resulting blood Cu^{64} activity and tissue deposition of Cu^{64}. There was some evidence indicating that Mo- and Mo + SO_4-supplementation might reduce the excretion of Cu^{64}, but this was not borne out in all groups. The excretion of Cu^{64} in the urine was quite variable and difficult to interpret.

The levels of Cu^{64} found in the blood when Cu^{64} was either injected or given orally to the animals are shown graphically in Figures 1 through 8. When Cu^{64} was injected, the Mo + SO_4-supplemented animals generally retained the highest levels of Cu^{64} in the blood, the

Table 21. Proportion of orally administered Cu^{64} recovered in excreta

Animal number	Treatment	Amount of Cu^{64} fed	Percentage of fed Cu^{64} recovered in excreta		
			Feces	Urine	Total excreta
		(cpm)		(%)	
610	LP, Control	210 x 10^7	84.29	0.14	84.43
611	LP, Mo	210 x 10^7	86.67	0.14	86.81
609	LP, Mo + SO_4	210 x 10^7	88.57	0.03	88.60
618	LP, Control	203 x 10^7	94.58	0.12	94.70
619	LP, Mo	203 x 10^7	85.22	1.47	86.69
620	LP, Mo + SO_4	203 x 10^7	92.60	1.24	93.84
613	HP, Control	169 x 10^7	90.53	0.01	90.54
614	HP, Mo	169 x 10^7	91.72	0.03	91.75
612	HP, Mo + SO_4	169 x 10^7	88.76	0.03	88.79
615	HP, Mo	200 x 10^7	99.50	0.10	96.60
616	HP, Mo + SO_4	133 x 10^7	96.24	0.57	96.81

Table 22. Proportion of injected dose of Cu^{64} recovered in excreta

Animal number	Treatment	Amount of Cu^{64} injected	Percentage of injected Cu^{64} recovered in excreta		
			Feces	Urine	Total excreta
		(cpm)	—	(%)	
610	LP, Control	164 x 10^6	2.71	0.47	3.18
611	LP, Mo	164 x 10^6	2.58	0.13	2.71
609	LP, Mo + SO_4	164 x 10^6	2.51	0.12	2.63
618	LP, Control	378 x 10^6	4.68	7.78[a]	12.46
619	LP, Mo	378 x 10^6	3.18	0.91	4.09
620	LP, Mo + SO_4	378 x 10^6	2.27	0.45	2.72
613	HP, Control	515 x 10^6	1.53	0.11	1.64
614	HP, Mo	515 x 10^6	1.89	0.33	2.22
612	HP, Mo + SO_4	515 x 10^6	1.31	0.29	1.60
615	HP, Mo	583 x 10^6	1.05	0.06	1.11
616	HP, Mo + SO_4	437 x 10^6	0.64	0.09	0.73

[a] The excretion pattern of Cu^{64} by this animal was not typical of the other animals. Over 90% of the Cu^{64} recovered in the urine was excreted during one 10-hour period.

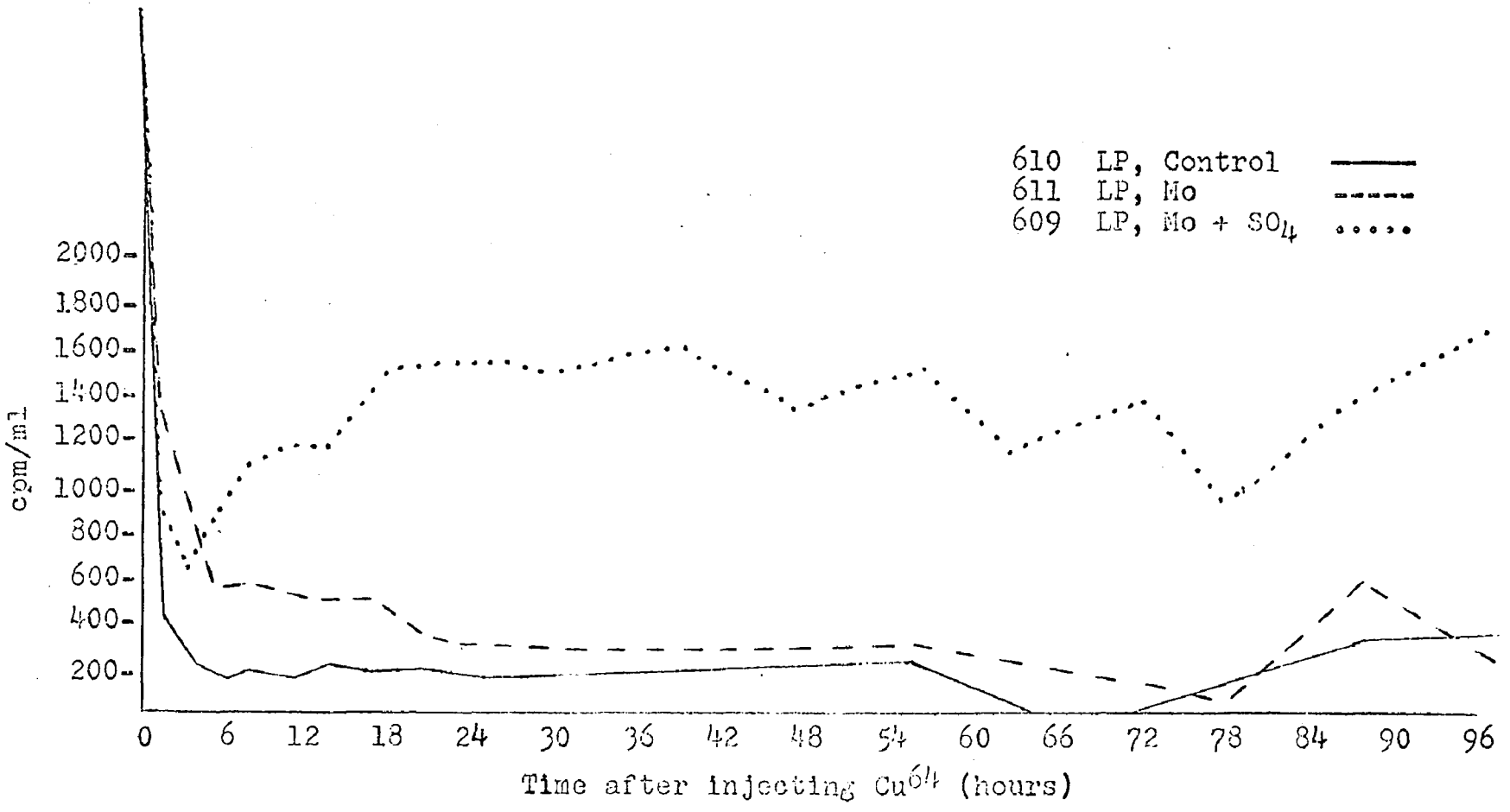

Figure 1. Levels of radioactivity at various time intervals in blood of animals injected with Cu^{64}

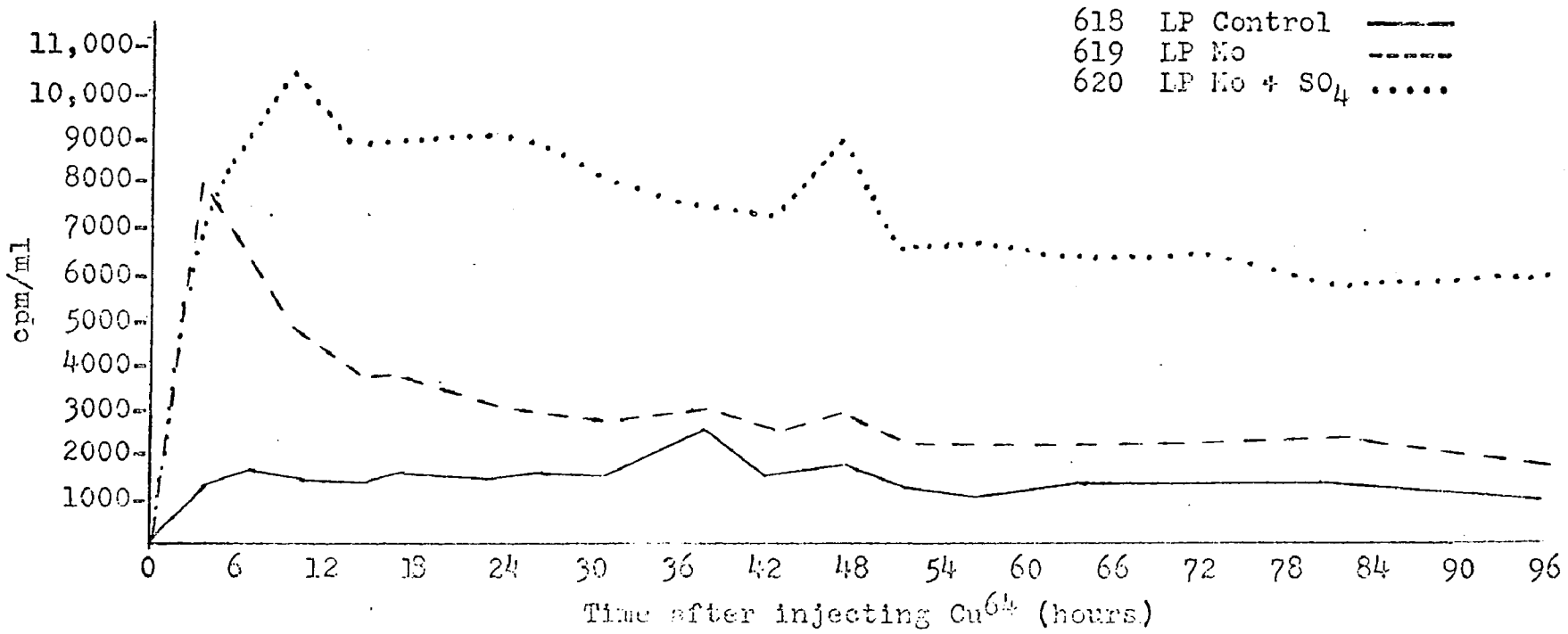

Figure 2. Levels of radioactivity at various time intervals in blood of animals injected with Cu^{64}

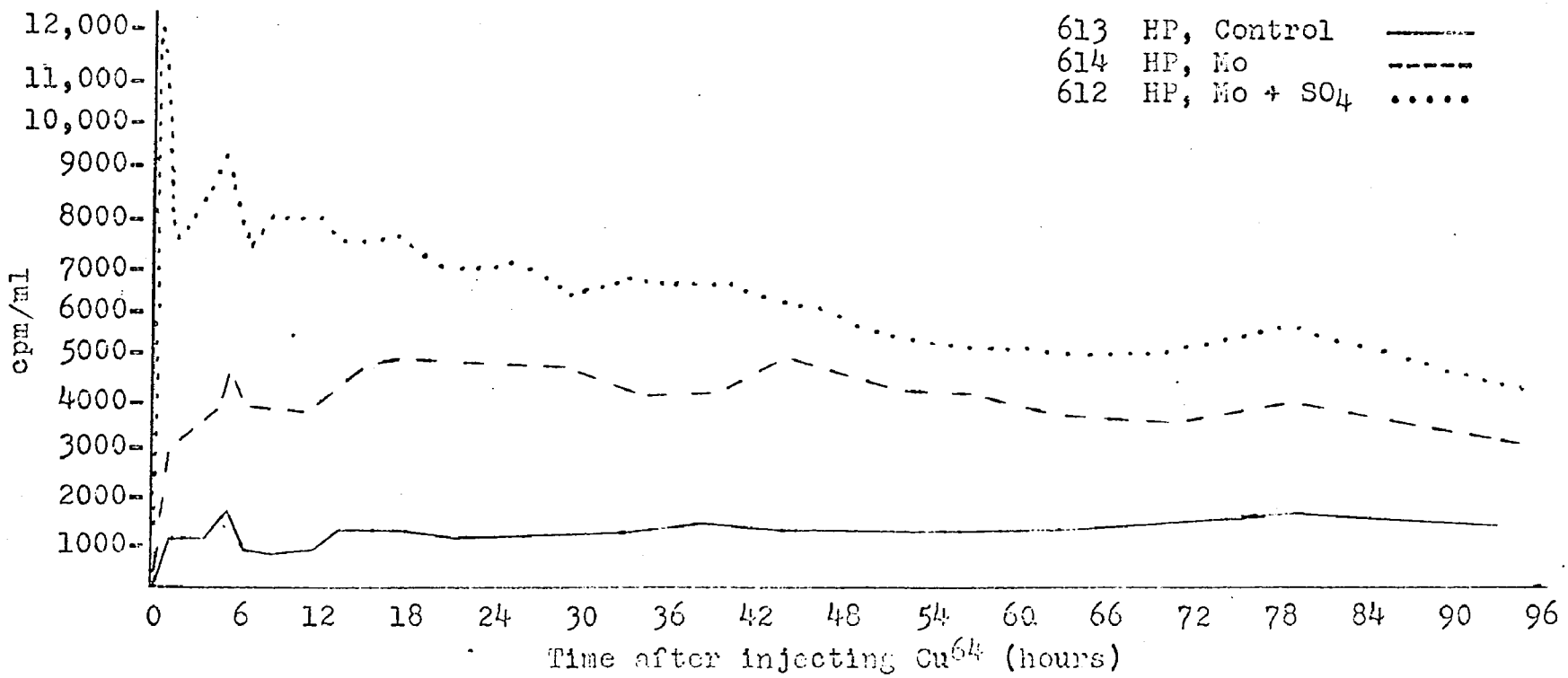

Figure 3. Levels of radioactivity at various time intervals in blood of animals injected with Cu^{64}

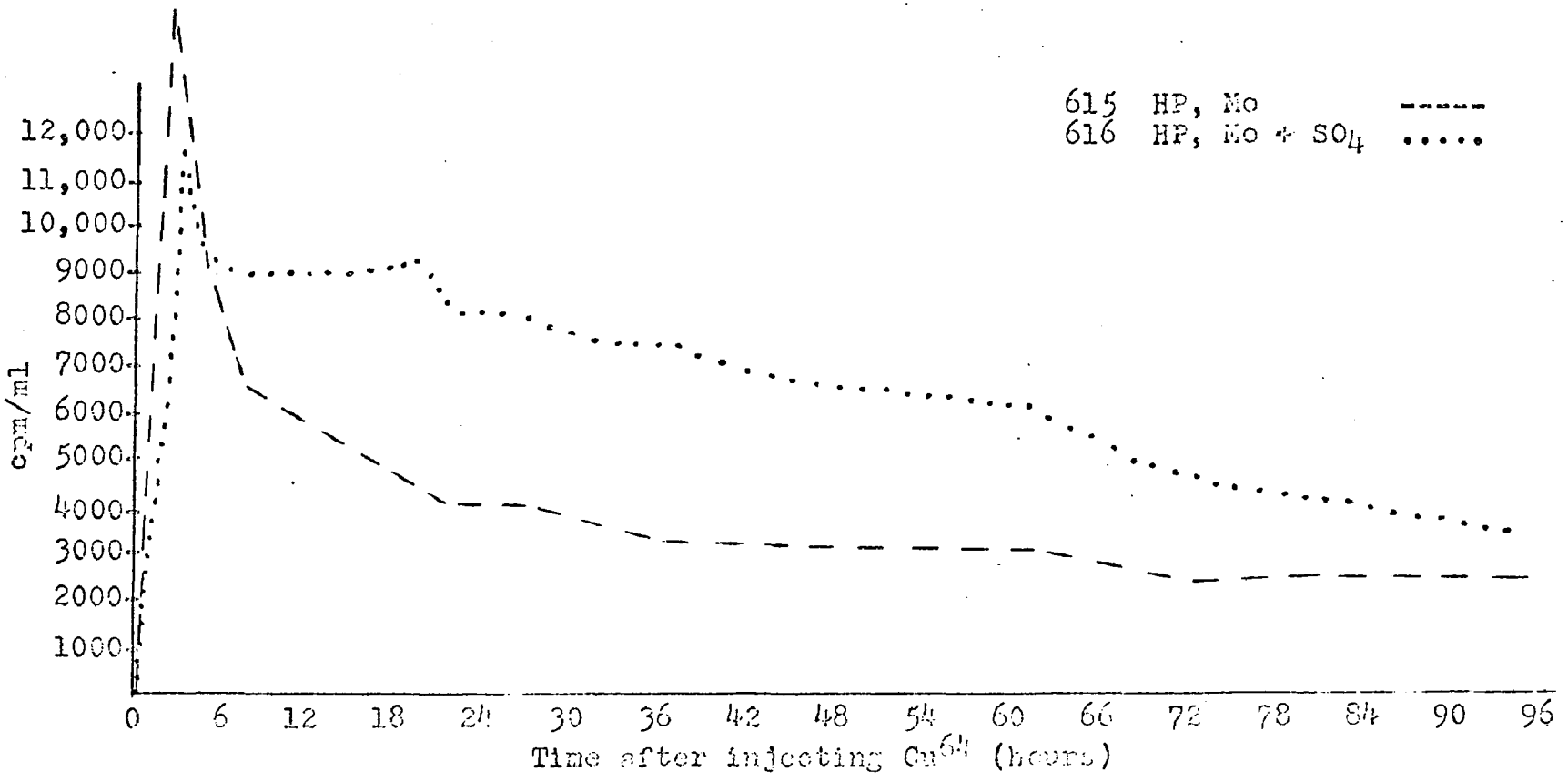

Figure 4. Levels of radioactivity at various time intervals in blood of animals injected with Cu^{64}

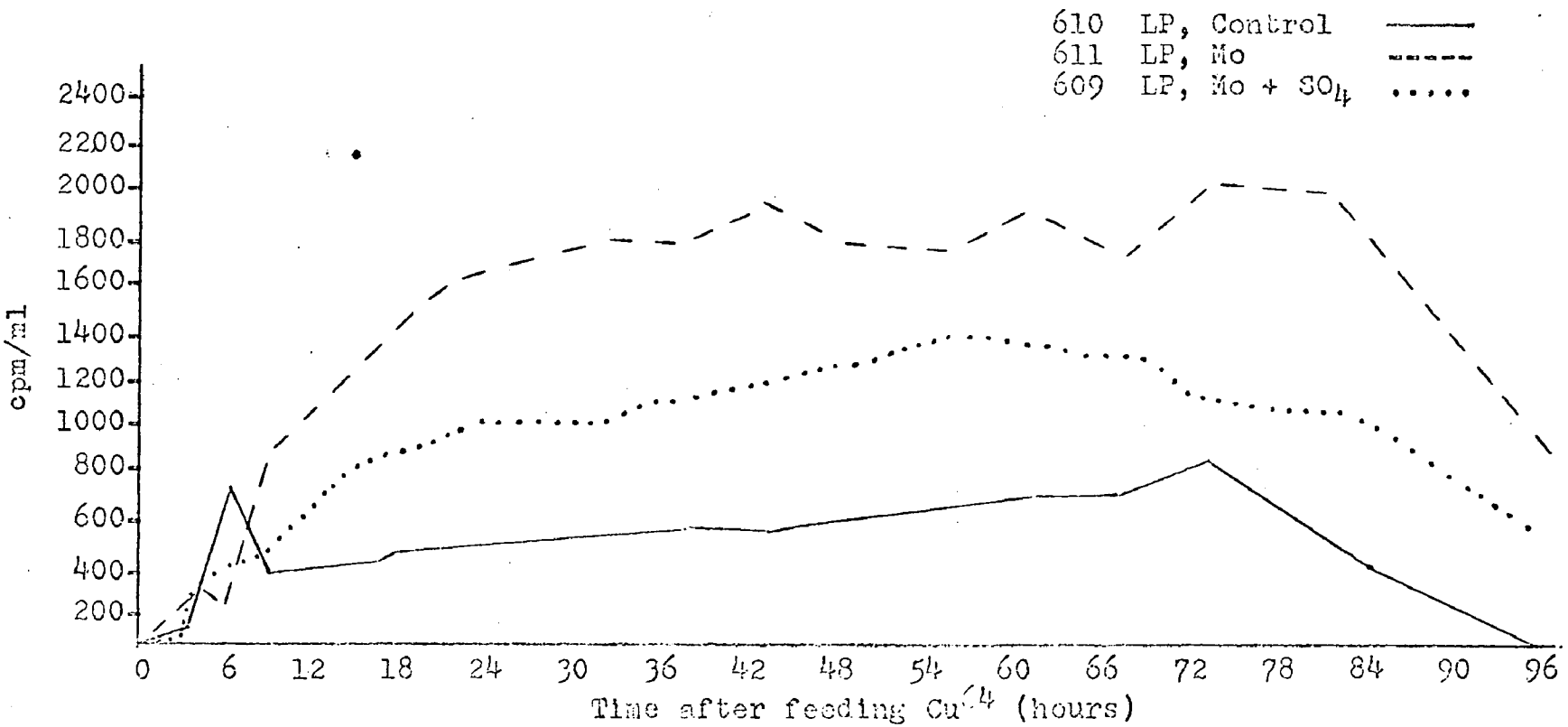

Figure 5. Levels of radioactivity at various time intervals in blood of animals fed Cu^{64}

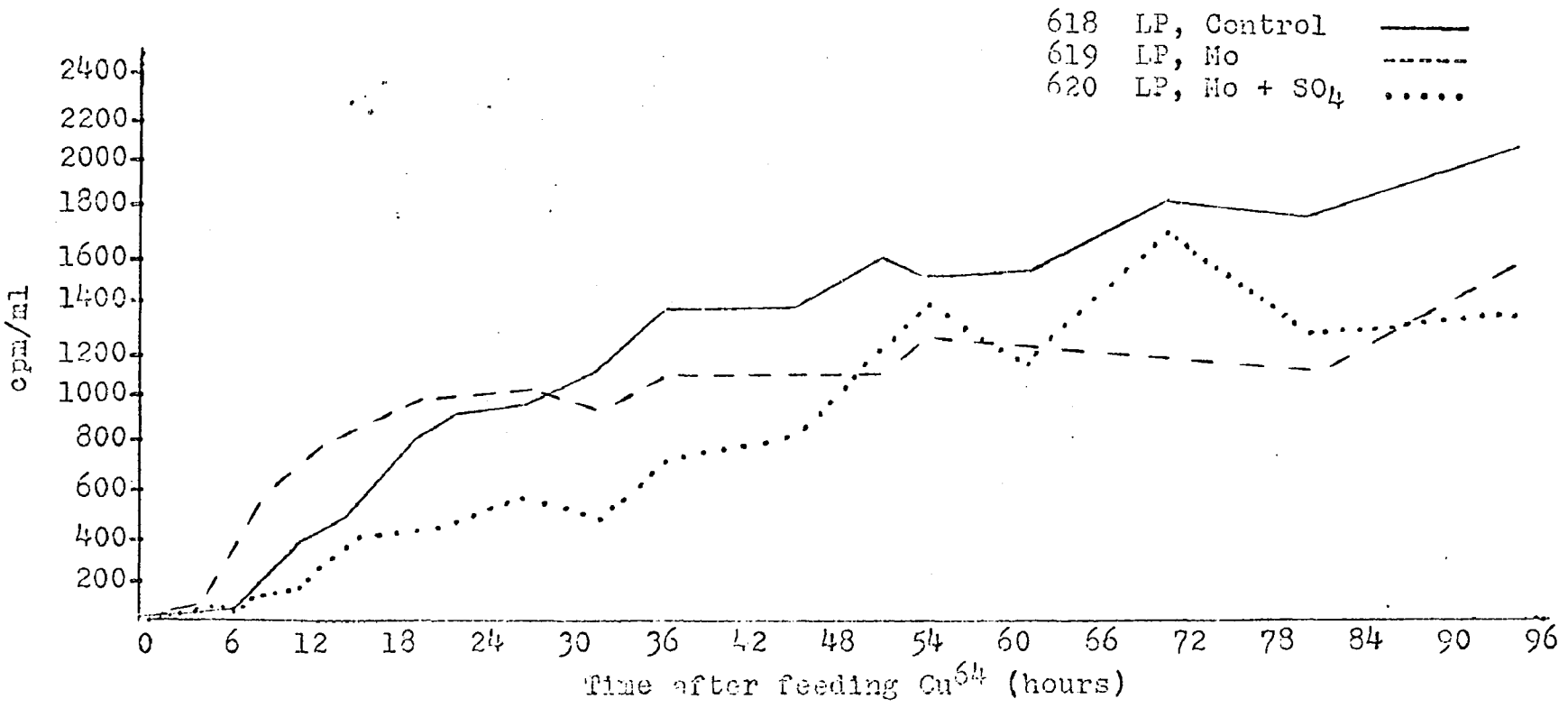

Figure 6. Levels of radioactivity at various time intervals in blood of animals fed Cu^{64}

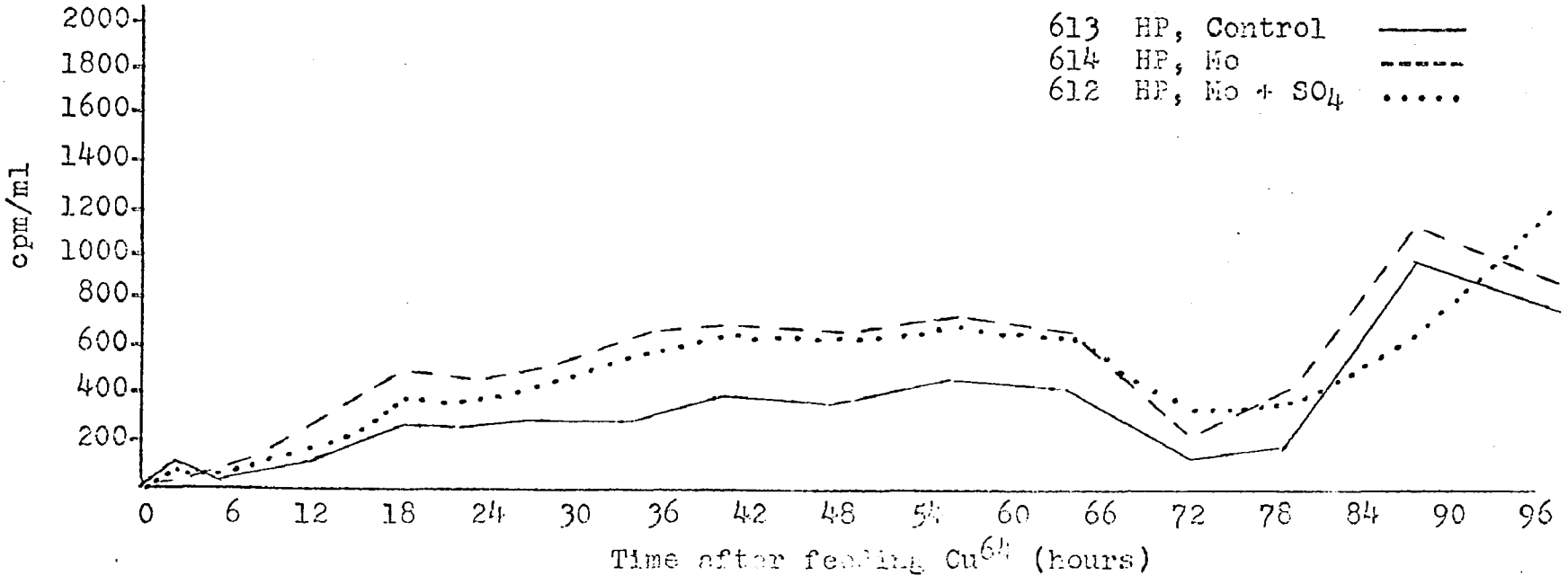

Figure 7. Levels of radioactivity at various time intervals in blood of animals fed Cu^{64}

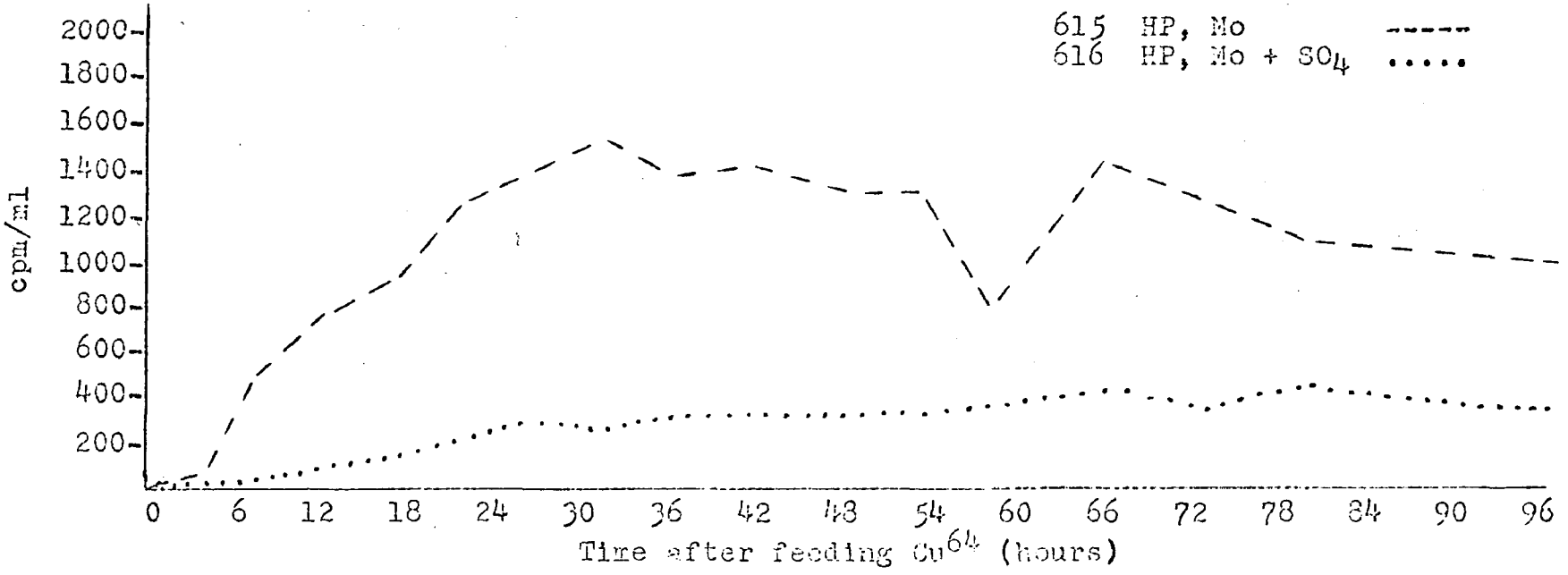

Figure 8. Levels of radioactivity at various time intervals in blood of animals fed Cu^{64}

Mo-supplemented animals second highest, and the controls the lowest. Although the blood Cu^{64} activity in the Mo-supplemented animals was generally higher than in the controls and lower than in the Mo + SO_4-supplemented animals, there also was an apparent treatment difference between the low and high phosphorus-fed animals in that their ability to utilize Cu^{64} injected into their blood stream was affected. In all cases the Mo + SO_4-supplemented animals showed an impaired ability to utilize the injected Cu^{64} as readily as did the control as indicated by the high blood Cu^{64} levels maintained by the former animals.

When Cu^{64} was given orally, the Mo-supplemented animals showed a tendency to have the highest blood Cu^{64} levels, and the Mo + SO_4-supplemented animals had the second highest blood Cu^{64} levels. This pattern was not consistent in all groups. The high blood Cu^{64} levels in the Mo- and Mo + SO_4-supplemented animals indicates that Mo- and Mo + SO_4-supplementation apparently did not prevent the absorption of Cu^{64} from the digestive tract. Similarly, high blood Cu^{64} levels in Mo- and Mo + SO_4-supplemented animals, as compared to the controls, were interpreted by Vanderveen (132) as evidence that absorption of Cu^{64} was not appreciably decreased by these supplements. When the amount of Cu^{64} deposited in the liver of these animals (Tables 23, 24) is taken into consideration, there seems to have been an apparent decrease in the absorption of copper

by the Mo + SO_4- and HP, Mo-supplemented animals as compared to the LP, Mo-supplemented and control animals even though the blood Cu^{64} concentrations were relatively high.

The radioactivity in selected tissues, fluids and excreta from animals injected with or fed Cu^{64} is recorded in Tables 21 and 22. There was a marked difference in the ability of the animals receiving various treatments to concentrate Cu^{64} in their tissues. The liver appeared to be the tissue most able to take up large quantities of Cu^{64}, with the kidney, rumen, reticulum and omasum also showing high Cu^{64} concentrations in several animals. Muscle and brain tissue showed low Cu^{64} concentrations even when blood Cu^{64} levels were high. The other tissues were usually similar in radioactivity to that of the blood. It should be noted that the animals showing a very limited ability to concentrate copper in the tissues had a high total copper, molybdenum and Cu^{64} concentrations in the ruminal, reticular and omasal tissues. These results suggest that the forestomach may be a major site for absorption of copper and molybdenum in ruminants. The Mo + SO_4-supplemented animals and, in addition, the HP, Mo-supplemented animal (612) showed a very high concentration of Cu^{64} and total copper in the forestomach tissues. This would seem to indicate that copper is being retained to some extent in these tissues. The high Cu^{64} concentrations in these tissues were not apparent in the LP, Mo-supplemented and control animals.

Table 23. Radioactivity in various tissues and excreta after feeding of Cu^{64}

Treatment	Low dietary phosphorus			High dietary phosphorus		
	Control	Mo	Mo + SO_4	Control	Mo	Mo + SO_4
Animal no.	613	614	612	618	619	620
Tissues	(cpm)					
Thigh	71	18	138	60	372	66
Shoulder	73	53	70	153	129	48
Loin	76	32	594	65	198	89
Heart	326	116	45	377	643	193
Spleen	327	152	77	590	798	204
Lung	333	109	121	540	783	220
Pancreas	885	163	101	662	1022	237
Brain	86	45	40	54	56	40
Kidney	2142	777	447	2117	3993	253
Rumen	2099	29273	13662	2485	5492	---
Reticulum	200	29942	9555	5830	7234	51000
Abomasum	1052	536	303	4984	4764	364
Omasum	2661	25913	22516	4415	6516	49944
Duodenum	182	736	552	2521	4778	479
Jejunum	1783	794	482	2217	4327	538
Ileum	1660	812	507	1234	3289	729
Cecum	1673	603	245	1746	3495	716
Colon	1242	1396	832	2983	2828	1130
Backfat	57	25	186	96	362	444
Liver	16533	602	517	17889	27589	1736
Blood	342	380	187	378	1073	601
Bone	132	16	913	41	86	38
Bonemarrow	0	0	247	33	257	200
Rumen & ret. contents	13000	12614	12946	13148	15380	12826
Abomasum contents	11520	14539	18047	14552	20124	13007
Omasum contents	35824	31806	39576	42808	34758	29812
Small int. contents	16838	19290	7555	20908	22652	9270
Large int. contents	43298	36388	33786	53542	68160	36320
Diaphragm muscle	211	76	55	225	278	132
Fat tissue	71	73	451	93	253	189
Bile	24	39	90	78	71	72

Table 24. Radioactivity in various tissues and excreta Cu^{64} injection

	Low dietary phosphorus			High dietary phosphorus	
Treatment	Control	Mo	Mo + SO_4	Mo	Mo + SO_4
Animal no.	610	611	609	615	616
Tissues	(cpm)				
Thigh	180	458	463	574	369
Shoulder	328	675	743	186	282
Loin	330	518	527	318	294
Heart	1495	3794	2151	1078	1061
Spleen	1743	2482	2688	743	3045
Lung	1648	3212	3106	1042	1908
Pancreas	2167	3361	2573	1363	1275
Brain	303	143	258	154	356
Kidney	8054	19803	18733	5329	12782
Rumen	2118	4309	2367	738	973
Reticulum	1505	2868	1550	1351	912
Abomasum	1443	4875	4785	1883	2515
Omasum	2204	2563	1832	1238	1657
Duodenum	6942	13292	8131	4454	3120
Jejunum	2728	8714	3991	2352	2179
Ileum	2884	5254	5293	2047	2391
Cecum	4066	4423	3970	1977	2260
Colon	2715	5513	3543	1460	1950
Backfat	--	1282	3213	752	126
Liver	56030	88142	29053	22954	8884
Blood	872	2098	5263	943	2363
Bone	104	1643	106	794	601
Bone marrow	65	227	1792	204	182
Rumen & ret. contents	11	0	0	26	17
Abomasum contents	47	77	52	9	19
Omasum contents	0	17	6	24	65
Small int. contents	477	1542	278	807	112
Large int. contents	196	496	291	94	227
Diaphragm muscle	887	2043	899	1058	552
Fat tissue	285	849	153	527	1697
Bile	247	141	83	38	23

Liver radioactivity appears to be the best indicator of treatment effects. Tables 23 and 24 show the percentage of the injected or fed dose of Cu^{64} recovered in the liver of the various animals. These data show that both the HP and LP, Mo + SO_4-supplemented animals and the HP, Mo-supplemented animal (614) had an impaired ability to concentrate Cu^{64} in the liver. The other HP, Mo-supplemented animal (616) showed a limited impairment in uptake of injected Cu^{64} in the liver when compared to the LP, Mo-supplemented animal (611). The LP, Mo-supplemented and the LP and HP control animals were able to store large quantities of liver Cu^{64}.

Considering the results of the radioactivity studies, it is apparent that molybdenum in the LP rations did not impede the deposition of radioactive copper in the tissues. The LP, Mo-supplemented animals had over 25% more total radioactivity in the liver tissues when injected with Cu^{64} and about 2% more when fed Cu^{64} than did the corresponding control animals. In both LP, Mo-supplemented animals, the radioactivity of the liver tissue was about 50% higher per gram of tissue than the corresponding controls, but in both animals the size of the liver was smaller. The blood Cu^{64} levels in the LP, Mo-supplemented animals were higher than in the controls when Cu^{64} was fed. Tissue Cu^{64} concentrations in the LP, Mo-supplemented animals fed Cu^{64} and sacrificed were higher than in the corresponding controls. All these findings seem to

indicate that high concentrations of molybdenum in LP rations did not decrease the absorption and deposition of Cu^{64} in the tissues but apparently enhanced them to some degree.

The HP, Mo-supplemented animals showed evidence of interference in their copper metabolism in contrast to the LP, Mo-supplemented animals. Phosphorus in the ration had a tendency to enhance the effect of sulfate. One HP, Mo-supplemented animal (614) had a low liver total copper concentration, low tissue Cu^{64} levels, a low liver Cu^{64} concentration and high blood Cu^{64} levels. All of these observations were similar to those found in the HP and LP, Mo + SO_4-supplemented animals, but dissimilar to the corresponding abservations in the LP, Mo-supplemented animals. The other HP, Mo-supplemented animal (615) did not appear to be affected to such a degree by this treatment. The liver copper concentration was fairly high in this animal but was lower than in either of the LP, Mo-supplemented animals. When this animal was injected with Cu^{64}, the liver tissues absorbed only about 25% of the injected dose as compared with over 60% by the LP, Mo-supplemented animal. It is difficult to determine how much the absorption of Cu^{64} was inhibited by the HP, Mo supplement in this animal, since the control in this group died. It is evident that when Cu^{64} was injected, the ability of the animals to absorb Cu^{64} into their tissues was not affected as much as when Cu^{64} was given orally.

No explanation can be given for the difference in reaction of the two high phosphorus, Mo-supplemented animals. The reaction could have been the result of animal differences, or differences in the quantity of supplement consumed. Animal 615 consumed less phosphorus than did 614, and this may have had an effect. The level of phosphorus was not particularly high in the HP rations, being only about three times greater than the minimum requirements generally accepted for cattle. High phosphorus levels in the feed of Mo + SO_4-supplemented animals appeared to depress liver copper and Cu^{64} concentrations, and caused a generally poorer physical well-being.

In this study, Mo + SO_4 supplementation apparently reduced absorption of radioactive copper and also appeared to decrease the ability of liver tissue to absorb Cu^{64}. This work supports Dick's (45) hypothesis that a cell membrane, whose permeability to molybdenum is impeded or blocked by sulfate, will be less permeable to copper. It is tempting to speculate, on the basis of these results, that the phosphate and the sulfate ion may have had a common role in their interference with copper metabolism. The two ions, in conjunction with molybdenum, probably interfere with the transport of copper across the cell membranes.

It is interesting to note the similarity in size and configuration in these ions. Sulfur and phosphorus have nearly the same atomic weight, both sulfate and

phosphate are ions of a strong acid, and both have four associated oxygen molecules. Since their configuration and chemical properties are similar, it is conceivable that the two ions, in conjunction with molybdenum, could inhibit the same enzyme system. If both phosphate and sulfate in the presence of molybdenum interfere with copper metabolism, such a theory might explain why it is so difficult to determine the true interrelationships between molybdenum, sulfate and copper.

SUMMARY AND CONCLUSIONS

This experiment was designed to investigate the effects of phosphorus on the absorption, deposition and excretion of copper when the animals were supplemented with molybdenum, molybdenum and sulfate or neither. Supplementary studies using radioactive copper were carried out during the experiment. Molybdenum was fed at the level of 75 ppm, sulfur as a sulfate at the level of 0.3 per cent, and phosphorus at 0.22 or 0.62 per cent of the ration. The levels of these various supplements actually consumed were recorded.

Molybdenum plus sulfate (Mo + SO_4) supplements in both the high and low phosphorus rations decreased the absorption and deposition of liver copper. High phosphorus (HP) levels in the ration appeared to decrease the liver copper and increase the physical manifestations of copper deficiency in the Mo + SO_4-supplemented animals. The physical manifestations of copper deficiency resulting from higher level of phosphorus included more intensive hair discoloration, diarrhea, loss of appetite, loss of weight, front leg deformities, and serious overall degeneration. The comparable low phosphorus (LP)-supplemented animals showed no physical signs of copper deficiency except the hair discoloration in one animal. Molybdenum plus sulfate supplements in both HP and LP rations increased whole blood

and plasma copper. It also increased blood cell zinc by a substantial amount in all but one of the Mo + SO_4-supplemented animals at both phosphorus levels. The addition of sulfate to the ration depressed the absorption of molybdenum and decreased molybdenum concentrations in all tissues analyzed except ruminal and omasumal tissues. The radioactivity studies showed that Mo + SO_4 supplements reduced the absorption of Cu^{64} but did not depress blood Cu^{64} values, especially in the blood plasma. The low Cu^{64} concentrations in the liver indicated that very little of this element was concentrated in this organ.

Molybdenum supplements decreased liver copper levels in animals receiving the HP rations but not in animals receiving the LP rations. There were no physical manifestations of copper deficiency in any of the molybdenum (Mo)-supplemented animals, although they had rough hair coats and appeared less thrifty than the controls. The HP, Mo-supplemented animals had lower liver copper values than the LP, Mo-supplemented animals. When they were injected with Cu^{64}, the LP, Mo-supplemented animals had blood Cu^{64} levels that were only slightly higher than those of the controls, while the HP, Mo-supplemented animals' blood Cu^{64} levels approached the high levels of the Mo + SO_4-supplemented group. This finding indicates that LP, Mo-supplemented animals were able to utilize the absorbed Cu^{64} more readily than were the HP, Mo-supplemented animals. There was a sharp decrease in the amount of Cu^{64} deposited in the liver

of one HP, Mo-supplemented animal similar to that in one HP, Mo + SO_4-supplemented animal in the same group. The other HP, Mo-supplemented animal (615) in the second group did not appear to be affected as much by this treatment as was Animal 614. Animal 615 did show a substantial decrease in its ability to concentrate injected Cu^{64} in the liver, when compared to the LP, Mo-supplemented animal (609) in Group I. It was difficult to interpret the significance of the liver Cu^{64} levels of this animal since there was no control in the group for comparison. Molybdenum supplementation in either HP or LP rations did not appear to increase the blood copper levels.

Nothing conclusive was learned from the pattern of excretion of Cu^{64}. A high level of phosphorus in the ration reduced the excretion of Cu^{64} in the feces by over 50%. There was little apparent treatment effect on the excretion of radioactive copper in the urine. Generally, the urinary excretion of Cu^{64} was very low, being less than 0.5% of that injected in nearly all cases. Generally, the animals on LP rations excreted larger amounts of Cu^{64} in their urine than did the animals on HP rations, but there were a number of exceptions. There appeared to be little effect from Mo or Mo + SO_4 supplements on urinary excretion of Cu^{64}.

The data show evidence that Mo + SO_4 supplementation in the ration decreases the absorption of Cu^{64} from the digestive tract. Although blood Cu^{64} levels of such

animals were usually slightly higher than those of the controls when Cu^{64} was fed, there was much less Cu^{64} deposited in liver tissue of Mo + SO_4-supplemented animals. When Cu^{64} was injected, the Mo + SO_4-supplemented animals had higher blood Cu^{64} levels than did the controls. This indicates that these animals were not able to absorb or utilize copper as readily as were controls. High phosphorus in the Mo + SO_4-supplemented ration appeared to further depress the deposition of Cu^{64} in the liver.

Molybdenum supplements in the LP ration did not appear to have any depressing effect on the absorption of Cu^{64} from the digestive tract and may have actually enhanced it. Concentrations of Cu^{64} in the liver of the LP, Mo-supplemented animals was 50% higher than in corresponding controls. Because both of the LP, Mo-supplemented animals had small livers, the total radioactivity in the liver tissue from these animals was only slightly higher than that of the corresponding controls. With higher levels of Cu^{64} in both blood and tissues, there was no indication that molybdenum decreased the absorption or deposition of Cu^{64} in these animals. When the Mo supplement was included in a HP ration, there was a decrease in the total liver Cu^{64}, and a relatively low blood Cu^{64} level indicating a decrease in the absorption and deposition of this element. The interference with the uptake and deposition of copper, whether it was the result of the HP or LP, Mo + SO_4, or HP, Mo supplementation, was apparently identical. These

supplements caused an apparent decrease in the absorption of Cu^{64} from the digestive tract and a definite interference in the utilization or uptake of Cu^{64} by the tissues. The red blood cells also showed a definite decrease in ability to take up Cu^{64} from the plasma. This is in general agreement with Dick's (45) hypothesis concerning the interaction of molybdenum and sulfate. He indicated that sulfate interferes with the transport of both molybdenum and copper across the membrane at the same time. This decreases the amount of Cu^{64} absorbed and prevents the absorbed copper from being utilized by the tissue, thereby bringing about copper deficiency.

This work suggests that the phosphate ion may act in conjunction with molybdenum to interfere with the absorption and deposition of copper. The HP, Mo-supplemented animals reacted in a manner similar to that of the Mo + SO_4-supplemented animals, while the LP, Mo + SO_4-supplemented animals did not show any interference in either the uptake or deposition of Cu^{64} in the tissues. This finding indicates that the sulfate and phosphate ion may act in a similar manner. This also increases the probability that only one enzyme system was affected because it is less likely that two different anions would interfere with a common group of enzymes. Increasing phosphorus in the ration did not increase blood copper or red blood cell zinc as did sulfate, suggesting that there may be other disturbances caused by the action of the

sulfate ion when it is fed with molybdenum.

It is difficult to describe the role of molybdenum in decreasing the body stores of copper and in the manifestation of copper deficiency. It was shown by Cunningham (35) that cattle are more sensitive to molybdenum supplementation than are sheep. It was further shown that cattle will lose body copper stores if dietary sulfate is 50% lower than the minimum reported necessary to decrease body copper stores in sheep. It is possible that in the experiment reported here the amount of sulfate in the feed was below the minimum necessary to reduce the body stores of copper. Because HP, Mo-supplemented rations caused the animals to react as though they were receiving a sulfate supplement in their ration, it appears that phosphorus added to the ration had the ability to act in place of the sulfate. This may explain some of the difficulties in interpreting the results of experiments on the interrelationship of sulfate, molybdenum and copper.

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