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CHANGES PRODUCED IN GROWTH, REPRODUCTION, BLOOD AND URINE OF RATS BY INGESTION AND ORAL ADMINISTRATION OF COBALT SALTS

by

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INTRODUCTION

At the end of the first transition group in the periodic arrangement of the elements, there occurs a group of elements which have become of considerable biological interest. These elements, atomic numbers twenty-five to thirty, are manganese, iron, cobalt, nickel, copper and zinc. With the exception of iron, which is needed in larger quantities than the other elements, the animal body apparently requires most of the other elements in very small amounts in order to maintain health. Accordingly, these elements are among those known as "trace elements" in animal nutrition.

Of these elements, iron is found chiefly in the heme portion of hemoglobin but it is also found as an indispensable constituent of all living cells in enzyme systems such as cytochrome, catalase, peroxidase and other oxidases; copper has been shown to be necessary in the diet to permit the body to utilize iron in hemoglobin formation; manganese deficiency in the diet of female rats leads to a high mortality rate of the young; a deficiency of zinc in the diet of rats causes a marked decrease in intestinal absorption and the growth rate decreases 50 per cent; nickel has never definitely been shown to be involved in animal nutrition, although it is found in traces in animal tissues; cobalt has recently been found to be an

indispensable element in the nutrition of cattle and sheep in order to prevent a deficiency disease which is usually fatal. In general, the functions of most of these elements in animal metabolism are not known. Thus the study of such trace elements offers a wide field of research opportunities.

Statement of the Problem

Although cobalt salts have been shown to have some physiological effects upon various species of animals, there is no report in the literature of any integrated investigation of these various effects of cobalt salts on any one species. Several studies of different aspects of the problem have been made on the albino rat, Mus norvegicus albinus, but some of these seem to be based on data obtained from experiments on only two or three rats. In view of the common use of the albino rat as an experimental animal, it seemed that a study of some of the various physiological effects of cobalt salts on this animal might produce some new knowledge leading to an understanding of the cobalt requirement of this animal or of the manner in which cobalt acts within the organism. To that end a nutritional and biochemical investigation was planned to furnish information on the following questions:

1. What is the effect upon the growth of rats when cobalt salts are added to an adequate basal ration?
2. Will cobalt salts added to the basal ration affect the reproduction of rats?

3. Will added cobalt salt in the diet affect the lactation of female rats as measured by the success in raising the young?
4. How is cobalt distributed in the tissues of normal rats and in the tissues of rats fed cobalt salts? Which tissues absorb the greatest proportion of the added cobalt?
5. How rapidly is cobalt deposited in those tissues where it is found to be most abundant?
6. What is the minimum lethal dose of cobalt salts when given to rats by stomach tube?
7. What is the minimum amount of cobalt in the diet of rats which will stimulate hematopoiesis?
8. Do ingested cobalt salts affect the concentration of such normal blood constituents as reducing sugar and non-protein nitrogen?
9. Does addition of cobalt salts to the diet affect the amount of reducing sugar in the urine?
10. Will amino acids other than cystine, cysteine and methionine detoxify cobalt within the organism?

HISTORICAL

Cobalt in Animal Nutrition

A "wasting disease" of cattle and especially of sheep has long been known in various parts of the world. Affected animals lose their appetites, show progressive emaciation and develop an anemia. The anemia, however, sometimes does not appear until very late in the course of the disease (3). Affected sheep become "dark and sunken in the wool", the face assumes a dirty gray color, there is a marked pallor of the visible membranes and the animals become depressed and listless (42). The fleece of affected sheep shows a discontinuity coincident with the onset of the disease, making the wool less satisfactory for use (74). The progressive emaciation generally terminates fatally, although not usually for many weeks after the first symptoms appear. In Scotland the disease is called "pine"; in Kenya, "Nakuruitis" (65). In New Zealand it is known as "Morton Mains ailment" or "bush sickness" (47). In Australia it is called "wasting disease", "enzootic marasmus" (55) or "coast disease" (95). In Florida affected sheep are "salt sick" or "hill sick" (120). In Michigan the ailment is known as "Grand Traverse disease", "Lake Shore disease" or "pica" (84).

Since one characteristic of the syndrome is that sheep suffer from the disease only when pasturedin certain areas and recover when moved to "healthy" pastures, it was long suspected that the disease might be due to some dietary deficiency. In view of the anemic condition of affected animals it was first thought that iron was the deficient factor and some workers found that administration of iron compounds cured the disease (13) (65). In 1934, however, Filmer (56), in Australia, was able to show that the curative factor was not iron but some contaminant in the iron compounds previously used to cure the disease. In addition, Underwood (142) found that the livers of bush-sick sheep contained more than five times as much iron as the livers of normal sheep, indicating that the disease was due to a deficiency of some substance necessary for the normal utilization of iron by the animal rather than a deficiency of iron itself. Underwood and Filmer (145) were soon able to show that the deficient factor was cobalt. Seven sheep suffering from bush sickness were cured by feeding them cobalt chloride solution at the rate of 0.1 to 2.0 milligrams of cobalt per day. Almost simultaneously, Lines (95), working in another part of Australia, announced the cure of two sheep suffering from coast disease by doses of cobalt nitrate given at the rate of one milligram of cobalt per day.

Since the initial discovery of the curative effects of cobalt salts on this syndrome, investigators in other parts of the world have reported similar success with such treatments.

These investigations are summarized in Table 1.

Table 1

Reports of Successful Treatment of Nutritional Deficiency Disease with Cobalt Salts

Location	Animal	Curative dose	Investigators
New Zealand	Sheep	4 mg. Co^{++} per week	Askew and Dixon (8)
New Zealand	Sheep	1-2 mg. $CoSO_4$ twice a week	Hopkirk (73)
Australia	Sheep	0.1 mg. Co^{++} per day	Filmer and Underwood (57)
South Australia	Sheep	45 mg. $CoSO_4$ + 45 mg. $CuSO_4$ every two months	Scott (133)
South Australia	Sheep	Both Co^{++} and Cu^{++} needed	Marston et al (110)
Scotland	Lambs	1 mg. Co^{++} per day	Corner and Smith (43)
Western Canada	Sheep	Not stated	Bowstead and Sackville (34)
Florida	Calves	5 mg. cobalt per day	Neal and Ahmann (120)
Michigan	Cattle	Not stated	Killham (84)

It has been found possible to treat the syndrome by feeding the animals salt licks containing cobalt chloride (42).

A close correlation has been found by a number of investigators (4) (74) between the incidence of bush sickness and the cobalt content of pastures (pasture plants) eaten by the animals. In areas where sheep were affected with bush sickness, the cobalt content of pastures ranged from 0.01 to 0.07 part per million while pastures from "healthy" areas contained 0.2 to 1.0 part per million of cobalt.

Cobalt deficiency in plant materials has been traced to a cobalt deficiency in the soils on which the plants were grown (80) (124) (135) (146) and investigators in different parts of the world have found it possible to increase effectively the cobalt content of such soils by treating them with fertilizers containing cobalt chloride or cobalt nitrate (10) (103) (139). Such treatment increased the cobalt content of pastures grown on the soils and prevented bush sickness in sheep pastured on them (7) (116).

Although some investigators seem to believe that cobalt deficiency is the sole cause of bush sickness, others maintain that it may be complicated by deficiencies of other elements such as copper or even iron. This may very well be true, considering the wide geographic distribution of the ailment and the consequent wide variation in soil deficiencies which might be encountered. In addition, the amount of cobalt necessary to cure the syndrome must be expected to vary since cobalt-deficient soils of various localities are not all equally deficient and varying amounts of cobalt would thus be found in plant materials grown on such soils. Consequently, the amount of cobalt needed in the diet for adequate nutrition of cattle and sheep cannot be stated precisely. It is probably of the order shown in the table on page 6.

Due to the extreme difficulty in preparing cobalt-free basal rations, the cobalt requirements, if any, of other animals are not known. Underwood and Elvehjem (144) fed rats on a milk

diet with added salts of copper, iron and cobalt. They found no hemoglobin increase or growth response over those of rats fed the diet without added cobalt. Analysis of the ration by the nitroso-R-salt method showed a cobalt content of eleven micrograms per liter, corresponding to a daily intake of 0.6 microgram of cobalt per rat. They concluded that if cobalt is required by the rat, it must be in amounts of 0.6 microgram or less per day.

Dixon (48) gave a drench of a cobalt salt at the rate of four milligrams of cobalt twice weekly to one cow each of seven pairs in a "sheep-sick" area and found that the milk yield was not altered significantly from that of the paired controls.

Bertrand and Nakamura (29) attempted to prepare a nickel- and cobalt-free ration (composition unstated) which they fed to mice, both with and without supplements of cobalt sulfate and nickel chloride. The mice receiving the supplement survived an average of 23.1 days while those not receiving the supplement survived an average of 19.7 days. These workers interpreted this data as indicating the physiological importance of cobalt and nickel. Their method of freeing the ration of nickel and cobalt, however, would also remove all the water-soluble vitamins and the growth curves they presented showed typical vitamin-deficient growth responses by the mice used in the experiments.

Physiological Effects of Cobalt and Cobalt Salts on Animals

Hematopoietic action

In 1929 Waltner and Waltner (150) observed that the hemoglobin and erythrocyte count of rats weighing 130 to 200 grams increased by 20 per cent within 24 hours after subcutaneous injection of 100 milligrams of cobalt nitrate in solution. The hemoglobin values and the erythrocyte count also were increased 20 per cent to 25 per cent when two per cent of metallic cobalt was added to the ration of rats. These animals died within two to three weeks, however. With 0.5 per cent of metallic cobalt added to the ration, rats survived six to seven months, on an average. The average erythrocyte count rose to 10,500,000 and the hemoglobin concentration increased to 165 per cent. These values persisted until the death of the rat. If cobalt was removed from the diet, both erythrocyte count and hemoglobin value decreased to normal levels. Later Waltner (149) fed daily doses of 10 to 60 milligrams of metallic cobalt and 50 to 300 milligrams of $CoAs_2$ to 21 children suffering from secondary anemia. The number of erythrocytes increased an average of 32 per cent in 14 cases. The other seven children were not benefited. The hemoglobin concentrations and the leucocyte counts remained unchanged.

Mascherpa (112) fed daily doses of ten milligrams of metallic cobalt to a normal dog, to a dog which had been bled

to produce anemia, to a dog which had been fed a milk diet and to a dog which had been bled and kept on a milk diet, each with another dog as a control, receiving the same treatment but not receiving cobalt. The dogs receiving cobalt showed a slightly higher hemoglobin concentration and erythrocyte count than the controls and Mascherpa considered this evidence of the hematopoietic action of cobalt.

Brewer (35) later discounted these experiments. He claimed that variations of 15 to 20 per cent in erythrocyte count are normal in dogs and that daily doses of powdered cobalt chloride furnishing 4.0 milligrams of cobalt ion per day did not produce erythrocytosis in any one of four dogs after 26 days of treatment. Later Brewer (36) reported that a dose in excess of five milligrams of cobalt (fed as cobalt chloride) per kilogram of body weight was necessary to increase significantly the hemoglobin concentration in dogs. Similarly, no regularly significant increase in erythrocyte count occurred until the amount of cobalt fed as cobalt salt exceeded ten milligrams per day per kilogram of body weight.

Davis (44) claimed that the erythrocyte count of normal dogs should not vary more than seven per cent from one day to the next. He found that feeding dogs a solution of two milligrams of cobalt chloride (in 1/1000 dilution) per kilogram of body weight per day increased the erythrocyte count 20 per cent in two weeks.

Polonovski and Briskas (126) fed cobalt chloride to dogs

made anemic by a milk diet. Ten to thirty milligrams of the salt were given every two or three days. The animals' weight decreased, the erythrocyte count increased from 2,000,000 to 5,000,000 but there was no effect on the hemoglobin concentration.

Davis (46) found that cobalt polycythemia in dogs is accompanied by an increase of blood volume due chiefly to an increased volume of cells.

In an investigation of the effect of cobalt on sheep, Bell (18) found that cobalt did not stimulate the formation of abnormal numbers of erythrocytes. Later Bell (19) reported that an anemia often accompanies bush sickness in sheep but that the sickness also occurs without anemia. Josland (77) gave a drench of cobalt sulfate to four sheep at the rate of one milligram of cobalt per 200 grams of body weight per day for seven weeks. One sheep developed a polycythemia and two sheep developed a mild anemia. Two hoggets drenched with five milligrams of cobalt (given as cobalt sulfate) per day for ten months became anemic. No data on the blood picture were presented.

Josland (76) fed two rats a stock diet with one per cent of anhydrous cobalt sulfate added. Two other rats received the stock diet only. He reported an intense polycythemia and loss of body weight within seven weeks in the rats receiving the cobalt sulfate. Josland and McNaught (78) found that one milligram of cobalt (fed as cobalt sulfate) given daily to

eight rats produced persistent polycythemia in only one rat and transient polycythemia in the others. The early hematopoietic effect was not sustained. After the first sixteen weeks, the erythrocyte count fell to the level of the controls. The hemoglobin concentration increased for 32 weeks and then decreased.

In a study of the effects of heavy metals on anemia, K. W. Schultze (131) reported that daily doses of 0.5 to 2.0 milligrams of cobalt added as cobalt chloride to a milk diet fed to rats increased the erythrocyte count from 6,000,000 to 9,000,000 within ten days while the hemoglobin concentration was unaffected. The rats died within ten days. Polonovski and Briskas (126) fed one milligram of cobalt chloride per day to rats made anemic by a milk diet and observed an average erythrocyte increase from 4,680,000 to 6,960,000. White cells increased slightly from 20,000 to 25,000. Hemoglobin rose from 60 per cent to 70 per cent. Feeding two milligrams of cobalt chloride per day increased these values somewhat.

The mechanism of the polycythemic action of cobalt is unknown, although several investigators have tried to attack the problem. Berwald, Arsenau and Dooley (30) found that splenectomy prevented daily injections of cobalt from increasing the erythrocyte count in rats. They pointed out, however, that this might be due to an anemia produced by Bartonella muris, a common infection in splenectomies. Orten (121) found that the proportion of reticulocytes increased somewhat preceding the

increase of hemoglobin and erythrocytes in cobalt polycythemia. He thought this to represent an increased rate of formation of erythrocytes rather than a decreased rate of destruction. He concluded that the effect of cobalt was due to an active stimulation of the hematopoietic organs rather than the enhancing of a passive accumulation of erythrocytes.

Barron and Barron (14) injected ten milligrams of cobalt sulfate subcutaneously into rabbits daily and found a polycythemia to appear in six to seven days, accompanied by the appearance of reticulocytes and erythroblasts. When 60 milligrams of ascorbic acid were injected intravenously simultaneously with the cobalt sulfate injection, the polycythemia failed to appear in ten days. When the ascorbic acid injections were discontinued, the polycythemia appeared within six to seven days. They suggested that the cobalt inhibits the respiratory function of immature erythrocytes, causing them to be thrown into the circulation as mature non-respiring cells. They thought ascorbic acid to function as one of the regulators of the level of red cells in the circulatory system.

Davis (45) found that while both cobalt and anoxia produce polycythemia in dogs, only the cobalt polycythemia is depressed when daily injections of ascorbic acid are given at the rate of eight milligrams per kilogram of body weight. He found that the ascorbic acid content of blood of the dogs fed cobalt was diminished. He suggested that cobalt might interfere with a respiratory function of vitamin C. Oral

administration of cobalt at the rate of two milligrams per day produced polycythemia in dogs but had no other apparent effect.

Anderson, Underwood and Elvehjem (2) fed whole liver powder, liver extract and pantothenic acid concentrate to rats with a cobalt polycythemia and found that these substances aided in producing and maintaining a high level of cobalt polycythemia when added to a milk diet containing a daily supplement of 0.7 milligrams of iron as the pyrophosphate, 0.07 milligrams of copper as the sulfate, 0.03 milligrams of manganese as the chloride and 0.7 milligrams of cobalt as the chloride.

Michelazzi and Saviano (118) investigated the polycythemia caused by cobalt compounds in rabbits and guinea pigs. They reported that 20 milligrams of cobalt chloride injected subcutaneously into rabbits or 15 milligrams injected subcutaneously into guinea pigs produced a numerical increase in erythrocytes. In the rabbit, the increase was as much as 3,000,000 above the original number. The erythrocyte count receded spontaneously, however, after 16 to 24 hours.

Since Hart (68) and Potter, Elvehjem and Hart (127) showed that copper was a necessary dietary factor in order for anemic rats and dogs, respectively, to utilize iron for hemoglobin regeneration, several investigators have studied the effects of cobalt salts on this phenomenon. Underhill and others (141) showed that cobalt salts, among other

compounds, could not replace the copper as a supplement to iron in curing the nutritional anemia of rats.

McGhee (98) placed an alloy of iron, cobalt, manganese and copper in milk in an ice box and fed the "metalized milk" so obtained to rats whose hemoglobin concentration had been lowered to 75 per cent. The rats to which the "metalized milk" was fed showed rapid hemoglobin regeneration. There was a lower mortality than when salts of these metals were added to the milk. No attempt was made to determine the amounts of these metals in the "metalized milk" fed nor were any experiments made with any other combinations of these metals.

Orten and others (122) added 0.5 milligram of cobalt chloride or cobalt sulfate per day to a milk diet containing 0.5 milligram of iron and 0.025 milligram of copper and fed this to young rats. They reported a marked polycythemia with the hemoglobin content and erythrocyte count increasing on an average about 50 per cent above the level of the controls not given the cobalt salt. The cell volume increased 25 per cent but the leucocyte count was not significantly changed. Although cobalt and copper together produced a polycythemia, when the copper was omitted from the diet the rats developed an anemia and died. Later these workers (123) reported that a milk-iron-copper-cobalt diet produced a true polycythemia in rats characterized by an actual increase in cell volume. They also found that added manganese alleviated the toxic condition due to long administration of cobalt.

Beard and Andes (16) fed rats a milk diet with 0.25 milligram of iron added daily. When 0.15 to 0.30 milligram of cobalt was added to this ration daily, a polycythemia occurred without any increase in hemoglobin values. Hemoglobin and erythrocytes were both increased when vitamin D and 0.30 milligram of cobalt ion per day were added to the milk-iron ration and also when 0.30 milligram of cobalt ion and 0.05 milligram of copper ion were added to the milk-iron ration. They concluded that copper, more than the amount present in the body of the rat and in milk, did not influence the production of cobalt polycythemia.

Marshall (107) fed rats a milk diet to which cod liver oil and yeast had been added, together with a daily supplement of 0.5 milligram of iron as the chloride, 0.025 milligram of copper as the sulfate, 0.1 milligram of manganese as the sulfate and 0.5 milligram of cobalt as the chloride. A polycythemia developed which was lowered only temporarily by daily injections of a concentrated liver extract.

Underwood and Elvehjem (144) fed rats a milk diet with added copper, iron and cobalt salts. They reported no increased hemoglobin or growth response over that of rats fed the milk, iron and copper without added cobalt salt. However, they found enough cobalt present in the milk used to furnish each rat with about 0.6 microgram of cobalt per day. They concluded that if cobalt is required in the nutrition of the rat, it must be in amounts less than 0.6 microgram per day.

Frost, Spitzer, Elvehjem and Hart (63) fed dogs on a milk diet with added salts of copper, iron and manganese. Adding three to six milligrams of cobalt ion per kilogram of body weight produced a temporary polycythemia. In addition to this transient effect, they found that feeding 40 milligrams of cobalt ion daily to puppies produced no polycythemia at all. When dogs made anemic by bleeding were fed cobalt ion prior to feeding iron and copper, the normal hematopoietic response to the iron and copper was inhibited. The hematopoietic activity was resumed when whole dry liver or liver extract was fed.

Frost, Elvehjem and Hart (62) reported that while iron and copper sufficed for normal hemoglobin regeneration in dogs made anemic with a milk diet, 0.1 milligram of added cobalt ion per day stimulated hematopoeisis in dogs where hemoglobin formation was unusually slow with only iron and copper added to the milk ration.

In summarizing the work on the hematopoietic effect of cobalt salts, it may be said that most investigators have found that cobalt compounds, in sufficient amounts, increase the erythrocyte count and the hemoglobin concentration in the blood of several species of animals when ingested or injected, although there seems to be considerable biological variation in hematopoietic response between individual animals. There is not much agreement in the literature as to the minimal amount of cobalt necessary to cause hematopoiesis. The mechanism by which the effect occurs is not definitely known,

although some investigators have made tentative suggestions.

Toxic effects

The physiological effects of ingestion of cobalt salts by various species of animals have usually been studied in connection with experiments on their hematopoietic effect or their use in treating the cobalt deficiency syndrome previously discussed. Josland (76), for example, in studying the hematopoietic action of cobalt sulfate on a pair of rats, observed that addition of one per cent of anhydrous cobalt sulfate to a normal ration produced loss of body weight and, in one rat, death, within seven weeks. He found no cirrhosis of the liver in either rat. Davis (44) found that feeding dogs two milligrams of cobalt per kilogram of body weight per day for five months produced no toxic symptoms other than polycythemia; similarly, giving dogs six milligrams of cobalt (as cobalt chloride) per kilogram per day by stomach tube produced no toxic symptoms in three weeks. Josland (77) noted no toxic effects in the organs of sheep which had been given a drench of cobalt sulfate daily for seven weeks at the rate of one milligram of cobalt per 200 grams of body weight.

Griffith, Pavcek and Mulford (66) fed cobalt sulfate to rats and supplemented the basal rations with cystine, choline and cysteine, both individually and in combination. They found that growth was decreased by adding 0.12 per cent of cobalt sulfate heptahydrate but that this toxic effect of the cobalt

salt was alleviated by the supplements of cystine and choline and was especially relieved by cysteine. Cobalt is known to form a complex with cystine and they believed that the detoxification of the cobalt by these substances operated through such a mechanism.

Sutter (137) subcutaneously injected small amounts of cobalt chloride in physiological salt solution, pH seven, into mice, frogs and guinea pigs. Besides observing a marked polycythemia, he determined the toxic dose of cobalt chloride to be about two to three milligrams per ten grams body weight of frogs and about one milligram per ten grams body weight of mice. Yosada (153) reported the lethal subcutaneous doses of cobalt chloride and cobalt sodium citrate to be 0.1 milligram of cobalt per ten grams of body weight in frogs and 0.3 milligram of cobalt per ten grams of body weight in mice. Caujolle (38) reported the lethal dose of cobalt chloride injected intravenously into dogs to be more than 30 milligrams of cobalt per kilogram of body weight. Massol and Breton (114) found that one milligram of cobalt sulfate injected into the brain of a guinea pig did not produce death. Villaret, Bertrand and Justin-Besançon (148) reported that liver cirrhosis resulted after hypodermic injections for five days a week of two millliliters of 0.5 per cent cobalt acetate in guinea pigs and four millliliters of one per cent cobalt acetate in rabbits. They presented little data on their experiments or observations, however. LeGoff, in a series of articles (90,

91, 92, 93), found that cobalt chloride, cobalt citrate and cobalt salicylate produced a vasodilating action in the head, ears and face of men when isotonic solutions were injected subcutaneously in doses of 0.01 to 0.05 gram of cobalt. The reaction was instantaneous and lasted five to fifteen minutes or more. There were no other toxic effects. Larger doses led to nausea and intestinal pain. In another study, LeGoff (88) injected 0.09 gram of cobalt chloride into rabbits daily for four months without observing any ill effects.

Elimination after ingestion or injection

Caujolle (37) injected 1/20 molar cobalt chloride solutions parenterally into a dog in whose bile duct a catheter had been introduced. He found that after 147.5 milligrams of cobalt chloride were injected parenterally, only 2.1 milligrams appeared in the bile. Caujolle and Lafitte (39) injected cobalt chloride solutions into dogs intramuscularly and intravenously and found only traces of cobalt in the bile. They found cobalt to be eliminated in both urine and feces under these conditions. They thought that urinary elimination occurred only slowly, but since their first urine analysis was not made until ten hours after the injection, it is difficult to see on what evidence this idea is based. Stuart (136) injected cobalt acid tartrate subcutaneously into several species of animals and found that the volume of urine was generally increased. He attributed this to an increased water

intake by the animals. LeGoff (88) injected cobalt chloride into rabbits and found that only 20 to 25 per cent of the cobalt was eliminated in the urine in the next 24 hours. The cobalt was determined electrolytically in the ashed residue from evaporation of the urine. Later LeGoff (89) injected 0.6 per cent cobalt chloride into men and women. After injections of 24 milligrams of cobalt chloride, one subject eliminated 6.8 milligrams and 6.4 milligrams in two tests. Another subject (a diabetic) eliminated 2.64 milligrams. The same electrolytic method was used as before in determining the amount of cobalt present. Duval and LeGoff (51) determined the amount of cobalt in human urine after intramuscular injection of one to two milliliters of isotonic cobalt chloride solution. They claimed that some people did not eliminate cobalt for 24 hours after injection. They concentrated the cobalt by electrolysis of the urine in a U-tube and determined the amount of cobalt with alphanitrosobetanaphthol in acetic acid. Mascherpa (111) gave dogs, by mouth, one milligram of metallic cobalt per kilogram of body weight and claimed that the urinary nitrogen excretion was increased, although his data do not seem to support this view.

In experiments on mineral metabolism with the use of radioactive isotopes, Copp and Greenberg (40) gave Co^{56} as cobalt chloride to two 250 gram rats, both intraperitoneally and by stomach tube. Ninety-six hours after giving doses of ten micrograms of cobalt ion, 0.46 microgram of cobalt was retained

after the intraperitoneal injection and 0.27 microgram was retained after administration by stomach tube. When parenterally administered, the chief path of excretion was by the urine, although there was a small continuous elimination in the feces. Over 60 per cent of the cobalt was recovered in the feces after administration by stomach tube. Most of the cobalt absorbed was rapidly excreted in the urine. In both cases 70 per cent of the cobalt was excreted in the first ten hours and more than 90 per cent was excreted within two days.

Askew and Josland (11) drenched sheep with four milligrams of cobalt chloride and determined the amount of cobalt present in the urine and feces by the nitroso-R-salt method. Within 24 hours of the drenching, a large proportion of the total cobalt found in the urine had already been excreted and only a small amount of cobalt appeared in the urine after 72 hours. The cobalt in the urine, however, represented only 2 per cent of the total given, the great bulk of it appearing in the feces passed during the first 48 hours after drenching. After 120 hours, no cobalt from the drench appeared in either urine or feces. Apparently some cobalt was stored in the tissues.

Effects on some enzyme systems

Several investigators have studied the effects of cobalt on various enzyme systems. Cori, Colowick and Cori (41), in a study of the enzymic conversion of glucose-1-phosphoric ester to the hexose-6-ester in tissue extracts, showed that the

activity of the phosphoglucomutase involved was increased up to fifteen times by the presence of cobalt ion, among other metallic ions tested. Lehmann (94) showed that both cobalt nitrate and reduced glutathione increased the formation of hexose-6-phosphate from glycogen but not the glucose-1-phosphate, the cobalt nitrate being effective at lower concentrations than the glutathione. A 1/850 molar cobalt nitrate solution added to an undialyzed rabbit muscle extract formed 0.1 milligram of glucose-1-phosphate and 3.6 milligram of the hexose-6-phosphate while a control run simultaneously without cobalt nitrate formed 0.1 milligram and 2.6 milligrams respectively. The cobalt could not act by the same mechanism as the reduced glutathione since it not only reacted at a much lower molarity but actually removed free -SH groups, as shown by negative nitroprusside tests.

Thannhauser, Reichel and Cratton (140) found that cobalt ion increased the ascorbic acid activation of serum β-glycerophosphatase. The cobalt ion and the ascorbic acid formed a complex.

Hellerman and Perkins (70), in an investigation of the role of metal ions in the activation of arginase, found that the hydrolysis of arginine was induced by the activating action of cobalt, nickel, manganous or ferrous ions on urease. The minimum concentration of cobalt ion necessary for maximum activation under conditions of the experiment was 1×10^{-5} molar. Hellerman and Stock (71) found that liver arginase action was

enhanced by nickel ion and especially by cobalt ion from pH values of 5 to 7.7. Edlbacher and Baur (53) found that liver arginase, inactivated by several weeks dialysis, was reactivated by cobalt sulfate and other metal sulfates in 0.001 molar concentration. Manganous sulfate was active at a concentration of 3.3×10^{-6} molar while other metal sulfates were not, so these workers believed manganese to be the coenzyme.

Jacoby and Shimizu (75) found that 0.2 to 1.0 gram of powdered metallic cobalt destroyed the activity of 25 milliliters of 0.3 per cent urease solution in 20 minutes. Adding five milliliters of five per cent glycocoll solution to ten milliliters of urease solution inactivated by cobalt or one milligram of potassium cyanide to 25 milliliters of inactivated urease solution reactivated the enzyme. The glycocoll was the more effective, however.

Michaelis and Stern (117) studied the effects of some heavy metals on the activity of calf spleen cathepsins against its own spleen proteins. Ferrous sulfate, zinc sulfate and cobalt sulfate increased the enzyme activity while mercuric sulfate depressed the enzyme activity. If the activity of the enzyme alone was called 100 per cent, then a mixture of enzyme and cobalt sulfate (9.6×10^{-2} milligrams of cobalt ion per milliliter) showed an activity of 124 per cent and a mixture of enzyme and cobalthexamminchloride had an activity of 158 per cent.

Bernheim and Bernheim (21) found that cobalt sulfate partially, and manganese chloride completely inhibited the oxidation of phospholipides catalyzed by the washed liver protein-vanadium system. Nickel, iron, titanium and chromium salts had no effect. Oxidation of cysteine to its sulfonic acid by washed liver protein was also inhibited by cobalt, manganese and titanium salts. Cobalt salts added to rat liver had little effect on the oxygen uptake.

Effects on the action of insulin

Several articles have appeared in the literature concerning a possible relationship between cobalt and the hypoglycemic action of insulin. Bertrand and Mâcheboeuf (24) reported finding 62 to 100 milligrams of cobalt in 200 gram samples of pancreas from five species--cow, calf, horse, sheep and pig, so feeling that there might be some relation between cobalt and insulin, they analyzed four different insulin preparations and found 15 to 42 milligrams of cobalt per 100 units of insulin. Later (25) they injected rabbits intraperitoneally with isotonic salt solutions containing cobalt (as chloride or sulfate) simultaneously with injections of insulin. Their data, presented in the form of graphs, show no great variation in the blood sugar curves for the rats receiving insulin alone and those receiving insulin with added cobalt salt, but nevertheless they concluded that cobalt did prolong the action of the insulin, although it did not increase the velocity of

the hypoglycemic action. The same investigators (26) later reported the same results in experiments on dogs. Insulin action was prolonged, the blood sugar remaining at low values for a longer time when cobalt was injected with the insulin.

Labbé, Roubeau and Nepreux (86) obtained similar results with a rabbit injected with three units of insulin and later with three units of insulin and 0.3 milligram of cobalt sulfate. Three hours after the injection, the blood sugar was 77 milligram per cent in the rabbit given insulin alone and 44 milligram per cent in the rabbit given insulin and cobalt sulfate. Three hours and forty-five minutes after the injection, the values were 94 and 54 milligram per cent respectively. Although these represent data from one experiment on one rabbit, the investigators concluded that cobalt sulfate increased the hypoglycemic action of insulin.

Magenta (106) reported later that cobalt nitrate given to dogs slightly increased the glycemia while cobalt nitrate given with insulin to dogs did not increase the hypoglycemic action of the insulin. He presented no data to substantiate these assertions.

Blatherwick and Sahyun (31) injected intravenously 0.041 milligram of cobalt (as cobalt salt) per kilogram of body weight into rabbits and found that there was no effect on the blood sugar concentration. They injected intravenously 0.009 milligram of cobalt ion per kilogram of body weight simultaneously with insulin into rabbits and determined the blood

sugar before injection and 1.5 and 3.0 hours after injection. A week later they determined blood sugar at the same intervals in the same rabbits after injection of the same amount of insulin but without any cobalt salt. By comparing the sets of data obtained, they found that three of the five rabbits tested had a somewhat greater hypoglycemia with the cobalt present, one rabbit had less hypoglycemia and the data for the fifth rabbit were inconclusive. Subcutaneous injection of 0.04 to 0.06 milligram of cobalt ion per kilogram of body weight in the same type of experiment enhanced the hypoglycemia in only one of four rabbits. They concluded that cobalt ion was without appreciable effect on insulin hypoglycemia in the rabbit.

Rathery and Levina (129) studied the effect of a mixture of cobalt and nickel salts on human diabetics. They claimed that injections of an isotonic salt solution containing 0.1 milligram of the metals at the rate of 2.5, 5 or 10 milliliters every two days exerted a favorable action in five cases and did not exert a favorable action in six cases. They presented no data and did not state what they considered a "favorable action".

Labbé, Roubeau and Nepreux (85) determined blood sugar concentrations in nine diabetics given nickel or cobalt salts at various levels. The cobalt solution injected at the rate of 1.0 milligram of cobalt chloride per kilogram of body weight had no hypoglycemic action either with or without simultaneous

insulin injections. Similar results were obtained for the nickel ion compounds. These workers concluded that these metals had no hypoglycemic effect in the human diabetic.

Sahyun, Nixon and Goodell (130) added 1.0 milligram of cobalt chloride to 1000 units of insulin and found that the physiological activity of the insulin was preserved after incubation at 52° for seven weeks or longer. They concluded that cobalt ion was as effective as zinc ion in preserving insulin.

Analytical Methods for Determination of Cobalt in Biological Material

It is obvious that no reliable data on the distribution of cobalt in plant materials or in animal organs or excretions can be obtained without a satisfactory analytical method for determining the amount of cobalt present. A special difficulty is that the amount of cobalt in such materials is usually very small--a few parts per million at the most and often only a few tenths of a part per million. Several workers have attempted to adapt known methods or devise new ones for making accurate determinations of such minute quantities of cobalt.

Bertrand (27) (28) and some of his co-workers, early investigators in this field, determined the cobalt present in soils, seeds, animal tissues, etc., by precipitating the cobalt as potassium cobaltinitrite. They claimed that five

micrograms of cobalt could be detected in this way. Even if this remarkable claim is true, this is greater than the amount of cobalt often found in any manageable weight of sample. Later (23) they developed another method of separating cobalt and determining it by the brown color developed with dimethylglyoxime. Caujolle and Lafitte (39) precipitated the potassium cobaltinitrite, oxidized it with standard potassium permanganate and titrated the unused excess of permanganate. They obtained data on the cobalt content of various animal tissues and excreta in this way.

LeCoff (88) determined cobalt in urine by an electrolytic method after evaporation of the urine and ashing the dry residue. Bayle and Amy (15) deposited traces of cobalt electrolytically on copper or zinc cathodes and determined the cobalt spectrographically. They claimed that this method would detect ten micrograms of cobalt. Dutoit and Zbinden (49, 50) using a spectrographic method, reported finding cobalt in the human pancreas and occasionally in human blood but never in human liver. They gave no data on the amounts of cobalt present. Fox and Ramage (60, 61) analyzed animal tissues spectrographically and reported a spasmodic distribution of cobalt. They found cobalt only in marine animals. Webb (151) was unable to detect cobalt in animal tissues by spectrographic means and concluded that the concentration of cobalt in such material was below the limit of sensitivity of the spectrographic method.

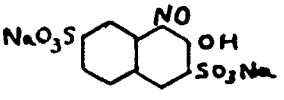
Wright and Papish (152) and Blumberg and Rask (32) were unable to find cobalt spectrographically in milk ashes although Underwood and Elvejhem (144), by means of a colorimetric method, later showed milk to contain small amounts of cobalt. Becker and Gaddum (17) applied a spectrographic method to the analysis of limonites. A sample which had been found effective in curing bush-sickness was found to contain 0.005 per cent of cobalt. No cobalt was found in an ineffective sample. No other element present in the effective ore was absent in the ineffective ore, leading these workers to conclude that cobalt was the effective element.

Of the colorimetric methods for determining cobalt, the alphanitrosobetanaphthol method was used by McHargue (100) who reported finding traces of cobalt in Kentucky blue grass. Paulais (125) claimed to be able to detect one to ten micrograms of cobalt per milliliter by this method and reported finding cobalt in the organs of a mollusk. Duval and LeGoff (51) claimed that 0.05 microgram of cobalt could be determined with alphanitrosobetanaphthol in acetic acid when this method was applied to the analysis of human urine previously concentrated electrolytically in a U-tube.

Bertrand and Mâchebœuf (22, 23) separated cobalt from ashed materials from animals and plants by extracting with ammonium hydroxide and hydrogen peroxide, precipitating with hydrogen sulphide, dissolving the nickel and cobalt and precipitating them with dimethylglyoxime. This was extracted

with chloroform, evaporated in a porcelain dish and the color obtained compared with standards. This procedure produced data whose maximum cobalt values were from 0.05 to 2.0 parts per million in animal organs.

In 1933 Stare and Elvehjem (134) adapted the colorimetric method of Van Klooster (147) for cobalt determinations on biological material. This method makes use of a reagent, nitroso-R-salt, whose structural formula is



This obviously is a derivative of the alphanitrosobetanaphthol reagent but instead of producing a red precipitate with cobalt, it forms a red color in solution. Stare and Elvehjem were able to detect 0.01milligrams of cobalt as the lower limit of the method but modifications of the method made by Kidson, Askew and Dixon (82) and McNaught (104) in New Zealand (where the influence of cobalt on bush sickness had just been discovered) soon improved the sensitivity of the method. McNaught (101) found that the smallest amount of cobalt which could be determined with this modified method was about 0.2 microgram. Underwood and Elvehjem (144) claimed that 0.5 microgram was about the smallest amount of cobalt that could be determined with any accuracy with the modified procedure. Kidson and Askew (81) examined the procedure critically and recommended a minor change in a neutralization step which gave

more consistent results. McNaught (102) found that this slight change enabled 0.05 microgram of cobalt to be detected. No interference with the cobalt determination was noted when 100 micrograms of copper and 1000 micrograms of iron were present. Ahmad and McCollum (1) used the method to analyze plant materials and reported that it was not necessary to remove iron or copper from these substances before determining the cobalt present.

The nitroso-R-salt method was found to be unsatisfactory in the presence of much calcium due to precipitation occurring at the neutralization step and carrying down cobalt, so Lugg and Josland (96) devised another modification in which calcium ion was effectively removed from solution by complex formation with added citrate salts.

Sylvester and Lampitt (138) found the nitroso-R-salt method unsuitable for plant materials where a high ash content was obtained. They treated the hydrochloric acid extract of the ash with alphanitrosobetanaphthol, extracted with dithizone reagent and finally determined the cobalt present with nitroso-R-salt, thus avoiding interference of other metal ions. Marston and Dewey (109) obtained a solution of plant and animal tissues by treating them with nitric, perchloric and sulfuric acids. The cobalt present was removed with dithizone and the nitroso-R-salt color developed in citrate-sodium carbonate buffer, the yellow color of the excess nitroso-R-salt being

destroyed by adding bromine. They were able to recover and determine two micrograms of cobalt with an error of about one per cent.

Hibbard (72) has suggested a procedure for estimating cobalt with choline in a dithizone extract. This method was particularly adapted to determinations of cobalt in soils.

To summarize the analytical methods for determining cobalt in biological materials, it is apparent that the colorimetric nitroso-R-salt method is the most satisfactory one available at the present time. Not only does it require less expensive equipment than the spectrographic method, but it is also more sensitive, as evidenced by the wide disagreement as to the cobalt content of various biological materials among investigators using the spectrographic method. This need not be surprising in view of the complexity of the cobalt spectrum and the relatively recent rise to prominence of very sensitive colorimetric methods for determination of many elements by means of various organic reagents.

Cobalt Content of Plant Materials

Bertrand and Mokragnatz (27, 28) analyzed twenty samples of food vegetables and found cobalt in most of them, ranging from 0.02 to 0.3 part per million. Cobalt was found in all plants examined in a later study. No data were presented in these articles, however. McHargue (99, 100) reported finding

traces of cobalt in Kentucky blue grass and in soy beans. Ramage reported the occurrence of cobalt in St. Ignatius beans.

The more reliable data in this field, however, have been obtained more recently with the nitroso-R-salt method, usually in connection with recent studies on bush sickness. Pasture plants, from both bush-sick and healthy areas, have been analyzed for cobalt by several workers. Their data are summarized in Table 2.

Table 2

Cobalt Content of Pasture Plants

Plant	Cobalt content (parts per million): From healthy area	From bush-sick area	Reference
"Pastures"	0.03-0.43	0.02-0.07	Underwood and Harvey (146)
Alfalfa hay	0.12	0.03-0.06	Killham (84)
"Pastures"		Less than 0.04	McNaught (103)
"Pastures"	0.04-0.37	0.005-0.17	McNaught and Paul (105)

Some investigators have noted a seasonal variation in the cobalt content of plants. Askew and Maunsell (12) found the cobalt content of pastures to be distinctly higher in the spring than later in the season. McNaught and Paul (105) found the cobalt content of pastures to increase in winter when growth was retarded and to decrease in the spring when growth was rapid. McNaught (103) claimed that the minimum cobalt content occurred

in December (a summer month in New Zealand where the study was made). Kidson and Maunsell (83) state that adding cobalt chloride to superphosphate fertilizers used on cobalt-deficient soils produced an increased cobalt content in fodder crops (turnips, swedes, rape, oats). Bonner, McNaught and Paul (33) found the cobalt content of pastures to increase from 0.06 to 1.03 part per million when cobalt sulfate was used as a soil top dressing.

Analyses of other plant materials reported in the recent literature are summarized in Table 3.

Table 3

Cobalt Content of Plant Materials

Material	Cobalt content (parts per million)	Reference
Wheat germ oil	0.007	Grimmett (67)
Corn starch	0.003	"
Casein	0.01	"
Dried yeast	0.038	"
Beans (dry)	0.18-0.475	Ahmad and McCollum (1)
Peas (dry)	0.14-0.42	"
Corn (dry)	0.06-0.08	"
Wheat	0.06-0.15	"
Peanuts	0.57	"
Pecans	0.25	"
Tea	0.12-0.19	Sylvester and Lampitt (138)
White flour	0.003	"
Coffee bean	0.028-0.049	"
Cacao bean	0.03-0.41	"

Cobalt Content of Animal Materials

Bertrand and Mâcheboeuf (23, 24) reported finding more cobalt in animal tissues than in plant materials by use of an analytical method utilizing potassium cobaltinitrite. They found that beef liver contained 0.20 part per million of cobalt while the pancreas contained 0.23 part per million. Pancreas of other animals seemed to contain less cobalt: cow, 0.075; calf, 0.070; horse, 0.100; sheep, 0.075; and pig, 0.062 part per million. They found that insulin preparations contained 0.015 to 0.042 part per million of cobalt. Caujolle and Lafitte (39) reported that much higher concentrations of cobalt were present in pancreas (94 to 198 parts per million) and liver (53 to 289 parts per million) of dogs which had previously been injected with solutions of cobalt salts. They reported no cobalt analyses on normal dogs, however.

Dutoit and Zbinden (49), using a spectrographic method, reported finding cobalt in the ash of animal organs and blood. Later (50) they reported finding cobalt in the pancreas but not in the liver. Fox and Ramage (60, 61) also determined cobalt spectrographically and reported finding 0.3 part per million of cobalt in the liver of Archidoris tuberculata. They reported finding cobalt only in marine animals.

A number of recent investigators have used the nitroso-R-salt method for determining cobalt in animal tissues.

Josland (77) gave a cobalt sulfate drench to sheep for seven weeks at the rate of one milligram of cobalt per 200 grams body weight and found that the cobalt content of the organs was much higher than in sheep which had not been fed cobalt. Josland (76) also investigated this effect on rats. Two rats were fed cobalt sulfate and the analyses of their organs were compared with those of organs from normal rats. These data are shown in Table 4.

Table 4

Cobalt Content of Rat Organs

Organ	Parts per million of cobalt in fresh tissue		
	Control rats (not fed cobalt)	Rats fed cobalt	
		Rat No. 1	Rat No. 2
Carcass	0.015-0.025	3.33	2.61
Spleen	0.63	17.9	6.2
Pancreas	0.75	18.9	16.7
Kidneys	0.3	21.2	17.1
Liver	0.36	57.5	43.6

It is apparent that the greatest increase in cobalt content occurred in the liver with the kidney taking up the next greatest amount.

Underwood and Harvey (146) analyzed livers of bush-sick and of healthy sheep. The livers of bush-sick sheep contained 0.03 to 0.14 part per million of cobalt (on the dry matter basis) while those of healthy sheep contained 0.08 to 0.58 part per million of cobalt. McNaught (104), in a critical study of the nitroso-R-salt method, found that rat livers

contained about 0.20 part per million of cobalt on the average. Stare and Elvehjem (134) fed rats on a milk-iron-copper-manganese diet and found that the entire bodies of the rats contained less than 0.01 milligram of cobalt (the limit of sensitivity of the analytical procedure they used). When polycythemia occurred 0.04 to 0.05 milligram of cobalt was found in the entire body. Josland and McNaught (78) reported that a polycythemic rat contained 0.050 milligram of cobalt in its entire body after being fed one milligram of cobalt (as cobalt sulfate) for 48 weeks. Rats which had been fed this amount of cobalt without developing polycythemia contained only 0.030 milligram of cobalt in the entire body. Control rats not fed cobalt had only 0.0047 milligram of cobalt in the entire body.

Askew and Dixon (9) analyzed the pancreas, liver and spleen from sheep that had been drenched with cobalt salts in bush sickness experiments and obtained the data shown in Table 5.

Table 5

Cobalt Content of Sheep Organs

Organ	Parts per million of cobalt in dried tissue	
	Sheep not given cobalt	Sheep given cobalt
Pancreas	0.02	0.07
Liver	0.02	0.20
Spleen	0.03	0.04

A number of food materials of animal origin have been analyzed for cobalt with the nitroso-R-salt method or modifications of it. These data are given in Table 6.

Table 6

Cobalt Content of Some Food Materials of Animal Origin

Food	Parts per million of cobalt	Reference
Butter	0.013	Grimmett (67)
Lard	0.007	"
Cod liver oil	0.004	"
Pancreas	0.18-0.24	Ahmad and McCollum (1)
Milk	0.001	Sylvester and Lampitt (138)

Addenda

Some investigators have studied the biological effects of metallic cobalt on certain animals. The work of Maschorpa (113) might be cited. He claimed that he had obtained cobalt-protein compounds by treating proteins with metallic cobalt and that when proteins from a particular animal tissue were used to make such a substance and the resulting compound injected into another animal, preferential deposit of cobalt occurred in the homologous tissue of the second animal. This whole idea has been categorically denied by Lazaris (87) who could not duplicate Maschorpa's work.

A number of reviews of the literature on the biological significance of cobalt have appeared in recent years. Among

the shorter reviews are those of Godden (64), Fairbanks (54), Schultze (132) and McCollum (97). More complete reviews have been made by Marston (108), Underwood (143) and Maynard (115).

EXPERIMENTAL

Plan of Experiment

In investigations such as those for this thesis it is not always possible to make a detailed plan of all the experiments to be made, since results of one experiment often suggest other studies which might be of interest. In general, however, it was planned to feed rats the laboratory's stock growing ration with cobalt salts added to furnish various concentrations of cobalt and to study the effects of these diets on growth, reproduction and lactation. It was also planned to investigate the composition of blood and urine with respect to a few of the more common constituents for which satisfactory analytical methods are available. These are limited by the very small blood samples obtainable from rats without causing serious injury to the animal. More specifically, it was planned to study the changes produced in the blood in hemoglobin concentration, erythrocyte count, non-protein nitrogen concentration and blood sugar concentration and to examine the urine for albumin and for reducing sugar after the rat had ingested simple cobalt salts, either fed in the diet or administered by stomach tube. It was thought best to restrict the investigation to these means of entry of cobalt

ion into the organism (through the digestive tract) since these are more closely related to nutritional problems than other means of injection of test solutions into animals. Of interest in this connection was the determination of the minimum lethal dose of cobalt (as cobalt salt) when given by stomach tube and the minimum concentration of cobalt salt in the diet necessary to stimulate hematopoiesis. It was also planned to make cobalt analyses of such tissues as liver, kidney, pancreas, spleen, heart, lungs, stomach, intestine and testes, both from rats which had been fed cobalt salt and from those which had not received added cobalt salt in the diet.

Materials and Methods

Rations

The basal ration fed all the rats in this study was the growing ration on which the stock rats of the colony of the chemistry department have been grown successfully for many years. It consisted of a mixture of natural feeds in the following parts per volume: ground hulled oats, 4 parts; ground yellow corn, 4 parts; ground wheat, 1 part; alfalfa meal, 1 part; tankage, 0.5 part; buttermilk powder, 0.5 part; linseed meal, 0.5 part. To this mixture 0.35 pound of bone meal and 0.5 pound of sodium chloride were added for each 100 pounds of ration. These materials were obtained from a dealer in Ames, Iowa, and were mixed every two weeks by the regular laboratory staff.

Analyses of the growing ration for cobalt by the nitroso-R-salt method showed a variation in the cobalt content from 0.27 to 0.42 part per million over a period of six months. The average cobalt content was 0.33 part per million and this is so much less than the lowest concentration of cobalt ion fed (5 parts per million) that it need not be considered in these experiments.

No investigator of the cobalt problem has described his method for incorporating small amounts of cobalt salts into the rations used in his experiments. Since failure to obtain a feed mixture of approximately uniform cobalt content is obviously important to the validity of the experiment, special attention was given to the method of adding the cobalt salts to the basal ration. The calculated amount of cobalt salt was weighed out on an analytical balance to at least three significant figures, transferred to a small wash bottle, dissolved in 10-15 milliliters of distilled water and sprayed over the surface of a weighed amount of basal ration in a large flat pan. This was then mixed by hand so that the clumps of feed moistened with the cobalt salt solution seemed evenly distributed and not larger than the size of a bean. The ration was allowed to stand until the clumps containing the cobalt salt were thoroughly dry. The entire ration was then sifted through a small flour sieve (holding about 200 grams of ration) into another large flat pan and the unsiftable residue from each sievefull was ground in a mortar and pestle. This

was then resifted over the contents of the pan, the unsiftable residue again ground in the mortar and resifted on to the contents of the pan as before. This always reduced the cobalt clumps to such a fine powder that none was apparent in the small amount of unsiftable residue remaining. This, consisting mostly of pieces of cracked corn and a few oat hulls, was added to the previously sifted material so that its composition might remain the same as the growing ration used for the control groups, except for the added cobalt salt. The entire sifted ration was next mixed thoroughly by hand and stored in glass jars with screw tops.

These rations were mixed in this way every two to three weeks for each group of rats and their homogeneity is demonstrated by the smooth growth curves obtained during experimental periods of as long as 40 weeks.

Care of animals

The rats used for the feeding experiments were selected from the young of the stock colony at weaning age (approximately 28 days), their initial weight being 45-55 grams when each experiment was started. They were assembled into groups of three males and three females and housed in metal cages measuring 24 inches long, 12 inches wide and 8 inches high. The cages were arranged in batteries and kept in the same room as the stock colony rats where the temperature was maintained at approximately 28° C. the year around. Each cage, except as

noted below, had a removable screen bottom with a sliding pan underneath. The bottom of the pan was covered with wood shavings and the pans were removed, cleaned and filled with fresh shavings each week. The wire screens were removed regularly, cleaned in running water and dried before being replaced.

Water was supplied to each cage from an inverted bottle fitted with a one-hole rubber stopper and a bent glass tube drinking fountain. The rats were allowed to drink as much water as they wanted. Fresh water was supplied at all times and the bottles were cleaned thoroughly with a brush every week.

Feed was supplied to each cage in a tin cup soldered to a tin plate and set inside a pan with high walls to prevent scattering of the food. All the groups (except certain control groups) were supplied with all the feed they would eat. At regular intervals the cups and pans were washed thoroughly and dried.

Pregnant females were segregated from their groups and placed in cages provided with wood shavings but no screen bottoms. In experiments where lactation was studied, the mothers were permitted to rear their young until 28 days after birth when the young were destroyed and the females were returned to the original group.

For experiments where cobalt salts and other substances were injected into the stomach, young or mature rats were

selected from the stock colony, placed in screen bottom cages and supplied with water but no food. They were fasted in this way for approximately 24 hours before use. Their weights were recorded prior to the experiment.

Operative methods

Collection of urine. Urine specimens were collected by placing rats individually in a circular metabolism cage made of galvanized screen wire with three meshes per inch. The cage was 8 inches in diameter and 8 inches high. It was equipped with two small circular openings through which the rat could put its head to obtain feed and water. The cage was set on an eight inch glass funnel which directed the urine excreted into a test tube graduated in tenths of a milliliter. A porcelain Gooch disc in the apex of the funnel prevented feces from falling into the urine. The feed and water receptacles were placed outside the rim of the funnel so that no feed or water could fall into the urine. Rats were placed in the cage in the morning and the urine collected was taken for analysis at the same hour the following morning. The cage and funnel were washed thoroughly with water and dried each time they were used.

Anesthesia. In order to pass a stomach tube into rats without exciting them, they were anesthetized with intraperitoneal injections of a nembutal solution containing 3 milligrams of nembutal per milliliter and injected at the rate

of one milliliter per 100 grams of body weight. In order to make the injection, the rat was placed on the table, covered with one thickness of a towel to avoid danger from biting and grasped with the left hand with the rat's head toward the wrist and the fingers encircling the body, the little finger encircling the throat. The rat was turned over, the head lowered to allow the gut to slip forward, and the needle of an injection syringe containing the calculated volume of nembutal solution inserted into the peritoneum near the midline of the body with the needle directed toward the posterior. The solution was injected, the needle withdrawn and the rat placed in a bucket until needed. The entire operation can be done rapidly and there is usually little struggling. Anesthesia was usually complete in 10-15 minutes and lasted one to two hours. The hypodermic syringe and the 22 guage needles used were obtained from Becton, Dickinson and Company.

Use of stomach tube. In order to inject test solutions into the stomachs of rats, a French No. 8 catheter about six inches long was used as a stomach tube. One end of the tube was fitted to the hub of a hypodermic needle cut off for this purpose and was attached by this means to a hypodermic syringe. The end of the catheter was dipped below the surface of some of the test solution in a small test tube and drawn into the syringe. The syringe was then inverted and enough of the contents expelled to remove all air bubbles from the syringe and catheter. With the desired volume of test solution in the

syringe, the catheter was passed into the rat's stomach by grasping the tongue and pulling it forward while the catheter, moistened with water, was inserted with a rotary motion down the esophagus. With a little experience it was found possible to insert the tube into the esophagus and not the trachea. The rat was raised into a vertical position, head up, the syringe emptied and the catheter removed. It will be noted that the catheter itself remained filled with test solution both before and after the injection, making possible the injection of precise volumes of solution from the syringe itself.

Blood sampling. Blood samples for counting erythrocytes and determining blood sugar were obtained from the tail. The larger samples (0.1 ml.) needed for determination of non-protein nitrogen were obtained from the external saphenous vein. In each case, however, blood was drawn from a free-flowing incision.

To draw blood from the tail, the rat was wrapped in a towel with the tail protruding, the tail wiped and stroked once or twice toward the tip. An incision was made with a sharp scalpel in the prominent vein near the tip of the tail and a sample of blood drawn into a pipette of suitable size. Trenner pipettes were used for the erythrocyte counts and pipettes marked at 0.05 milliliter were used for the blood sugar samples.

To draw blood from the external saphenous vein, the rat was rolled in a towel with one hind leg extended outside, the inside of the leg was shaved and the saphenous vein punctured with a lancet. Blood was drawn from the free-flowing puncture into a 0.1 millliliter pipette. Care was taken to avoid severing the vein and producing blood clots under the skin.

Analytical methods

Hemoglobin. Hemoglobin was determined by the acid hematin method as described by Hawk and Bergein (69). Readings were made in a hemoglobinometer obtained from the Bausch and Lomb Optical Company.

Erythrocyte counts. Erythrocyte counts were made with a Trenner diluting pipette and a Levy counting chamber with the improved Neubauer ruling. Physiological salt solution was used as the diluting fluid.

Determination of pH. The pH of the cobalt chloride solutions and the pH of the hydrochloric acid solution were measured on a Cameron pH Meter manufactured by the Webster Electric Company, Racine, Wisconsin. The current was turned on and the instrument allowed to operate long enough before making the pH determinations so that there was no drift in the meter reading.

Blood sugar. Blood sugar was determined by the method of Folin and Malmros (59) as modified by Keil and Nelson (79).

Non-protein nitrogen in blood. Non-protein nitrogen was

determined in blood by the method of Folin and Wu described by Hawk and Bergeim (69) as modified by V. B. Fish (58) for small samples of blood.

Reducing sugar in urine. Reducing sugar in the urine was determined by Sumner's method as described by Hawk and Bergeim (69).

Cobalt. Cobalt was determined by the colorimetric nitroso-R-salt method of Stare and Elvehjem (134) as modified by Kidson, Askew and Dixon (82) and Kidson and Askew (81). The procedure outlined in the following paragraphs was found to give consistent results and showed excellent recoveries of cobalt salts added to animal and plant tissues.

Mince the tissues with a scalpel and ash the weighed sample in a muffle furnace, raising the temperature gradually to avoid spattering. Finally maintain at a red heat for three hours to insure complete destruction of organic matter.

Dissolve the residue in 15 ml. of HCl (specific gravity 1.12), warm till dissolved, cool and extract with ether using 20 ml. of ether for each 10 ml. of solution. Warm to expel ether from the water phase and extract again with ether. Evaporate the water phase to dryness.

Take up the residue with 0.5 ml. of HCl (specific gravity 1.12) and six drops of HNO_3 (1:1). Add enough water to bring the volume to 15 ml. and boil to oxidize any reducing substances. Cool. Add two ml. of 0.1 per cent nitroso-R-salt solution and then two grams of sodium acetate. Heat to 70°.

Add five drops of phenolphthalein and then ten per cent KOH drop by drop with shaking until pink. Add three more drops of phenolphthalein and just destroy the pink color with 0.5 N HCl. Boil exactly two minutes, keeping the solution just acid to phenolphthalein. Add 20 drops of HNO_3 (1:1) and boil two minutes more. Cool under the tap and make up to volume in Nessler tubes, filtering into the tubes if there is any precipitate or cloudiness. Compare with standards prepared the same day using known (and comparable) amounts of cobalt according to the procedure of this last paragraph

Chemicals

Cobaltous chloride. The cobalt chloride used was Baker and Adamson's reagent quality $CoCl_2.6H_2O$. The maximum limits of impurities stated on the label were: SO_3, 0.0025 per cent; Cu, 0.000 per cent; Fe, 0.005 per cent; Pb, 0.000 per cent.

Cobaltous nitrate. Cobalt nitrate, $Co(NO_3)_2.6H_2O$, absolutely chemically pure and nickel free, was obtained from the City Chemical Corporation, New York, N. Y.

Cystine. The cystine used was originally prepared from hair in these laboratories. It was dissolved in 20 per cent hydrochloric acid and reprecipitated with a hot concentrated solution of sodium acetate. The precipitate was allowed to stand over night in a refrigerator, filtered, washed with water and then with alcohol and finally allowed to dry in air on a

porous plate. A fine white powder was obtained which consisted of typical hexagonal cystine crystals when examined under a microscope. A hot saturated solution of the powder, when allowed to cool, formed similar typical hexagonal crystals easily visible to the naked eye.

Glucose. The glucose used for standard solutions and for injection solutions was Baker and Adamson's reagent quality anhydrous glucose.

Glycine. Glycine, ammonia free, was obtained from the Eastman Kodak Company, Rochester, N. Y.

Nitroso-R-salt. The nitroso-R-salt used was obtained from the Eastman Kodak Company, Rochester, N. Y.

Sodium tungstate. The sodium tungstate used for preparing blood filtrates was obtained from the Mallinckrodt Chemical Works and was free of molybdenum.

Presentation of Data

Growth

Since the literature is not clear with respect to the amount of added cobalt salts which rats can tolerate in their diet, a preliminary experiment was planned to determine the maximum level of cobalt salt in the ration which rats can ingest without dying. To the growing ration used for the rat colony of the physiological chemistry section of the chemistry department, cobalt chloride was added in amounts to give 1.0 per cent,

0.5 per cent, 0.1 per cent and 0.05 per cent of cobalt and each one of these rations was fed to a group of six rats. A fifth group of rats was fed growing ration only, with no added cobalt salt, to serve as controls. In these feeding experiments, as well as in all others, except where otherwise noted, each group of rats consisted of three males and three females, 45-55 grams in weight at the beginning of the experiment, kept together in metal cages with feed and water supplied ad libitum. The lethal effects of these rations are shown in Table 7.

Table 7

Lethal Effects of Rations Containing Added Cobalt Chloride

Cobalt ion content	Time necessary for rats to die
500 parts per million (0.05%)	3-5 weeks
1,000 parts per million (0.1%)	2-3 weeks
5,000 parts per million (0.5%)	1-2 weeks
10,000 parts per million (1.0%)	1 week

It is apparent that rations containing 500 parts per million (0.05%) or more of cobalt (as cobalt chloride) are lethal to young rats.

Growth experiment A. Three groups of six rats each were fed growing ration with cobalt nitrate added to give levels of 200, 100 and 50 parts per million of cobalt (0.02 per cent, 0.01 per cent and 0.005 per cent cobalt respectively), with a control group of six rats receiving growing ration without added cobalt

salt. The rats were weighed each week, feed consumption records were kept for each group and a dated record was kept of all pregnancies, the number and weight of young in each litter at birth and at 28 days of age and the weight of each female before and after parturition. Growth data are shown in Table 8. Growth curves for each level of cobalt (as cobalt nitrate) are plotted in Figure 1. Feed consumption by these groups of rats is shown in Table 9.

Growth experiment B. It was noted in growth experiment A that the feed consumption of groups of rats receiving cobalt salt was always less than that of the controls receiving no added cobalt salt, so another series of growth and reproduction experiments was planned where the control groups receiving no added cobalt salt were fed only the weight of ration consumed on the previous day by the rats receiving added cobalt salts and supplied with all the feed they desired to eat. Cobalt was fed at a level of 50 parts per million to a group of twelve rats (six males and six females) and at a level of 200 parts per million to a group of six rats (three males and three females). Each group was paired with a control group consisting of similar numbers of rats whose feed was restricted as described above. Cobalt nitrate was used as the source of cobalt, as in growth experiment A. Growth, feed consumption and reproduction records were kept as before. In addition, since these rats were ingesting equivalent quantities of ration, the urine of both groups and their controls was

Table 8

Growth Experiment A
Average Weight in Grams of Groups of Six Rats Fed Growing Ration with Added Cobalt Nitrate

Weeks Fed ration	Concentration of added cobalt			
	None	50 p.p.m.	100 p.p.m.	200 p.p.m.
0	57	52	54	50
1	74	70	64	50
2	88	80	74	52
3	101	91	86	51
4	114	106	99	50
5	144	119	104	54
6	151	130	111	55
7	165	136	120	59
8	194	157	138	72
9	202	158	143	76
10	210	161	145	83
11				
12				
13	216	170	149	94
14	217	176	164	103
15	251	184	161	108
16	255	201	179	116
17	266	200	179	128
18	266	199	181	131
19	279	215	190	141
20	282	218	187	140
21	295	229	185	136
22	300	233	191	144
23	309	237	196	150
24	313	248	198	155
25	317	234	205	162
26	321	241	211	166
27	327	244	217	168
28	331	251	222	169
29	330	257	221	179
30	338	263	232	179
31	338	273	233	189
32	357	257	246[a]	208[a]
33	342	258	259	221
34	338	250	254	233
35	336	258	266	229
36	338	263	265	248
37	334	261	266	248
38	326	261	270	255
39	348	280	272	267

[a]Fed growing ration without added cobalt salt from thirty-second week until the end of the experiment.

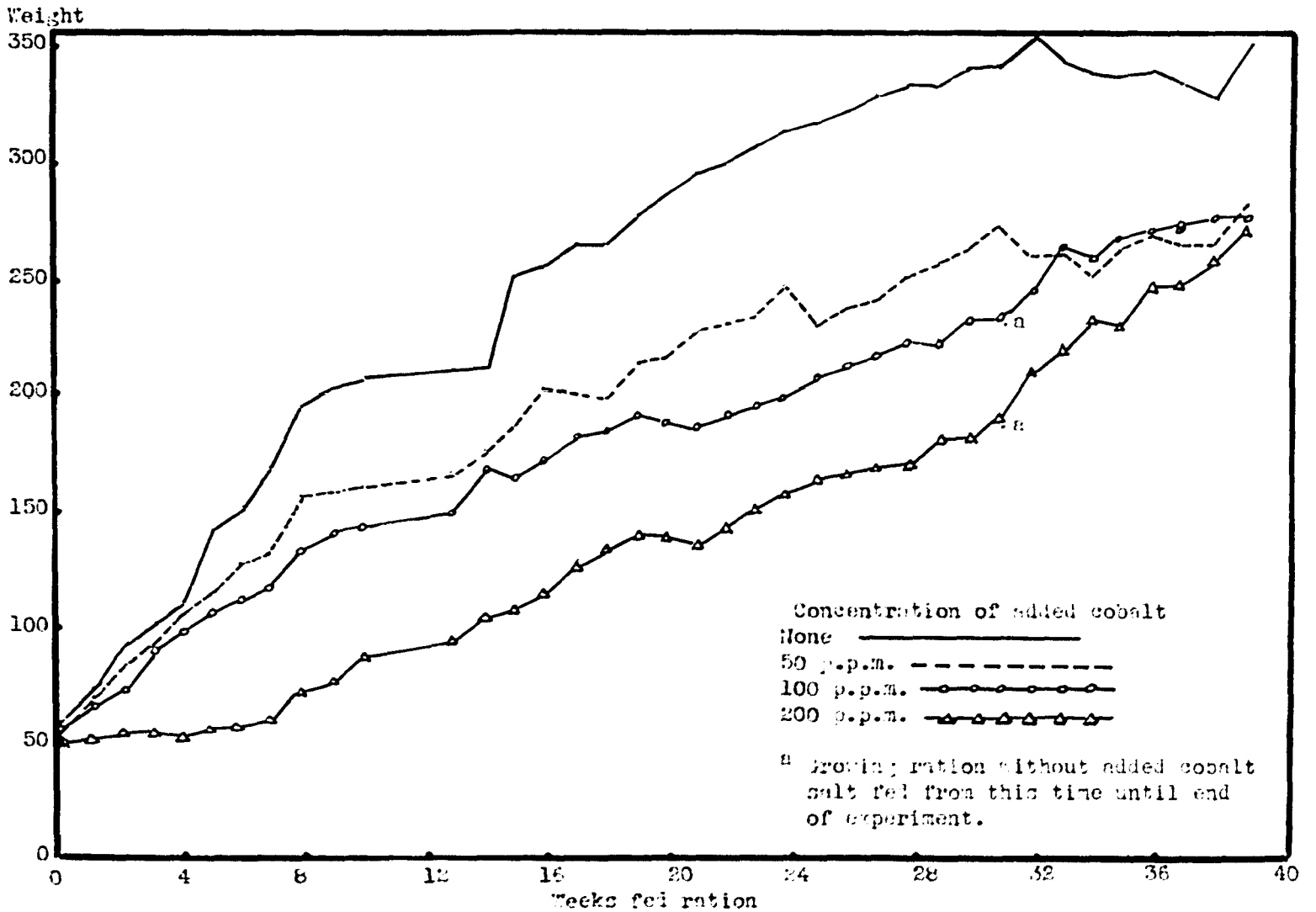


Fig. 1. Growth experiment A. Average weight of groups of six rats fed growing ration with added cobalt nitrate

Table 9

Feed Consumption in Grams by Groups of Six Rats in Growth Experiment A (Table 8)

Weeks fed ration	Concentration of added cobalt			
	None	50 p.p.m.	100 p.p.m.	200 p.p.m.
1	470	385	350	260
2	500	390	370	340
3	460	460	400	290
4	600	300	470	350
5	500	430	340	300
6	530	470	420	310
7	540	470	440	360
8	620	530	410	310
9	620	400	450	480
10	500	460	400	480
11	820	400	380	400
12	500	410	400	510
13	600	405	420	390
14	1130	515	600	540
15	1100	430	400	500
16	750	515	390	430
17	770	530	440	535
18	830	640	450	500
19	670	500	460	535
20	880	550	390	600
21	720	650	480	470
22	620	480	420	530
23	540	550	430	600
24	640	520	420	650
25	600	530	490	570
26	700	660	410	680
27	700	700	580	500
28	600	790	470	600
29	600	730	580	540
30	650	790	460	560
31	600	620	440	700
32	700	630	560[a]	750[a]
33	850	500	540	750
34	1200	600	500	500
35	1300	800	600	800
36	800	1000	660	1100
37	600	1000	640	800
38	700	1000	700	500
39	900	900	700	800

[a]Fed growing ration without added cobalt salt from thirty-second week until the end of the experiment.

analyzed quantitatively for reducing sugar and tested qualitatively for albumin. The blood of the rats receiving 50 parts per million of cobalt ion and that of their controls was analyzed for non-protein nitrogen. At the end of the feeding experiment, tissues were pooled and analyzed for cobalt by the nitroso-R-salt method. Growth data are shown in Table 10 and are plotted in Figures 2 and 3.

After being fed these rations for 23 weeks, the rats receiving 200 parts per million of added cobalt and their control rats had consumed an average of 1.38 kilograms of feed each. The rats receiving only 50 parts per million of added cobalt ion and their controls had each consumed an average of 1.92 kilograms of feed in the same length of time.

Growth experiment C. In order to observe any effects of smaller concentrations of added cobalt nitrate than used in the preceding experiments, a ration containing 25 parts per million of added cobalt nitrate was fed to a group of fifteen males and fifteen females while another group of fifteen males and fifteen females, receiving growing ration without added cobalt nitrate, served as controls. Records were kept as before on growth, feed consumption and reproduction. Tissues from these rats were analyzed for cobalt by the nitroso-R-salt method at the close of the experiment. Growth data are presented in Table 11 and are plotted in Figure 4. Feed consumption data are shown in Table 12.

Table 10

Growth Experiment B
Average Weight in Grams of Groups of Rats Fed Growing Ration with Added Cobalt Nitrate. Control Groups Fed Only the Weight of Ration Consumed by the Paired Group Receiving Added Cobalt Salt

Weeks fed ration	Concentration of added cobalt			
	None	50 p.p.m.	None	200 p.p.m.
	12 rats	12 rats	6 rats	6 rats
0	47	48	54	51
1	66	64	64	60
2	70	81	75	66
3	112	99	93	75
4	131	124	96	86
5	146	138	109	92
6	165	159	122	102
7	182	175	133	118
8	202	187	149	123
9	213	200	148	132
10	207	202	159	136
11			176	139
12	221	204	173	146
13	225	211	186	157
14	230	227	184	155
15	240	229	186	159
16	237	231	196	159
17	238	244	192	163
18	248	239	192	164
19	255	248	199	162
20	253	248	202	171
21	257	266	203	173
22	263	248	207	172
23	261	261	221	179
24	278	267	218	178
25	280	266	218	178
26	286	275	228	173
27	292	282		
28	288	271		
29	295	281		
30	288	281		
31	293	279		
32	298	283		
33	293	288		

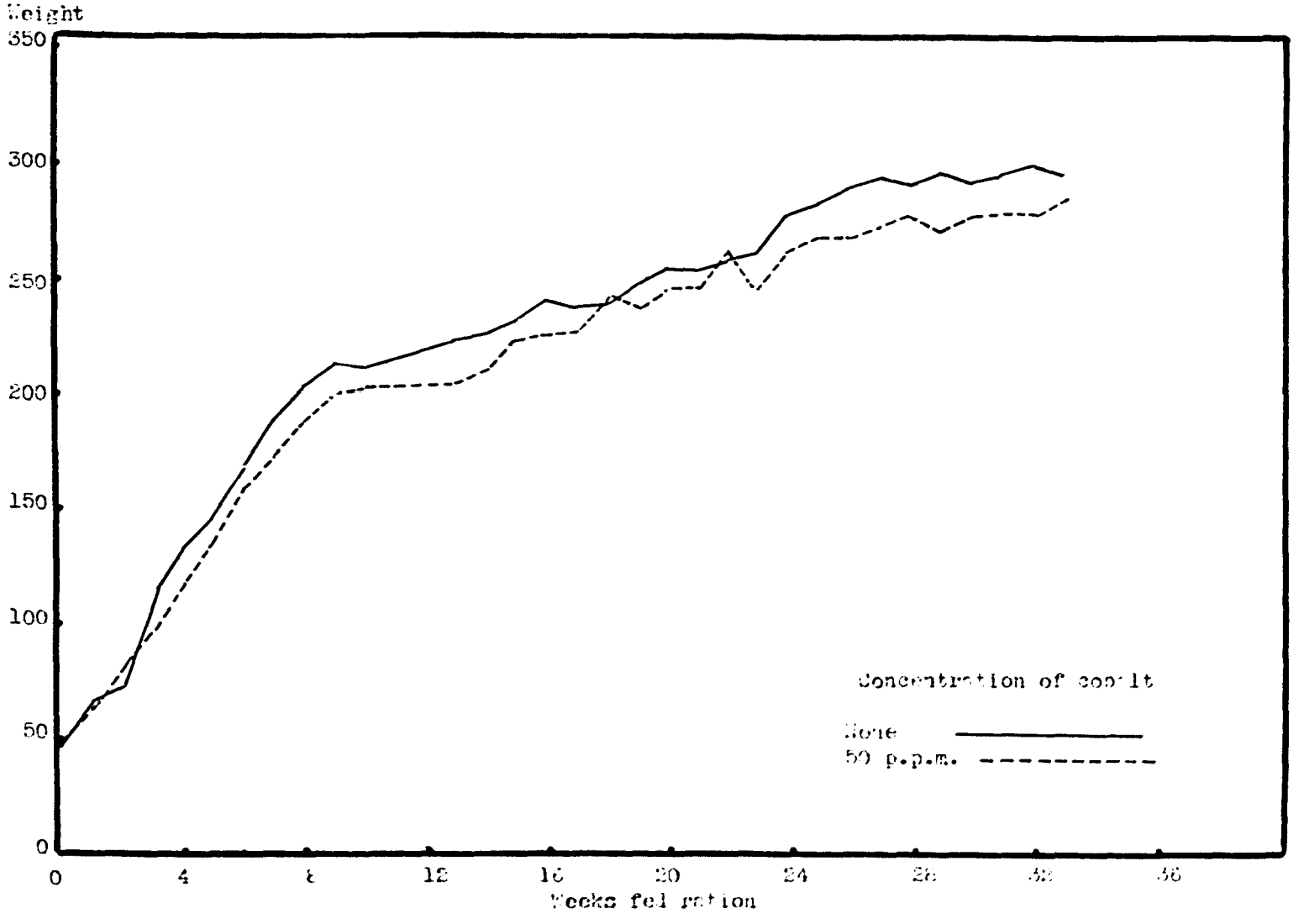


Fig. 2. Average weight in grams of groups of rats fed practical ration with added cobalt nitrate. Control group fed only the weight of ration consumed by the rats fed added cobalt salt.

Fig. 3. Average weight in grams of groups of rats fed grain ration with added cobalt nitrate. Control group fed only the weight of ration consumed by the rats fed added cobalt salt.

Table 11

Growth Experiment C
Average Weight in Grams of Groups of Thirty Rats Fed Growing Ration Alone and Growing Ration with 25 Parts Per Million of Added Cobalt as Cobalt Nitrate

Weeks fed ration	Concentration of added cobalt	
	None	25 p.p.m.
0	73	67
1	95	85
2	119	105
3	142	121
4	162	141
5	179	158
6	192	181
7	209	203
8	220	209
9	224	220
10	222	221
11	228	227
12	228	230
13	235	230
14	255	244

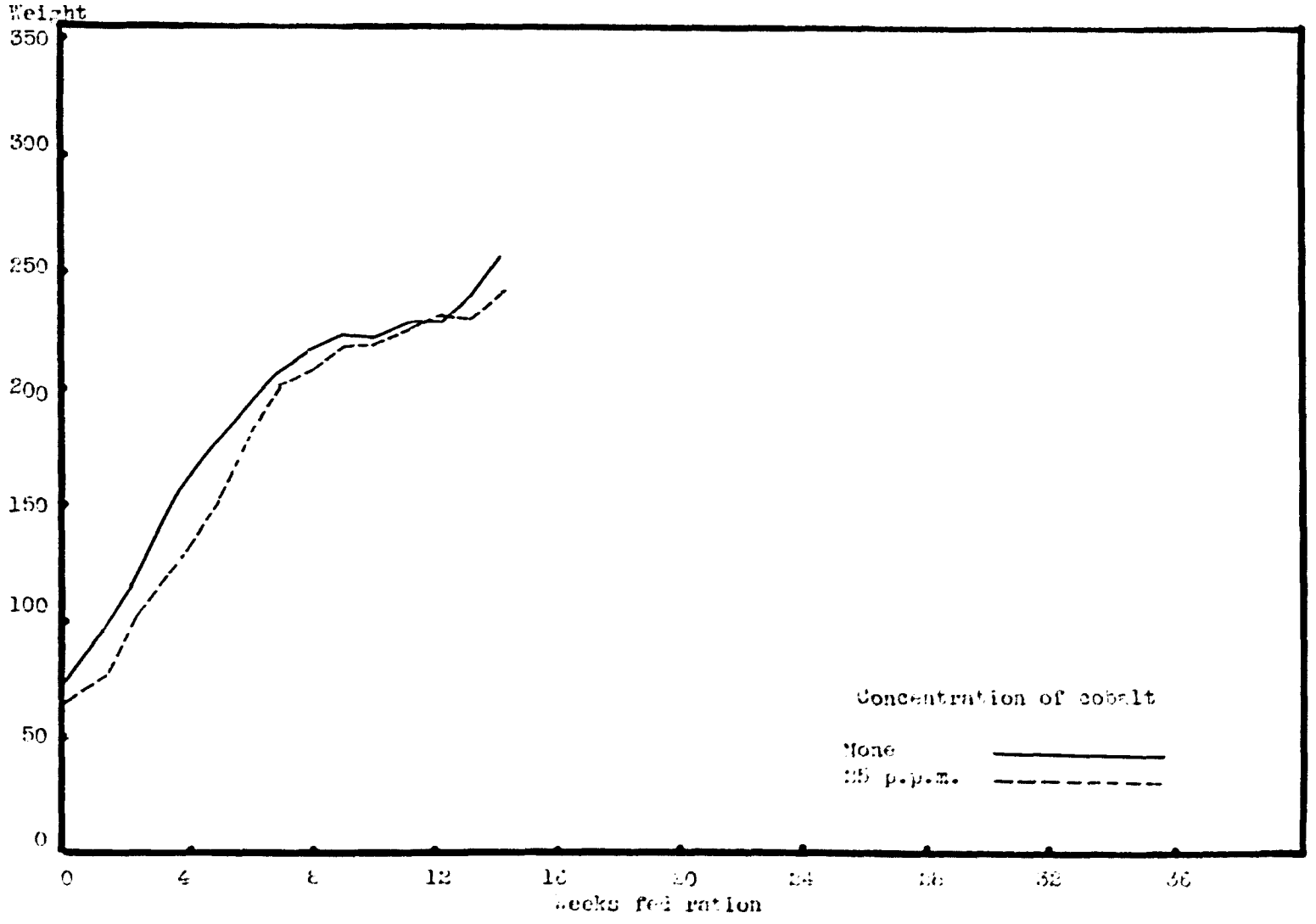


Fig. 4. Growth experiment 3. Average weight in grams of groups of rats fed growing ration with added cobaltous nitrate.

Table 12

Feed Consumption in Grams by Groups of Thirty Rats in Growth Experiment C. (Table 11)

Weeks fed ration	Concentration of added cobalt	
	None	25 p.p.m.
1	3800	3000
2	4200	3100
3	4200	3000
4	3700	3200
5	3500	3500
6	4100	3000
7	4300	3800
8	3300	2200
9	3000	2000
10	3200	3800
11	3900	3500
12	4100	3000
13	4100	4000
14	4700	4500

Growth experiment D. Groups of six rats each were fed growing ration with 5 and 10 parts per million of added cobalt as cobalt nitrate, respectively, and a control group of rats receiving no added cobalt salt was grown simultaneously. Hemoglobin determinations were made on these rats at regular intervals and growth, feed consumption and reproduction records were kept as before. Some of the tissues of the group receiving 10 parts per million of added cobalt and some tissues from the controls were analyzed for cobalt as in the preceding experiments. Growth data are given in Table 13 and plotted in Figure 5.

Reproduction

Reproduction data were recorded for all the rats in growth experiments A, B, C and D but some variations in the conditions of the different experiments should be noted. In all cases, however, when litters of more than six young were produced, the litters were reduced to six before the females were allowed to rear them to weaning age (approximately 28 days).

In growth experiment A, where cobalt nitrate was added to the ration to give cobalt concentrations of 0, 50, 100 and 200 parts per million, the females were allowed to raise six of their young to weaning age. In the case of the controls (receiving no added cobalt ion), the females were not returned to the males for some time after weaning of their first litters.

Table 13

Growth Experiment D
Average Weight in Grams of Groups of Six Rats
Fed Growing Ration with Added Cobalt Nitrate

Weeks fed ration	Concentration of added cobalt		
	None	5 p.p.m.	10 p.p.m.
0	51	47	49
1	80	56	75
2	101	78	102
3	121	109	125
4	143	129	159
5	164	143	173
6	185	172	190
7	196	187	211
8	206	203	207
9	224	213	219
10	247	234	223
11	250	235	238
12	269	250	252
13	268	258	262
14	290	256	280
15	285	264	268
16	299	274	281
17	307	284	293
18	305	286	304
19	316	296	288
20	321	302	300
21	341	292	319
22	329	302	311
23	338	306	313
24	352	304	315
25	349	298	317
26	341	312	317
27	354	327	

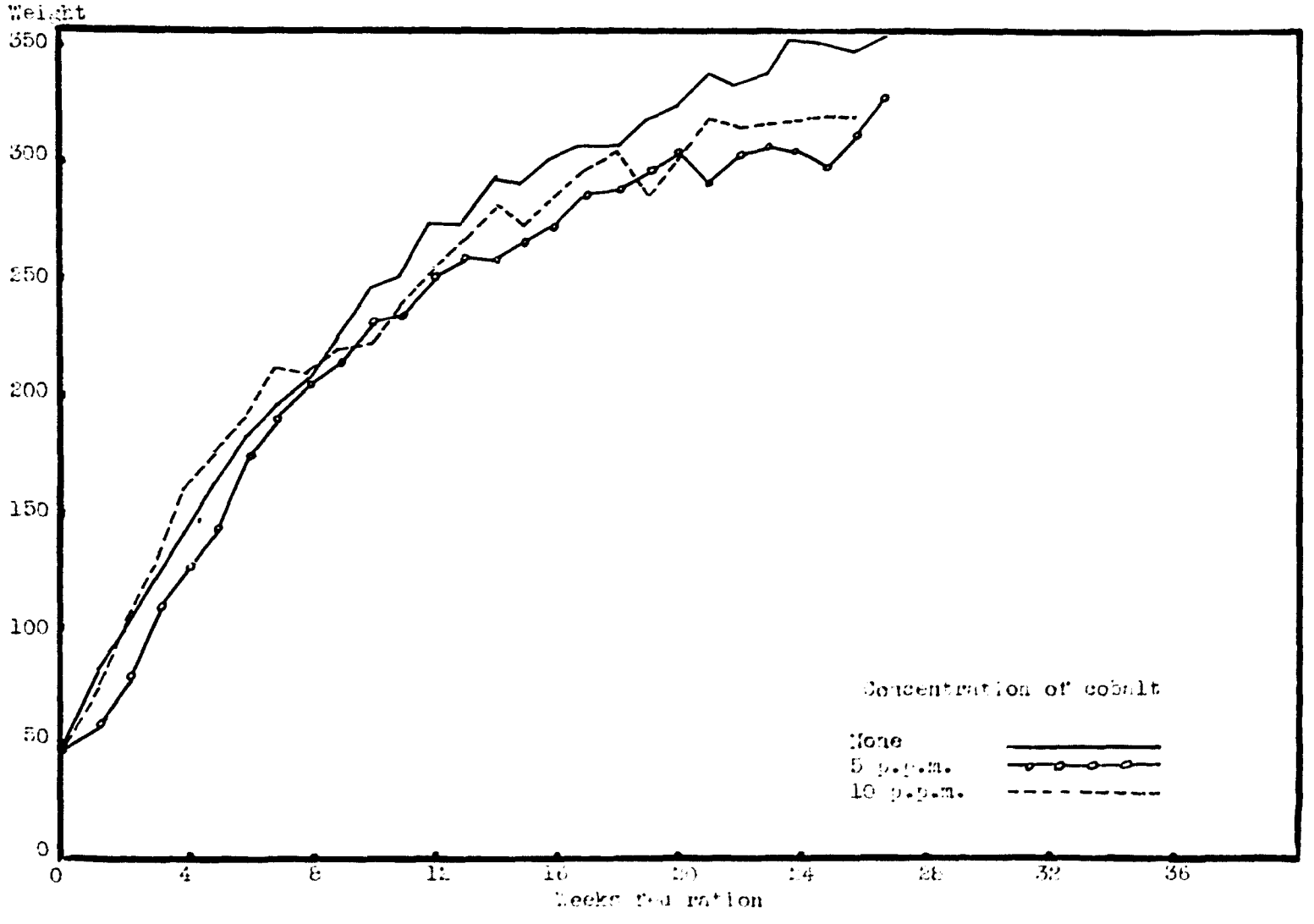


Fig. 5. Growth experiment 2. Average weight in grams of groups of six rats fed growing ration with added cobalt nitrate.

When the control females were returned to the males, however, they promptly reproduced again.

In growth experiment B, where the feed intake of the control groups was restricted to the amount eaten by the paired groups receiving 50 and 200 parts per million of added cobalt ion, respectively, the females were not allowed to raise their litters but were returned to the males after each parturition.

In growth experiment C, reproduction data included only the first litters raised by the females, since these rats were intended primarily for tissue analysis studies.

In growth experiment D in which added cobalt ion was fed at concentrations of 0, 5 and 10 parts per million, reproduction data were kept throughout the entire experiment (27 weeks) and each female was allowed to raise litters of six or less to weaning age after each parturition.

It is apparent that due to the different conditions of the experiments, the data from all four main groups are not all strictly comparable. However, the conditions up to the production of the first litter in each group are comparable and these data are shown in Table 14. Table 15 shows a similar comparison of data for the second litters from growth experiments A and D only. Table 16 shows a compilation of data for growth experiment B for the entire duration of the experiment (25 weeks).

Table 14

Comparison of Data on Production of First Litters

(Data for control groups are underlined.)	Growth experiment A				Growth experiment B				Growth experiment C		Growth experiment D		
	No Co	50 ppm	100 ppm	200 ppm	No Co	50 ppm	No Co	200 ppm	No Co	25 ppm	No Co	5 ppm	10 ppm
		Co	Co	Co		Co		Co		Co		Co	Co
Number of females	3	3	3	3	6	6	3	3	15	15	3	3	3
Number reproducing	3	3	1	0	6	5	3	0	13	13	3	3	3
Number of young	32	16	1	0	52	31	23	0	105	81	17	26	22
Total weight of young	139	43	3	0	255	167	111	0	381	329	47	83	61
Average number of young per litter	10.7	5.3			8.7	6.2	7.6		8.1	6.2	5.3	8.7	7.3
Average weight of each litter	46	14			43	33	37		29	25	15	28	20
Average weight of each young	4.3	2.7			4.9	5.4	4.8		3.6	4.0	2.8	3.2	2.8
Average age of females in days at first parturition	113	126			92	93	121		97	94	87	103	104

Table 15

Comparison of Data on Production of Second Litters

(Data for control groups are underlined.)	Growth experiment A: No Co	50 ppm Co	100 ppm Co	200 ppm Co	Growth experiment D: No Co	5 ppm Co	10 ppm Co
Number of females	3	3	3	3	3	3	3
Number producing second litters	3	2	0	0	3	3	2
Total number of young	28	13	0	0	26	23	22
Total weight of young	112	49	0	0	91	98	74
Average number of young per litter	9.3	6.5			8.7	7.7	10.5
Average weight of each litter	37	25			30	33	37
Average weight of each young	4.0	3.8			3.4	4.3	3.5

Table 16

Comparison of Reproduction Data for Growth Experiment B (25 Weeks)

(Control groups for each concentration of cobalt ion were fed only the weight of growing ration consumed by the paired froup fed added cobalt ion.)

(Data for control groups are underlined.)	Concentration of added cobalt ion			
	None	50 ppm	None	200 ppm
Number of females	6	6	3	3
Number reproducing	6	5	3	0
Total number of litters	33	25	8	0
Total number of young	279	167	65	0
Total weight of young	1500	797	306	0
Average number of litters	5.5	5.0	3.7	
Average number of young per litter	8.5	6.7	7.9	
Average weight of each litter	45	32	38	
Average weight of each young	5.3	4.7	4.8	

Lactation

Success of lactation was judged by the per cent of litters raised from birth to an age of 28 days and by comparison of the average weights of the young at birth and at 28 days of age. These data were obtained during the course of growth experiments A, C and D for the groups of rats fed rations with a sufficiently low concentration of added cobalt ion to permit reproduction to occur. The data are summarized in Table 17.

Hemoglobin formation

Since it is well established in the literature that administration of sufficient amounts of cobalt salts stimulates hemoglobin formation in rats, an attempt was made to determine the minimum amount of added cobalt necessary in the diet of rats to produce such a stimulating effect.

The blood of the rats in growth experiment D, receiving 0, 5 and 10 parts per million of added cobalt (as cobalt nitrate) was analyzed for hemoglobin at regular intervals for 25 weeks. Feed consumption records were kept. The data from the hemoglobin determinations are summarized in Table 19 and plotted in Figure 6. The feed consumption at representative periods is shown in Table 18.

Table 17

Summary of Data on Lactation
Per Cent Success in Raising Litters. Average Weights of Each Young at Birth and at 28 Days of Age

(Data for control groups are underlined.)	Growth experiment A		Growth experiment C		Growth experiment D	
	No Co	50 ppm Co	No Co	25 ppm Co	No Co	5 ppm Co
Number of females	3	3	15	15	3	3
Number reproducing	3	3	13	13	3	3
Number of litters born	6	7	13	13	10	8
Number of litters raised	6	5	12	12	9	8
Per cent success in raising litters	100%	71%	92%	92%	90%	100%
First litter — Weight of each young at birth	4.3	2.7	3.8	3.8	2.8	3.1
First litter — Weight of each young at 28 days	34	26	30	30	43	52
Second litter — Weight of each young at birth	4.0	3.5			3.6	4.2
Second litter — Weight of each young at 28 days	40	36			65	55
Third litter — Weight of each young at birth		4.0			3.7	2.9
Third litter — Weight of each young at 28 days		29			63	57

Table 18

Average Feed Consumption (Grams of Feed Per Rat Per Day) of Rats in Growth Experiment D at Representative Periods

Week	Concentration of added cobalt		
	None	5 p.p.m.	10 p.p.m.
5	12	13	13
9	16.6	14	15.5
14	16.6	16.6	16.6

Distribution of cobalt in the tissues

The rats of growth experiment C receiving 0 and 25 parts per million of added cobalt were raised primarily for tissue analysis so after 15 weeks feeding on these rations, the animals were killed and the liver, kidney, spleen, heart, lungs, stomach and intestine of each rat were removed, rinsed free of blood and hair with distilled water, blotted on clean filter paper and placed in individual tared casseroles, weighed and analyzed for cobalt by the nitroso-R-salt method previously described. The contents of the stomach and intestine were removed before analysis. At the same time, tissues from four rats from growth experiment A which had been fed 50 parts per million of added cobalt ion for 40 weeks were analyzed individually in the same way. The data obtained are shown in Table 20.

Table 19

Average Hemoglobin Concentrations (Milligrams per 100 ml. of Blood) of Groups of Six Rats Fed Growing Ration with 0, 5 and 10 Parts per Million of Added Cobalt

Week	Concentration of added cobalt		
	None	5 p.p.m.	10 p.p.m.
0	11.7	10.0	11.0
1	11.7	11.4	10.6
2	11.9	11.9	11.8
3	11.6	12.2	12.5
4	12.7		12.8
5	12.0	12.2	13.2
6	11.9		13.2
7	12.7		14.2
8		13.0	
9			14.6
10	12.6	13.2	
11	12.6	12.7	13.3
12		12.0	
13		12.2	12.8
14		12.3	
15	12.9		12.4
16	12.7	12.4	12.2
17	12.7	12.6	12.3
18	12.6	12.8	12.4
19	12.7	12.9	12.9
20	12.5	13.1	12.9
21	12.3	13.2	12.8
22	12.1		13.1
23	12.3		13.3
24	12.4		13.4
25	12.3		13.5

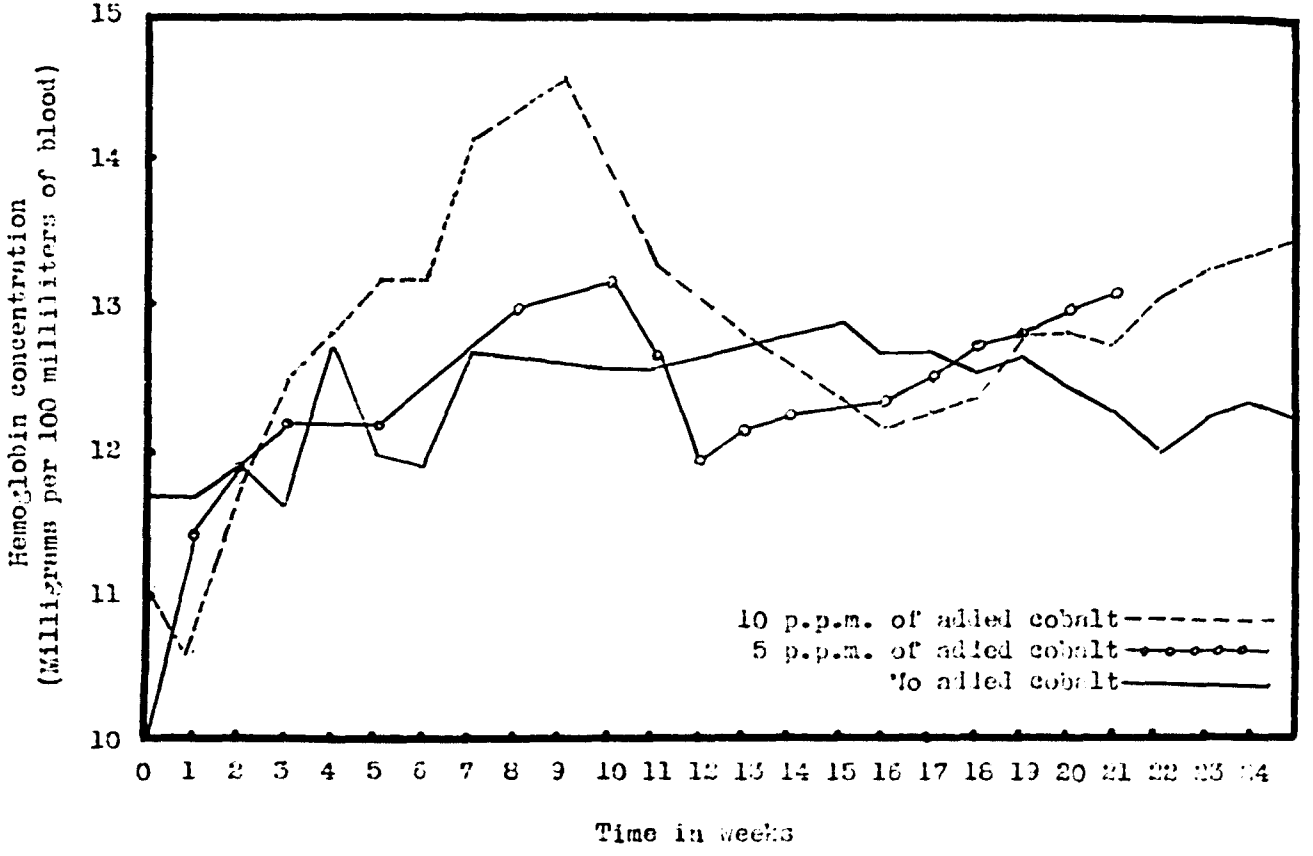


Fig. 6. Average hemoglobin concentrations of groups of six rats fed growing ration with 0, 5 and 10 parts per million of added cobalt

Table 20

Distribution of Cobalt in the Tissues of Rats Fed Cobalt Nitrate. Parts Per Million of Cobalt in Fresh Tissue

Amount of Co added to ration	Rat	Sex	Liver	Heart	Lungs	Spleen	Intestines	Kidney	Stomach
	20	M	0.6	1.5	1.0	1.4	1.2	1.2	0.9
	21	M	0.4	0.9		trace	0.3	1.1	1.0
	22	M	0.5	1.4	0.7	0.0	0.7	2.2	0.8
	23	M	0.5	0.0	0.8	0.0	0.6	0.5	0.5
None	11	M	0.4	0.0	0.0	0.0	0.3	0.5	0.8
	19	F	0.6	2.8	0.8	0.0	0.3	2.0	1.9
	24	F	0.5	0.0	0.3	0.0	0.6	1.2	0.9
	25	F	0.5	0.0	0.0	0.0	0.5	2.4	0.8
	26	F	0.2	0.0	0.2	0.0	0.4	0.8	0.9
	Average		0.5	0.8?	0.5	0.0	0.6	1.5	0.9
	6	M	1.6	0.0	0.0	0.0		1.1	
	7	M	1.9	0.0?	0.0?	0.0	1.4	0.5	0.0
	8	M	1.6	trace	0.0	0.0	1.0	2.8	0.7
25	9	M	1.2	0.0	0.0	0.0	1.1	1.5	0.7
parts	10	M	2.0	0.0	0.0	0.0	0.8	1.8	0.8
per	12	F	1.0	0.0		0.0	0.6	1.6	0.5
million	13	F	1.6	0.0	0.0	0.0	0.9	1.0	0.0
	14	F	1.8	0.0	0.6	0.0	0.9	0.7	1.4
	15	F	0.9	0.0	0.0	0.0	0.5	1.5	0.6
	16	F	1.7	0.0	0.0	0.0	1.1	1.8	1.5
	17	F	2.3	0.0	0.0	0.0	1.2	2.7	2.7
	Average		1.7	0.0	0.0	0.0	0.9	1.7	0.9
50	27	M	1.4	0.0	0.8	1.4	0.6	1.5	0.6
parts	28	M	1.6	2.7	0.6	1.5	0.6	2.0	0.7
per	29	F	1.9	0.6	0.0	0.0	0.6	2.0	1.5
million	30	F	1.6	2.7	1.6	2.3	0.3	2.0	1.7
	Average		1.6	1.5	0.7	1.4	0.5	2.0	1.1

Tissues of three rats which had been fed 10 parts per million of added cobalt in growth experiment B were next analyzed individually as before, along with tissues from six of the control rats which had not been fed added cobalt salt. Tissues analyzed were liver, kidney, spleen, pancreas and heart. These data are shown in Table 21.

A third series of analyses was made on the tissues of 34 rats of growth experiment B in which added cobalt ion was fed in concentrations of 50 and 200 parts per million along with control groups receiving no added cobalt ion. Organs analyzed were liver, kidney, spleen, pancreas, heart, lungs and testes. In these analyses, similar organs from three rats receiving the same concentration of added cobalt salt were pooled so that the cobalt present might be more easily determined due to the increased amount in each sample analyzed. For these reasons (the greater number of rats used, the increased amount of cobalt in each sample) as well as the fact that these analyses were made after much valuable experience with the method had been obtained, these data must be considered the most accurate of all the data shown for distribution of cobalt in the tissues. These data are presented in Table 22.

Table 21

Distribution of Cobalt in the Tissues of Rats Fed Cobalt Nitrate. Parts Per Million of Cobalt in Fresh Organ

Amount of cobalt ion added to ration	Rat	Sex	Liver	Kidneys	Spleen	Pancreas	Heart
	W	M	0.9		1.0	trace	1.0
	G	F	1.0	1.6	1.0	1.5	1.0
	BS	M	0.8	1.4	0.0	0.0	1.0
None	W	M	0.9	1.5	1.0	trace	1.0
	W	F	1.6	2.0	2.0	0.0	0.5
	BS	F	1.1	1.8	1.5	0.0	1.0
	Average		1.0	1.6	1.1	traces?	0.9
10 parts per million	W	M	0.9	2.0	1.5	1.4	1.7
	BS	M	1.1	2.0	1.8	0.6	1.5
	W	M	1.4	1.9	1.2	1.0	0.3
	Average		1.1	2.0	1.5	1.0	1.1

Table 22

Distribution of Cobalt in the Tissues of Rats Fed Cobalt Nitrate. Parts Per Million of Cobalt in Fresh Organs

Amount of Co added to ration	Rat Sex	Liver	Kidneys	Spleen	Pancreas	Heart	Lungs	Testes
50 parts per million	3 males	1.7	2.0	1.1	1.0	1.7		0.3
	3 males	1.7	2.4	1.5	0.5	1.5		0.3
	4 females	1.8	3.0	1.1	0.8	1.9	0.8	
	Average	1.7	2.5	1.2	0.8	1.7	0.8	0.3
None	3 males	0.4	0.7	0.0	0.8	0.8		0.2
	3 males	0.2	0.7	0.5	0.0	0.3		0.2
	3 females	0.3	1.0	0.4	0.7	0.7	0.0	
	3 females	0.2	0.8	0.2	0.2	0.2	0.0	
	Average	0.3	0.8	0.3	0.4	0.5	0.0	0.2
200 parts per million	3 males	6.0	4.8	1.4	1.2	1.2		0.9
	3 females	7.8	4.3	2.4	1.4	1.7	1.3	
	Average	6.8	4.5	1.9	1.3	1.5	1.3	0.9
None	3 males	0.5	0.7	0.0	0.0	0.4		0.2
	3 females	0.5	0.8	0.0	0.0	0.2	0.2	
	Average	0.5	0.7	0.0	0.0	0.3	0.2	0.2

N. B. Each control group was fed the same weight of growing ration without added cobalt as was consumed by the paired group of rats receiving added cobalt nitrate in the ration.

Rate of deposition of cobalt in the liver

The study on the distribution of cobalt in the tissues showed that cobalt was deposited in greater proportion in the liver than in any other organ. For this reason it was thought of interest to determine the length of time necessary for the cobalt concentration to reach a maximum in the liver when rats were fed a diet containing added cobalt salt.

Young rats (80-100 grams in weight) were fed growing ration to which 100 parts per million of cobalt had been added in the form of cobalt nitrate. One male and one female were killed each day, the livers removed and analyzed for cobalt in duplicate. To serve as controls, the livers of two males and two females were analyzed for cobalt before the feeding of added cobalt salt was started. Food consumption and weight records were kept. The data obtained are shown in Table 23.

Toxicity of cobalt salts administered by stomach tube

Since there is little agreement in the literature as to the minimum lethal dose of cobalt ion for rats, it was thought advisable to determine the toxicity of the cobalt salts used in previous experiments when given by stomach tube in solution in varying amounts to rats of different weights.

Table 23

Rate of Deposition of Cobalt in the Liver of
Ration with 100 Parts Per Million of Ad

Number of days rats were fed ration with added cobalt nitrate	0				1		2		3		
Average food consumption per rat (grams)					12		13		10		
Cobalt intake (micrograms)					1200		1300		1000		
Sex	F	F	M	M	F	M	F	M	F	M	
Weight (grams)	88	80	88	98	106	126	109	120	84	146	
Weight of fresh liver (grams)	3.07	2.99	3.11	3.04	(part) 1.30	3.39	3.66	4.84	2.83	4.35	
Cobalt found (micrograms)	7.0	5.0	6.8	4.7	4.5	12.3	18	23	21	14.5	2
Cobalt concentration, parts per million	2.2	1.7	2.2	1.5	3.5	3.6	4.9	4.6	7.5	3.4	
Average cobalt concentration, males and females	1.9				3.6		4.8		5.4		

Liver of Rats Fed Growing
ion of Added Cobalt

3	4				5		6		7		8	
	10				11		12		13		14	
	1000				1100		1200		1300		1400	
M	F	F	M	M	F	F	F	M	F	M	F	M
146	112	62	120	106	92	114	93	138	101	143	118 (part)	118
4.35	3.37	2.08	4.22	3.39	3.16	3.36	2.82	3.67	3.17	5.13	2.42	3.64
14.5	22.5	20	17	23	21.5	32	29	20	27	21.5	13.5	23.5
3.4	6.9	9.6	4.4	6.8	6.8	9.9	10.3	5.5	8.7	4.2	7.5	6.6
.4	7.0				(8.4) (females only)		7.9		6.5		7.0	

Solutions of cobalt nitrate and cobalt chloride were prepared ranging in concentration from 20 to 60 milligrams of cobalt per milliliter. Rats selected from the stock colony were anesthetized by intraperitoneal injection of nembutal solution at the rate of 3.0 milligrams of nembutal per 100 grams of body weight. When the rat was unconscious, a stomach tube was passed into the stomach and exactly 1.0 milliliter of the cobalt test solution passed into the stomach from a hypodermic syringe fitted to the stomach tube. In some cases the rats were fasted for 24 hours before the experiment. The data obtained are shown in Tables 24 and 25.

Effect of cobalt salt in the diet on reducing sugar and albumin in the urine

Rats from growth experiment B which had been fed cobalt nitrate added to the growing ration for six months in concentrations of 50 and 200 parts per million of cobalt and control rats which had been fed the same weights of growing ration with no added cobalt salt were placed individually in metabolism cages with food and water and the urine collected for 24 hours. The 24-hour volume of urine was recorded. The urine was tested qualitatively for albumin with Roberts' reagent and analyzed quantitatively for reducing sugar by Sumner's method. Each rat was left in the metabolism cage for two consecutive days so that check analyses might be made.

Table 24

Effects of Administering Cobalt Chloride Solution to Rats by Stomach Tube

(None of the rats in this experiment were fasted.)

Rat		Milligrams of cobalt ion injected	Milligrams of cobalt ion per 100 grams of body weight	Effect
Sex	Weight			
M	220	30	14	Recovered
M	277	40	14	Recovered
M	274	40	15	Recovered
M	273	40	15	Recovered
F	166	30	18	Recovered
F	165	30	18	Recovered
F	154	30	20	Died
M	270	60	22	Died
M	250	60	24	Died
M	244	60	25	Died
F	160	40	25	Died
F	163	40	25	Died
M	220	60	27	Died
F	144	40	28	Died

Table 25

Effects of Administering Cobalt Nitrate Solution to Rats by Stomach Tube

Rat		Milligrams of cobalt ion injected	Milligrams of cobalt ion per 100 grams of body weight	Treatment	Effect
Sex	Weight				
M	250	20	8	Fasted	Recovered, 1 hour
M	234	20	9	Fasted	Recovered, 2 hours
M	230	20	9	Fasted	Recovered, 2 hours
M	310	40	13	Fasted	Recovered, 1 hour
M	254	40	16	Fasted	Recovered, 2 hours
M	210	40	19	Not fasted	Died, 2 hours
F	196	40	20	Not fasted	Recovered, 2 hours
M	284	60	21	Not fasted	Recovered, 2 hours
M	190	40	21	Fasted	Died, 30 minutes
M	270	60	22	Not fasted	Recovered, 2 hours
M	180	40	22	Not fasted	Died, 24 hours
M	255	60	22	Not fasted	Died, 20 minutes
M	250	60	24	Not fasted	Died, 2 hours
M	240	60	25	Not fasted	Died, 30 minutes
F	154	40	26	Fasted	Died, 30 minutes
F	142	40	28	Not fasted	Recovered, 48 hours
M	208	60	29	Not fasted	Died, 30 minutes
M	180	60	33	Not fasted	Died, 30 minutes

Albumin was found present in all urine specimens tested, both from rats fed cobalt salt and from rats not fed cobalt salt. The data for the reducing sugar determinations in the urine are presented in Tables 26 and 27.

Effect of cobalt salt in the diet on non-protein nitrogen in the blood

Non-protein nitrogen was determined in the blood of the rats of growth experiment B which had been fed 50 parts per million of cobalt (in the form of cobalt nitrate) in the growing ration for a period of five months. Similar determinations were made on the control group of rats fed the same weight of growing ration with no added cobalt salt as was consumed by the paired group fed cobalt nitrate. For most of the rats a second series of analyses was made to check the first results.

The determination was made by the Folin-Wu method as modified by V. B. Fish for small volumes of blood. Blood was drawn from the saphenous vein for the analyses. The data obtained are presented in Table 28.

Effects on blood sugar of peroral administration of cobalt chloride solutions alone and with urea, glucose and glycine

The study of the toxicity of solutions of cobalt salts given by stomach tube was extended to an investigation of the changes in blood sugar concentration brought about by

Table 26

Reducing Sugar in Urine of Rats Fed Cobalt Nitrate for Six Months

Concentration of added cobalt ion	Rat	24-hour volume of urine (ml.)	Per cent reducing sugar	Milligrams of reducing sugar excreted in 24 hours
None	WM3	5.8	0.050	2.9
		3.0	0.036	1.1
	WML4	4.4	0.027	1.2
		3.2	0.047	1.5
	WMR4	3.9	0.035	1.4
		3.2	0.028	0.9
	WF3	3.2	0.035	1.1
		5.2	0.040	2.1
	WFL3	2.7	0.025	0.7
		2.3	0.039	0.9
	WF4	1.7	0.021	0.4
		3.4	0.050	1.7
	Average	3.5 ml.	0.036%	1.3 mg.
	Variation	(1.7-5.8)	(0.021-0.050)	(0.4-2.9)
50 parts per million	WM1	2.4	0.026	0.6
		2.2	0.055	1.2
	WM2	2.5	0.030	0.7
		3.6	0.077	2.8
	GM2	4.3	0.120	5.2
		4.7	0.070	3.3
	BSF1	3.2	0.105	3.4
		2.0	0.085	1.7
	GF1	5.8	0.047	2.7
		2.2	0.105	2.3
	WF2	1.1	0.085	0.9
		1.7	0.075	1.3
	Average	3.0 ml.	0.073%	2.2 mg.
	Variation	(1.1-5.8)	(0.026-0.120)	(0.6-5.2)

N. B. Rats of the group receiving no added cobalt salt were fed the same weight of ration consumed by the rats receiving added cobalt.

Table 27

Reducing Sugar in Urine of Rats Fed Cobalt Nitrate for Six Months

Concentration of added cobalt ion	Rat	24-hour volume of urine (ml.)	Per cent reducing sugar	Milligrams of reducing sugar excreted in 24 hours
None	WP	3.2	0.045	1.5
		2.2	0.050	1.1
	GM	2.5	0.040	1.0
		3.9	0.038	1.5
	WM	5.2	0.064	3.3
		4.5	0.090	4.0
	WPL	3.0	0.065	2.0
		5.0	0.042	2.1
	WPL	3.0	0.033	1.0
		5.0	0.047	2.3
	WPR	5.2	0.090	4.6
		5.8	0.087	5.0
	Average	4.0 ml.	0.058%	2.4 mg.
	Variation	(2.2-5.8)	(0.033-0.090)	(1.0-5.0)
200 parts per million	GM	2.8	0.080	2.2
		6.4	0.053	3.4
	WP	1.4	0.085	1.2
		1.9	0.094	1.8
	WM	3.0	0.097	2.9
		6.2	0.100	6.2
	BSM	3.9	0.109	4.3
		3.6	0.140	5.0
	GP	1.8	0.070	1.3
		2.0	0.090	1.8
	BSF	3.8	0.105	4.0
		4.8	0.130	6.2
	Average	3.5 ml.	0.096%	3.4 mg.
	Variation	(1.4-6.4)	(0.053-0.140)	(1.2-6.2)

N.B. Rats of the group receiving no added cobalt salt were fed the same weight of ration consumed by the rats receiving added cobalt.

Table 28

Non-protein Nitrogen Concentration in the Blood of Rats Fed 50 Parts per Million of Added Cobalt Ion

Concentration of added cobalt ion	Rat	Milligrams per cent of non-protein nitrogen: First determination: Actual values	First determination: Average	Second determination: Actual values	Second determination: Average
50 parts per million	BSM1	49.7		47.7	
		50.0	49.8	47.3	47.5
	GM1	43.4	43.4	41.5	
				46.3	43.9
	GF1	50.3		41.1	
		51.1	50.7	41.0	41.0
	WM1	50.3		42.1	
		51.6	50.8	42.5	42.3
	BSF1	51.1	51.1	46.6	
				47.7	47.1
	WF2	52.4		51.1	
		53.0	52.7	50.3	50.7
	WM2	47.7		46.6	
		49.2	48.5	45.5	46.0
	MFL2	47.2			
		46.3	46.8		
	Average		49.2		45.5
	Variation		(43.4-53.0)		(41.0-50.3)
None	WFL3	48.2		44.4	
		45.5	46.9	43.4	43.9
	WML3	39.8	39.8	39.8	
				39.8	39.8
	WM3	38.2	38.2	43.4	
				41.5	42.5
	WMR3	41.5		47.2	
		47.7	44.6	46.6	46.9
	WF3	38.2		50.3	
		39.0	38.6	48.9	49.6
	WMR4	43.4			
		41.5	42.5		
	WM4	39.8			
		40.3	40.0		
	WF4	42.5	42.5		
	Average		41.6		44.5
	Variation		(38.2-48.2)		(39.8-50.3)

administration of less than the minimum lethal dose of cobalt. Changes in blood sugar concentration often reflect fundamental metabolic changes within the animal. An additional consideration was the availability of a suitable analytical method, worked out by Keil (79) in these laboratories, for determining blood sugar in such small samples of blood as can be obtained from rats without inflicting permanent injury.

Rats weighing 200 to 400 grams were fasted 24 hours. A blood sample was then drawn from the tail and analyzed for glucose. The rat was next anesthetized by intraperitoneal injection of nembutal at the rate of 3.0 milligrams of nembutal per 100 grams of body weight. Anesthesia was usually complete in 15 minutes so that a stomach tube could be passed into the stomach and 1.0 or 2.0 milliliters of the solution to be tested could be injected into the stomach from a hypodermic syringe attached to the stomach tube. The stomach tube was withdrawn and the tail blood analyzed for glucose at intervals of 15, 30, 60, 90 and 180 minutes after the time of injection of the test solution. Cobalt chloride solutions were used in concentrations of 20 milligrams of cobalt in 1.0 milliliter, 20 milligrams of cobalt in 2.0 milliliters and 10 milligrams of cobalt in 1.0 milliliter.

In order to show that the effects produced were due to the cobalt and were not caused by the slight acidity due to hydrolysis of the cobalt chloride, the pH of the cobalt chloride solutions used was measured on a Cameron pH Meter, a

very dilute hydrochloric acid solution was made up to approximately the same pH, this solution was injected into the stomach and the glucose concentration in the blood determined as before. With 20 milligrams of cobalt per millliliter, the pH of the solution was found to be 4.9. With 10 milligrams of cobalt per millliliter, the pH of the solution was 5.3. The hydrochloric acid solution used was made up to a pH of 5.0 by adding 0.1 normal hydrochloric acid drop by drop to a liter of distilled water until the desired pH was reached.

The blood sugar data obtained in these experiments are presented in Table 29 and plotted in Figure 7.

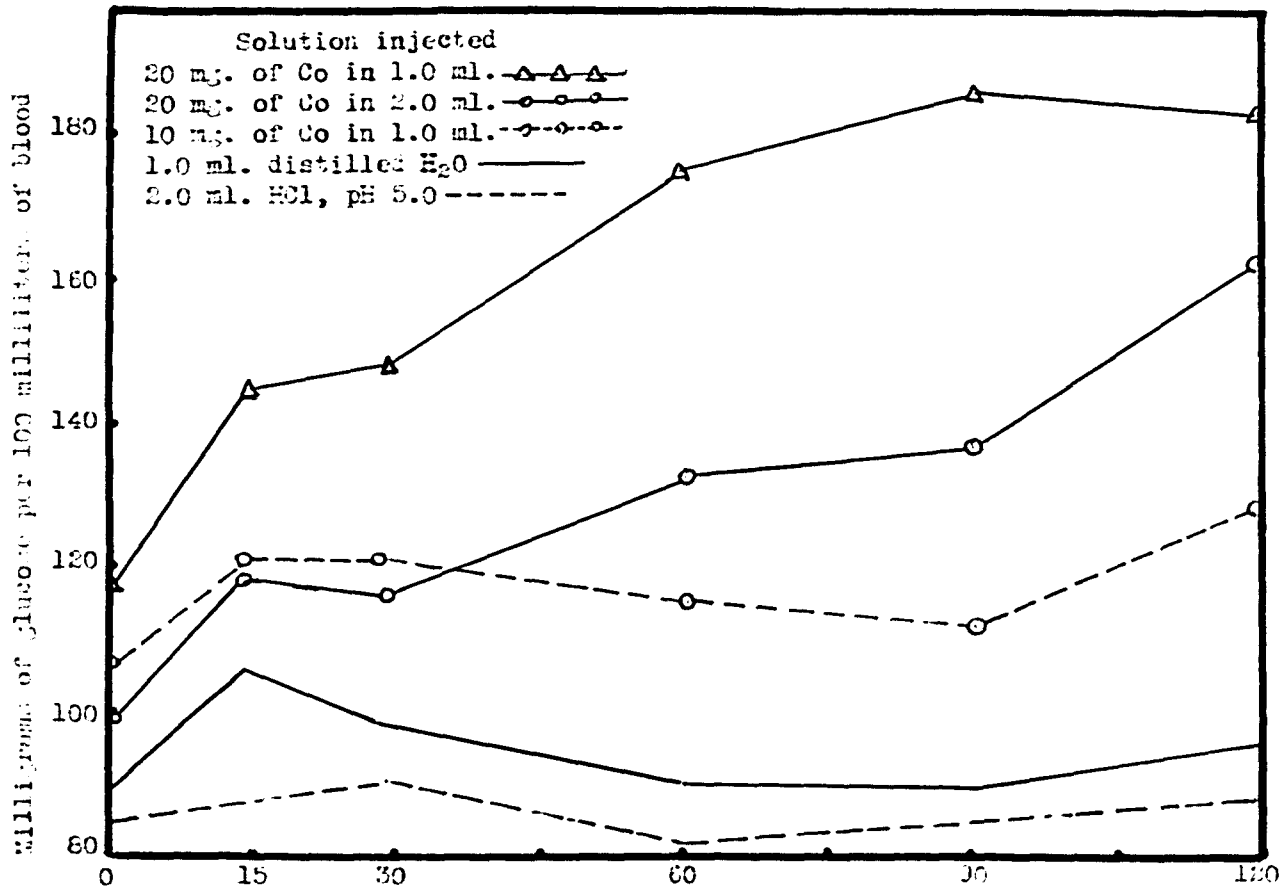
Since some amino acids have been shown to detoxify cobalt salts, cobalt chloride solutions were administered by stomach tube simultaneously with a solution of urea and with a solution of glycine to study the effect of these compounds on the hyperglycemia produced by cobalt ion. These data are shown in Table 30 and plotted in Figure 8, together with the data from Table 29 for the effect of distilled water injections and injections of 20 milligrams of cobalt in 2.0 millliliters of solution for purposes of comparison.

The effect of cobalt ion on glucose tolerance was studied by simultaneous injection of cobalt chloride and glucose solutions into the stomach in varying concentrations. As before, blood sugar was determined at intervals after the injection. Data are presented in Table 31 and are plotted in Figure 9.

Table 29

Changes Produced in Blood Sugar Concentration by Administration of Cobalt Chloride Solutions by Stomach Tube

Time in minutes after injection of cobalt solution			0	15	30	60	90	120
Solution injected	Rat Sex	Rat Weight	Milligrams of glucose per 100 ml. of blood					
1.0 ml. distilled water	M	370	91	107	86	83	86	102
	M	300	82	111	101			
	M	380	93	104	91	88	95	87
	F	200	96	102	107	98		98
	Average		90	106	99	90	90	96
20 mg. cobalt ion in 2.0 ml.	M	440	118	127	109	97	124	
	M	412	100	112	95	140	132	123
	M	290	94	115	127	143	133	182
	M	290	85		133	154	160	180
	Average		99	118	116	133	137	162
20 mg. cobalt ion in 1.0 ml.	F	220	115	131	137	194	208	185
	F	230	116	126	126	164	187	200
	F	250	112	155	156	157		
	F	342	109	154	140	180	210	227
	M	400	125	136	138	190	138	121
	M	180	124	167	192	167		182
	Average		117	145	148	175	186	183
10 mg. cobalt ion in 1.0 ml.	F	222	113	127	123	124	111	107
	M	280	98	108	122	102	111	117
	M	140	101	112	125	120		135
	F	150	114	141	132	114		140
	M	150	108	117	105	114		136
	Average		107	121	121	115	111	127
2.0 ml. HCl, pH 5.0	M	300	72		95	84	89	91
	F	200	72		83	77	80	89
	F	195	80		92	84	90	
	F	190	98		87	82	88	87
	M	240	94		91	78	80	81
	Average		83		90	81	85	87



Time in minutes after injection of test solution into stomach

Fig. 7. Changes produced in blood sugar concentration by administration of cobalt chloride solutions to stomach tube

Table 30

Changes Produced in Blood Sugar Concentration by Simultaneous Administration of Cobalt Chloride, Urea and Glycine Solutions by Stomach Tube

Time in minutes after injection of cobalt and glycine solutions			0	15	30	60	90	120
Solution injected	Rat Sex	Weight	Milligrams of glucose per 100 ml. of blood					
Co, 20 mg. Urea, 80 mg. in 2.0 ml.	M	300	96	114	101	110	163	192
	M	235	90	116	109	129	133	129
	M	310	78	92	95	108	122	95
	Average		88	107	102	115	139	139
Co, 20 mg. glycine, 200 mg. in 2.0 ml.	M	275	79	103	97	92	95	90
	M	275	74	103	98	96	95	
	M	350	86	97	95	100	90	104
	M	365		111	89	96	96	109
	Average		80	103	95	98	94	101
Co, 10 mg. glycine, 100 mg. in 2.0 ml.	F	220	84	101	87	100	102	109
	F	200	90	102	95	105	109	
	Average		87	108	91	102	105	109
Co, 20 mg. glycine, 100 mg. in 2.0 ml.	F	200	105	133	138	154	160	177
	M	220	93	95	111	118	111	103
	M	290	95	111	105	102	92	100
	M	275	86	111	111	143	161	182
	M	280	77	100	105	123		
	M	265	87	125	125	146	170	200
	M	270	83	104	108	108	111	105
	M	285	83	107	111	140	154	148
	M	240	90	100	106	112	124	125
	M	275	87	115	114	138	128	110
	M	270	74	108	108	142	144	156
	M	220	82	93	88	92	95	92
	Average		87	109	111	126	132	136

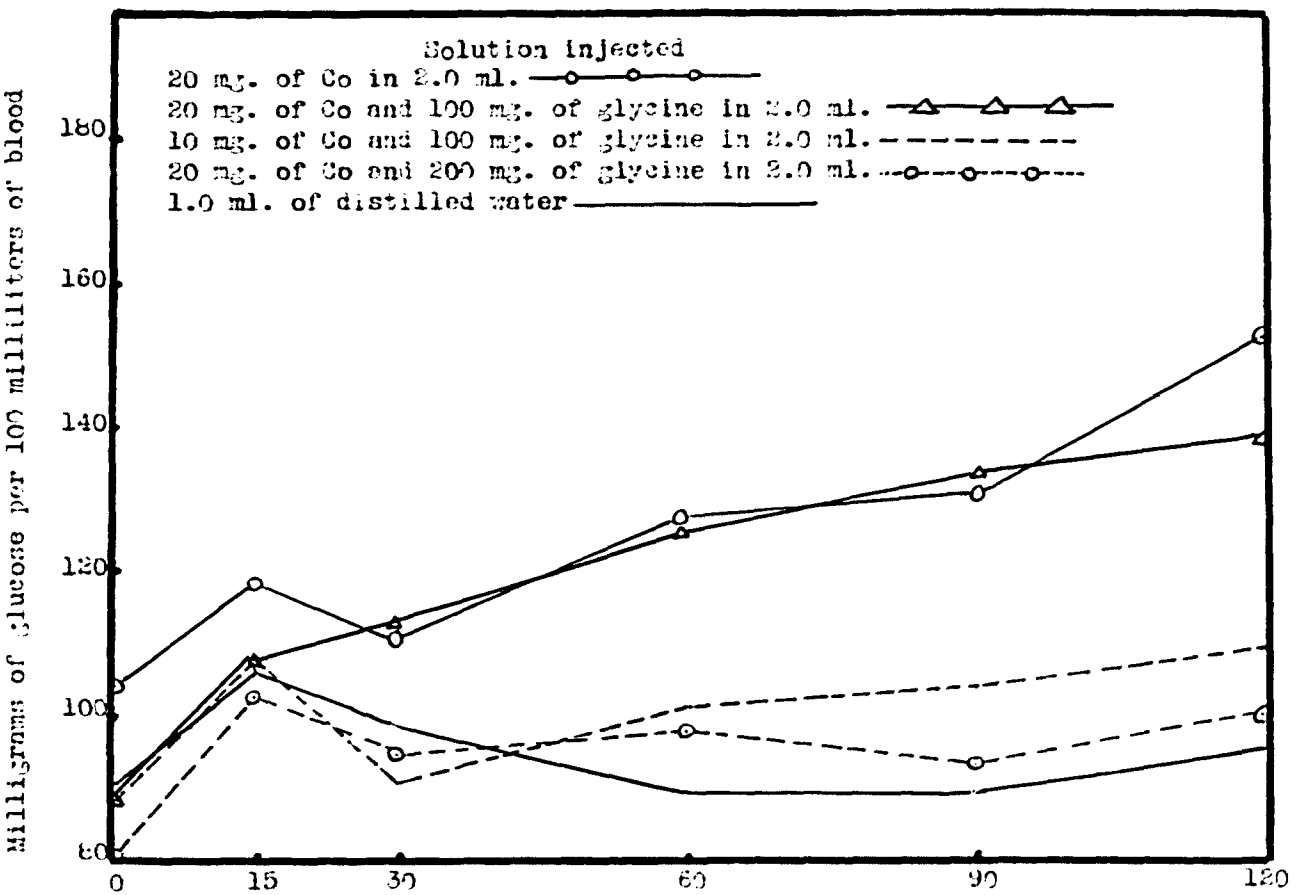


Fig. 6. Changes produced in blood sugar concentration by simultaneous administration of cobalt chloride and glycine solutions by stomach tube

Table 31

Changes Produced in Blood Sugar Concentration by Simultaneous Administration of Cobalt Chloride and Glucose Solutions by Stomach Tube

Time in minutes after injection of cobalt and glucose solutions			0	15	30	60	90	120
Solution injected	Rat Sex	Rat Weight	Milligrams of glucose per 100 ml. of blood					
	M	365	102	133	121	133	117	105
	M	317	119	125	124	145	120	
250 mg.	M	370	105	154	147	143	140	130
glucose	M	350	111	152	151	150	130	124
	M	320	107	180	143	138	126	126
	Average		109	149	137	142	127	121
20 mg.	M	310	129	160	143	139	132	125
cobalt	F	250	98	145	167			
250 mg.	M	367	113	161	147	156	135	159
glucose	M	365	106	141		160	177	178
in 1.0 ml.	M	360	143	178	156	136		122
	Average		118	157	153	148	148	146
	F	254	130	161	171	152		
20 mg.	M	330	83	107	121	131	154	158
cobalt	M	360	84	115	121	151		
250 mg.	M	350	106	131	115			100
glucose	M	406	103	167	147	150	222	200
in 2.0 ml.	M	355	97	148	158			
	M	305	137	154	154	138		138
	Average		106	141	141	144		149
	M	370	95	131	126	120		115
10 mg.	F	255	90	133	133	120		110
cobalt	F	230	98	115	100	117	108	125
125 mg.	F	230	109	134	135	125	123	110
glucose	M	217	91	140	127	129	171	177
in 1.0 ml.	F	180	96	182	206	238		267
	Average		97	139	138	141		151

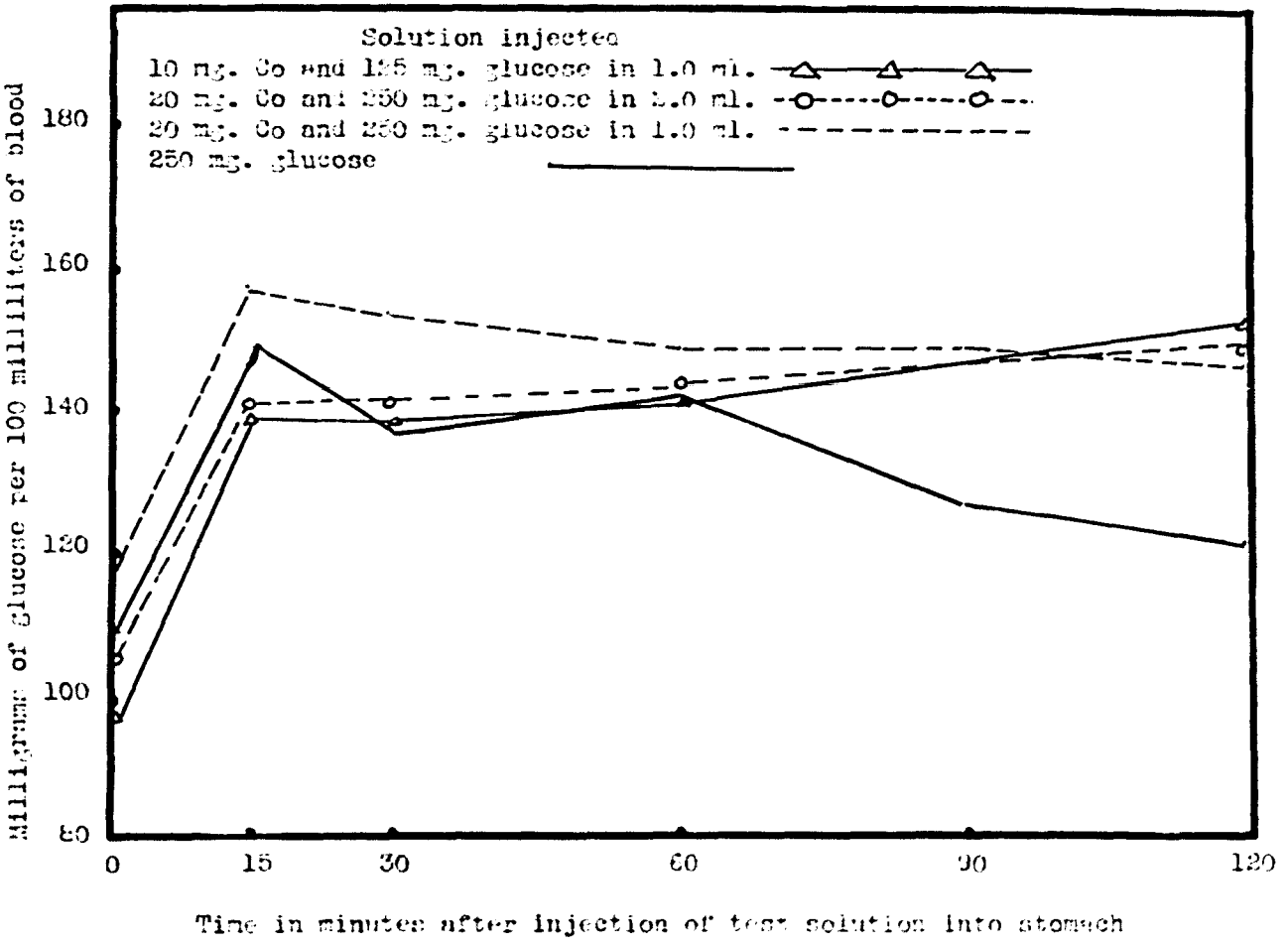


Fig. 9. Changes produced in blood sugar concentration by simultaneous administration of cobalt chloride and glucose solutions by stomach tube

Effects of feeding glycine and cobalt chloride simultaneously to rats

The data presented in the previous experiment indicated that glycine was able to detoxify the cobalt in some way which seemed to prevent the hyperglycemia produced by cobalt salt alone. It was thought of interest to investigate the effects of feeding cobalt chloride and glycine simultaneously to growing rats to observe the effects upon growth, reproduction, the hemoglobin concentration in the blood and the erythrocyte count. Groups of six rats of weaning age were fed growing ration with 200 parts per million of added cobalt (as cobalt chloride) with supplements of 0, 0.05 per cent, 0.1 per cent and 0.2 per cent glycine. In addition, a control group received only the growing ration. Hemoglobin determinations and erythrocyte counts were made on all the rats in this experiment after they had been receiving these diets for 13 weeks. These data are shown in Table 32.

After the rats had been receiving these rations for 17 weeks and it was apparent that the glycine was having no detoxifying effect upon the added cobalt chloride, it was decided to feed cystine in place of the glycine in two of the diets. Accordingly the 0.05 per cent glycine was replaced with 0.3 per cent cystine and the 0.1 per cent glycine was replaced with 0.5 per cent cystine. All other diets remained unchanged. All growth data is shown in Table 33 and is plotted in Figure 10.

Table 32

Erythrocyte Counts and Hemoglobin Data for Rats Fed Cobalt Chloride and Glycine

Diet fed	Rat: Sex	Rat: Weight	Hemoglobin, milligrams per 100 ml.	Erythrocyte count
No cobalt, no glycine	M	303	13.0	7,845,000
	M	278	12.5	8,405,000
	M	295	13.4	8,735,000
	F	237	13.4	9,065,000
	F	232	11.1	7,270,000
	F	262	11.2	8,405,000
	Average	268	12.4	8,287,500
Cobalt ion, 200 p.p.m. no glycine	M	184	16.2	12,490,000
	M	164	17.6	17,080,000
	M	162	14.0	13,365,000
	F	145	13.5	9,400,000
	F	127	16.7	12,080,000
	F	131	14.8	11,620,000
	Average	152	15.5	12,672,500
Cobalt ion, 200 p.p.m. glycine 0.2%	M	200	16.8	17,090,000
	M	140	18.5	17,900,000
	M	170	19.0	14,140,000
	F	162	15.8	13,040,000
	F	146	17.0	13,870,000
	F	161	14.5	12,030,000
	Average	163	16.9	14,680,000
Cobalt ion, 200 p.p.m. glycine 0.1%	M	150	18.2	14,600,000
	M	170	14.0	8,420,000
	M	150	14.5	8,444,000
	F	183	15.0	8,650,000
	F	120	15.4	13,040,000
	F	132	15.0	9,000,000
	Average	153	15.4	10,359,000
Cobalt ion, 200 p.p.m. glycine 0.05%	M	160	16.8	10,810,000
	M	151	17.0	15,870,000
	F	180	15.3	5,500,000[a]
	F	143	16.0	12,810,000
	F	130	15.4	8,430,000
	Average	153	16.1	11,684,000
[a]Ear infection			(16.3) Averages, neglecting (a)	(13,230,000)

Table 33

Average Weight in Grams of Groups of Six Rats Fed Cobalt Chloride with Supplements of Glycine or Cystine

Weeks fed ration	Supplement fed: None	Co, 200 p.p.m.	Co, 200 p.p.m.; glycine 0.2%	Co, 200 p.p.m.; glycine 0.1%	Co, 200 p.p.m.; glycine 0.05%
0	52	49	50	54	49
1	80	70	68	67	68
2	96	69	70	73	72
3	121	83	83	83	89
4	143	94	96	91	94
5	162	99	105	101	97
6	187	106	113	106	104
7	206	122	130	112	113
8	226	125	135	121	118
9	219	132	139	119	116
10	240	142	145	137	128
11	244	139	144	135	137
12	263	148	160	148	149
13	268	152	163	153	153
14	285	153	156	158	157
15	294	160	169	160	156
16	279	157	168	164	160
17	299	157	153	161	159
18	303	167	156	173[a]	155[b]
19	310	175	164	186	168
20	303	179	166	200	179
21	304	180	171	205	177
22	313	185	180	214	194
23	307	188	177	217	194
24	334	190	184	227	206
25	317	185	195	231	206
26	312	186	185	231	210
27	330	181	179	238	212
28	316	174	178	236	209
29	315	180	189	241	216
30	320	188	177	251	223

[a]Glycine replaced by 0.5 per cent cystine.
[b]Glycine replaced by 0.3 per cent cystine.

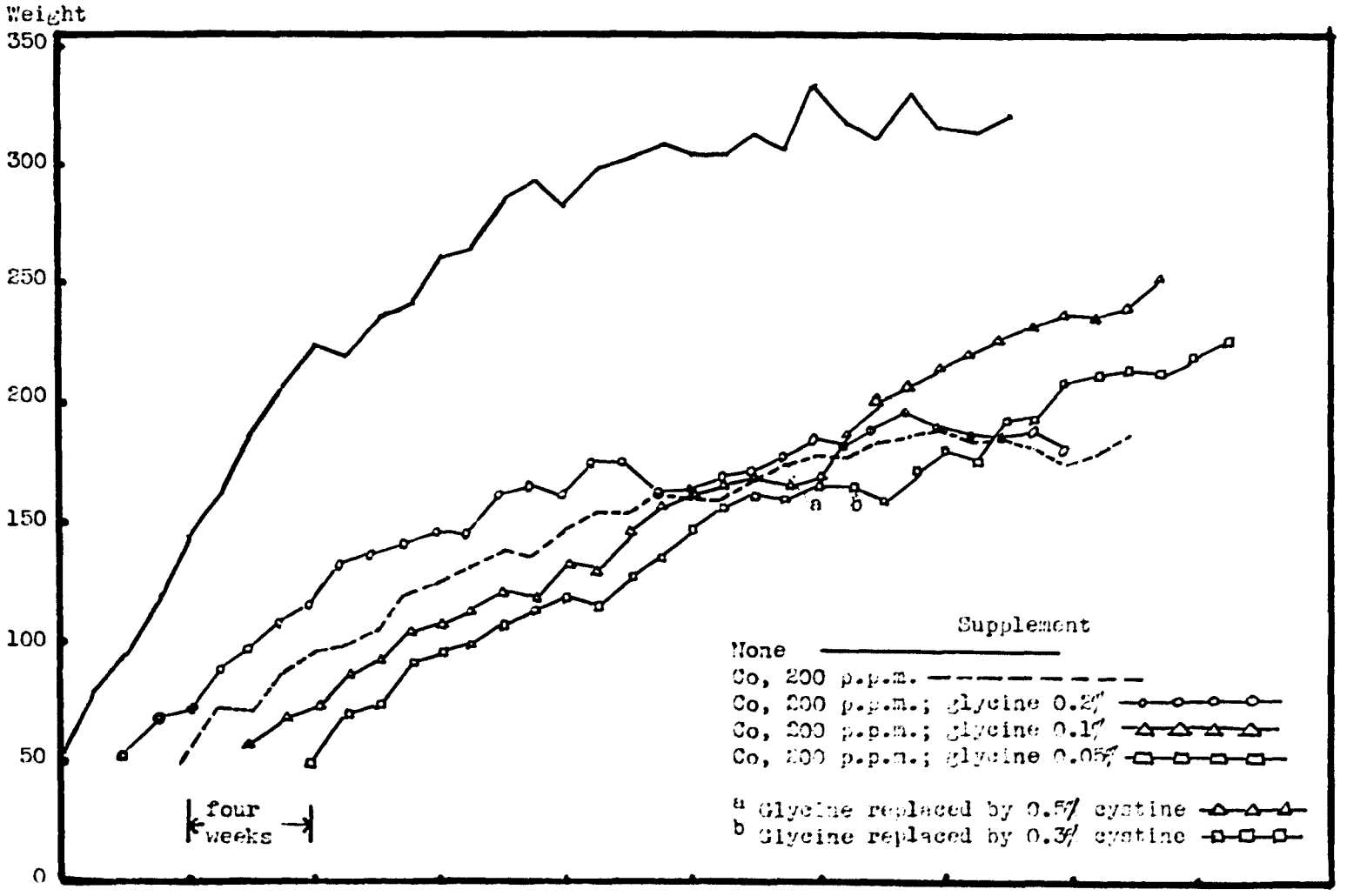


Fig. 10. Average weight in grams of groups of six rats fed cobalt chloride with supplements of glycine or cystine

No reproduction occurred in any of the groups fed cobalt chloride with or without added glycine or cystine except for two litters produced 53 days apart by one of the females receiving 200 parts per million of cobalt and 0.2 per cent glycine. The first litter was not produced until all the control group females had reproduced three times.

DISCUSSION

Growth

It is apparent from the data of Tables 11 and 13 and from Figures 4 and 5 that rations containing 5, 10 or 25 parts per million of added cobalt (as cobalt nitrate) do not appreciably affect the growth of rats. The curves of Figure 5 show that the growth rate of the rats receiving added cobalt ion in these concentrations is very similar to that of the control group not fed added cobalt salt. Table 8 and Figure 1 show that rats receiving 50 parts per million of cobalt (as cobalt nitrate) do not grow as rapidly as the controls when the controls are allowed to eat all the ration they want. Table 10 and Figure 2 show that when the control animals not fed cobalt nitrate are fed only as much ration as eaten by the rats consuming the ration with 50 parts per million of added cobalt, no appreciable difference in growth is apparent between the two groups. Neither group, however, grows as well as rats eating all the growing ration they want--compare tables 8 and 10 or Figures 1 and 2.

This indicates that at least a large part of the growth decrease caused by the presence of 50 parts per million of added cobalt in the diet is due to the restriction of feed

intake by the rats.

Table 8, Figure 1 and Table 10, Figure 3 show the effects of cobalt (as cobalt nitrate) at the 200 part per million level. In addition to the reduced growth rate due to restricted feed intake, there is an additional toxic effect within the organism, since rats receiving 200 parts per million of cobalt grow very appreciably slower than the controls eating the same weight of growing ration without added cobalt salt.

Feed consumption of rats receiving 200 parts per million of cobalt was considerably less than that of rats fed only 50 parts per million of cobalt (an average of 1.38 and 1.92 kilograms of feed in 23 weeks per rat for each group, respectively). This accounts for at least part of the difference in growth rates of the two groups.

The greatly decreased growth rate occurring on rations containing 200 parts per million of cobalt is apparently not due to any permanent injury to the rat, since the growth rate increased at once when the cobalt was omitted from the growing ration. This is shown in the lower curve of Figure 1.

Figure 1 shows that the decrease in growth resulting from adding cobalt nitrate to the ration is approximately proportional to the amount of cobalt added.

Reproduction

It is readily apparent from the data of Tables 14, 15 and 16 that when cobalt is present in the ration (as cobalt salt) in concentrations of 100 parts per million or more, reproduction ceases entirely.

Table 14 shows that when 5, 10 or 25 parts per million of cobalt ion are added to the ration, there is little difference in the reproduction of first litters by rats receiving cobalt salt and those receiving no added cobalt salt. Apparently cobalt in the diet at the 25 parts per million level or less exerts little harmful influence on reproduction as far as the first litter is concerned. Table 15 shows that cobalt in the ration at the 5 or 10 parts per million level does not affect reproduction of the second litter.

When 50 parts per million of added cobalt are present in the diet, the effect on reproduction seems to be more complex. Inspection of the data in Table 14, growth experiment A, shows the marked decrease in the average number of young and the average weight of the first litter when 50 parts per million of cobalt are added to the ration. The reproduction data of growth experiment B, Table 14, (where the control animales were fed only the weight of ration consumed by the rats receiving added cobalt ion) show a much less striking decrease in the number of young produced. It is probable that as far

as first litters are concerned, the decreased reproduction caused by 50 parts per million of added cobalt in the diet may come about through the effect of this concentration of cobalt on growth, which in turn depends mainly upon the decreased feed intake of rats fed cobalt salts at this level. Table 15 shows a decrease in the average number and weight of the second litter of rats fed 50 parts per million of cobalt, not quite so striking as that of the first litter. Table 16 shows that for longer periods of time, the average number of young and the weight of each litter are slightly smaller for rats receiving 50 parts per million of added cobalt than for the control animals fed the limited amounts of ration described previously. It should be noted that of the six females fed cobalt salt at this concentration, only five reproduced, one dying in first pregnancy. All of the control females reproduced.

It seems, then, that the initial decrease of reproduction caused by 50 parts per million of cobalt added to the diet as cobalt nitrate may be due in part to decreased growth but that over longer periods of time, when the more slowly growing rats receiving added cobalt salt have had an opportunity to mature, the differences in reproduction become smaller. The delay in attaining maturity and the decreased reproduction caused by restricted feed intake alone are clearly shown in the data for the control groups of growth experiment B, Tables 14 and 16.

Lactation

The data of Table 17 show that the per cent success in raising young from birth to 28 days of age is practically identical for the control rats receiving no cobalt salt and rats receiving 5 and 25 parts per million of added cobalt. In addition, the weights of young at birth and at 28 days show little difference between control groups and groups receiving cobalt. It must be concluded that 5 or 25 parts per million of cobalt ion in the ration do not affect lactation of rats.

When cobalt ion is fed at a concentration of 50 parts per million, only five of seven litters born were successfully raised. The average weights of the first litter at birth and at 28 days are smaller for the rats receiving cobalt. The second litters show no great differences. It may be concluded that a concentration of 50 parts per million of cobalt in the diet affects lactation of rats to a small extent but that lower concentrations of cobalt are without influence on lactation.

Hemoglobin Formation

The rats fed 10 parts per million of cobalt (as cobalt nitrate) showed a definite stimulation of hemoglobin formation during the first ten weeks of the experiment as shown in Table 19 and Figure 6. This early increase in hemoglobin

concentration in the young rats caused by the cobalt salt is not permanent and the values decrease to the level of the controls after the fourteenth week.

The feed consumption data of Table 18 show that this stimulation of hemoglobin formation was brought about by an average cobalt intake of 0.15 milligrams per rat per day. An average intake of 0.07 milligrams of cobalt ion per day by the rats fed 5 parts per million of cobalt ion failed to stimulate hemoglobin formation above the general level of the controls.

It is apparent that the minimum amount of cobalt in the diet which is necessary to stimulate hemoglobin formation in young rats lies between 0.07 and 0.15 milligrams of cobalt per day.

Distribution of Cobalt in the Tissues

The third series of analyses of rat organs for cobalt presented in Table 22 must be considered the most accurate since these analyses were made last, after much experience with the analytical method had been obtained. In addition, organs of three rats were pooled for these determinations, thus making the estimation of low concentrations of cobalt more accurate.

In general, the data of Tables 20, 21 and 22 indicate that the amount of cobalt present in the tissues of rats fed

growing ration without added cobalt salt is less than one part per million. Adding cobalt salt to the diet increases the cobalt content of most of the organs studied. Liver and kidney show the greatest increase in deposition of cobalt under these conditions while stomach and intestine show the least. Spleen, pancreas, heart, lungs and testes show some increased cobalt deposition but the increase is much less than proportional to the concentration of cobalt salt fed. Only in the liver does the increased deposition of cobalt seem to be proportional to the concentration in the diet. The deposition of cobalt in the kidneys, while less than proportional to the amount of cobalt fed, is still considerably greater than that of other organs except that of the liver. Since other workers have shown that a portion of the ingested cobalt ion is excreted in the urine, this deposition in the kidneys might be expected. The most striking observation from the data of Table 22, however, is the great increase in the cobalt content of the liver that occurs when cobalt salt is added to the diet.

Rate of Deposition of Cobalt in the Liver

Allowing for the biological variations inherent in the experiment, it is apparent from the data of Table 23 that the concentration of cobalt in the liver reaches a maximum average value of about 7.0 parts per million in about four days when young rats are fed a ration containing 100 parts per million of cobalt as cobalt nitrate. It should be noted in

Table 22 that the cobalt concentration in the livers of rats fed 200 parts per million of cobalt for 26 weeks was 6.8 parts per million, agreeing very well with the maximum value found in this experiment.

It is of interest that the livers of female rats showed a uniformly greater concentration of cobalt than the livers of male rats under the conditions of this experiment.

It is seen from the food consumption data of Table 23 that the amount of cobalt deposited in the liver in any one day (12-15 micrograms maximum) is only about one per cent of the daily intake of cobalt (1000-1400 micrograms).

Toxicity

It is apparent from the data of Tables 24 and 25 that the minimum lethal dose of cobalt salt for rats, when injected into the stomach of the anesthetized animals, is approximately 20 milligrams of cobalt per 100 grams of body weight.

There is little apparent difference between the toxic effects of cobalt nitrate and cobalt chloride.

Effect of Cobalt Salt in the Diet on Reducing Sugar and Albumin in the Urine

Comparing the data in Tables 26 and 27 for each level of cobalt with that of its own control group, it is evident that addition of cobalt ion to the diet at levels of 50 and 200

parts per million approximately doubles the per cent of reducing sugar in the urine. The actual number of milligrams of reducing sugar excreted in 24 hours is increased by adding cobalt nitrate to the diet but the values are not affected to the same extent as the per cent reducing sugar since the 24-hour volume of urine tends to be slightly lower for the rats receiving added cobalt nitrate in the ration. The average per cent reducing sugar, excretion of reducing sugar per 24 hours and the limits of variation of these values are all greater for the rats fed 200 parts per million of cobalt ion than for those fed 50 parts per million.

The lower limits of the variation of the values are about the same for rats fed cobalt nitrate and the controls receiving no added cobalt salt. The upper limits of the variation are invariably greater for the rats fed cobalt salt.

Qualitative tests for albumin with Roberts' reagent showed albumin present in urine of rats fed cobalt salt as well as in the urine of the rats fed growing ration with no added cobalt.

Effect of Cobalt Salt in the Diet on Non-Protein Nitrogen in the Blood

Considering the variation in the values for the determinations of non-protein nitrogen in the blood of individual rats and for all rats shown within one group shown in Table 28, it is probable that the differences between the non-protein

nitrogen concentration in the blood of rats fed cobalt salt and those not fed cobalt salt are not great enough to indicate any change caused by the additional cobalt in the diet. It may be concluded that cobalt fed at the 50 part per million level does not affect the blood non-protein nitrogen.

Effects on Blood Sugar of Peroral Administration of Cobalt Chloride Solutions Alone and with Urea, Glucose and Glycine

The data of Table 29 plotted in Figure 7 show that 20 milligrams of cobalt (as cobalt chloride) injected into the stomach by stomach tube causes the blood sugar concentration to increase markedly, especially in the period from 30 to 120 minutes after the injection. The hyperglycemia is much greater if the 20 milligram sample of cobalt is injected in 1.0 millliliter of solution than if it is injected in 2.0 millliliters. The anesthesia and manipulation probably cause the initial increase in blood sugar since such an increase is noted under these conditions when only distilled water is injected into the stomach. The hyperglycemia caused by the cobalt salt does not appear until 30 to 60 minutes after injection of the cobalt solution. The hyperglycemia is not due to the slight acidity caused by the hydrolysis of the cobalt chloride since injection of a hydrochloric acid solution of the same pH as the cobalt chloride solutions did not increase the blood sugar concentration at all. It had approximately the

same effect on the blood sugar concentration as did injection of distilled water.

Table 30 and Figure 8 show that when glycine in sufficient amounts is injected into the stomach simultaneously with the cobalt salt, the blood sugar concentration does not rise but remains approximately at the same level as when distilled water is injected. Urea, on the other hand, in two experiments of the three made, did not prevent cobalt from causing the hyperglycemia noted with injections of cobalt salt alone.

It should be noted from Table 30 that glycine depresses the hyperglycemia caused by cobalt only with injections of ten times as much glycine as cobalt by weight. The mole ratio for these weights is approximately eight glycine molecules to one cobalt ion. With injections in a ratio of four glycine molecules to each cobalt ion (100 milligrams of glycine and 20 milligrams of cobalt), it should be noted that approximately half of the values for the glucose concentration in the blood 120 minutes after the injection show the marked hyperglycemia typical of injections of cobalt salt alone. The other half of these values tends to approximate the low level produced by glycine and cobalt in the eight to one mole ratio. The five higher values lie between 148 and 200 milligram per cent while the five lower values lie between 92 and 110 milligram per cent. Only two values, 125 and 136 milligram per cent, lie in the range between. This concentration of blood sugar values in relatively narrow ranges,

especially at the lower values, apparently indicates that the mole ratio of four glycine molecules to each cobalt ion approaches the limiting value for detoxification of the cobalt in its hyperglycemic action.

Table 31 and Figure 9 show the effects on glucose tolerance of injecting cobalt salt into the stomach by stomach tube. The blood sugar curve after injection of glucose only starts to fall 60 minutes after the injection while all the curves for the effects of injecting cobalt salt and glucose into the stomach simultaneously do not fall after 60 minutes but remain at a practically constant value. Inspection of the curves of Figure 9 suggests that the normal decrease of blood sugar beginning 60 minutes after injection of glucose solutions into the stomach is apparently just offset by the increased blood sugar formed by the toxic action of cobalt salt which appears in the period from 30 to 60 minutes and especially in the period from 60 to 120 minutes after injection of the test solutions into the stomach.

Effects of Feeding Glycine and Cobalt Chloride Simultaneously to Rats

Table 33 and Figure 10 show that glycine, when fed in the diet, does not prevent added cobalt salt from inhibiting growth, the growth curves for rats fed both cobalt chloride and glycine at various concentrations not only being almost identical but also following closely the growth curve for rats

fed cobalt chloride without added glycine. Similarly, added glycine in the diet does not markedly alleviate the inhibiting effects of cobalt on reproduction, no reproduction occurring among any of the nine females receiving added cobalt chloride with added glycine with the exception of two litters produced by one female receiving 0.2 per cent glycine in addition to the 200 parts per million of cobalt.

Table 32 shows the effects of added cobalt chloride and glycine in the diet on the erythrocyte count and hemoglobin concentration in the blood of rats which had received these rations for about 13 weeks. The striking increases in erythrocyte count and hemoglobin concentration caused by adding 200 parts per million of cobalt to the diet were not lowered by adding 0.2 per cent glycine to the ration. Addition of 0.1 per cent glycine to the ration with 200 parts per million of cobalt apparently caused the erythrocyte count to decrease in some of the animals but not in others while the hemoglobin concentration remained at the higher level associated with cobalt feeding. A still lower concentration of glycine, 0.05 per cent, lowered the erythrocyte count in only one rat out of four. Anomalous results in cobalt polycythemia have been reported by others in the literature and apparently must be attributed to the biological variation to be expected in such experiments. With this in mind, it may be concluded that added glycine in the diet of rats probably has little effect in lowering the elevated hemoglobin concentrations and

erythrocyte counts caused by adding cobalt chloride to the ration.

When the rats had been fed rations with added glycine and cobalt chloride for 17 weeks and it was apparent that glycine was not detoxifying the cobalt, the glycine was replaced with larger concentrations of cystine. Table 33 and Figure 10 both show the immediate increase in growth rate which occurred when cystine feeding was begun. This agrees with the recently published work of Griffith, Pavcek and Mulford (66) who found that cystine and other sulfur containing amino acids were able to detoxify cobalt salts within the organism.

CONCLUSIONS

From the data presented in this thesis the following conclusions have been drawn:

1. Rations containing 500 parts per million of cobalt (as cobalt chloride) are lethal to young rats in 3-5 weeks. Greater concentrations of cobalt salt are lethal in a shorter time.

2. Rations containing 25 parts per million of cobalt (as cobalt nitrate) or less do not appreciably decrease the growth rate of rats.

3. When cobalt nitrate is fed at the rate of 50 parts per million of cobalt in the growing ration, decreased growth of rats results primarily because of decreased feed intake.

4. When cobalt nitrate is fed at the rate of 200 parts per million of cobalt, decreased growth of rats results both because of decreased feed intake and because of an additional toxic effect of the cobalt salt within the organism.

5. The decreased growth caused by 200 parts per million of cobalt in the diet is not due to any permanent injury to the rat as far as growth is concerned, since the growth rate increases immediately when the added cobalt salt is omitted from the diet.

6. The decreased growth of rats caused by adding cobalt salt to the diet is approximately proportional to the amount of cobalt added.

7. Reproduction of rats ceases entirely when 100 parts per million of cobalt or more are added to the diet as cobalt nitrate.

8. When 25 parts per million of cobalt are in the diet (as cobalt nitrate),reproduction of the first litter is not affected in any way.

9. When 50 parts per million of cobalt are added to the diet (as cobalt nitrate), the number of young per litter, the number of litters born and the weight of each young are all decreased. The effects are probably due in large measure to the decreased growth of the mothers caused by this concentration of cobalt salt in the diet.

10. When 50 parts per million of cobalt are fed in the ration, successful lactation, as judged by the successful raising of the young, is decreased to a small extent. Lower levels of cobalt salt do not affect lactation.

11. The amount of cobalt present in the tissues of rats fed growing ration without added cobalt salt is, in general, less than one part per million.

12. Adding cobalt salts to the diet increases the amount of cobalt most markedly in the liver and kidneys. Spleen, pancreas, heart, lungs and testes show some increased cobalt

deposition while intestine and stomach show the least increase.

13. Only in the liver is the deposition of cobalt proportional to the amount of cobalt salt fed, this organ taking up far more of the cobalt than any other. Deposition of cobalt in the kidneys is less than in the liver but is greater than that of other organs.

14. When cobalt nitrate or cobalt chloride solutions are injected into the stomach of anesthetized rats by stomach tube, the minimum lethal dose is approximately 20 milligrams of cobalt per 100 grams of body weight. There is little apparent difference in toxicity of the two salts under these conditions.

15. When young rats are fed 100 parts per million of cobalt (as cobalt nitrate), the deposition of cobalt in the liver reaches a maximum in four to five days. The maximum amount of cobalt deposited per day is only about one per cent of the total cobalt intake.

16. Ten parts per million of cobalt in the diet (as cobalt nitrate) is the lowest concentration that will stimulate the formation of hemoglobin in young rats. The stimulation is not permanent at this concentration of cobalt, the hemoglobin concentration decreasing to that of the controls after about 14 weeks. The intake of cobalt to cause this initial stimulation of hemoglobin formation lies between 0.07 and 0.15 milligrams per day.

17. Addition of cobalt nitrate to the diet at levels of 50 or 200 parts per million of cobalt approximately doubles the per cent of reducing sugar in the urine. Part of this effect is due to a small decrease in the volume of urine of rats fed the added cobalt salt but the major part of the effect is due to an increase in the amount of reducing sugar excreted.

18. Albumin is present in the urine of rats fed cobalt nitrate as well as in the urine of rats not receiving added cobalt salt in the diet.

19. Addition of cobalt nitrate to the diet does not appreciably affect the amount of non-protein nitrogen in the blood.

20. When a solution containing 20 milligrams of cobalt (as cobalt chloride) is injected into the stomach of rats, the blood sugar concentration is markedly increased in 30 to 60 minutes after the injection. The effect is more pronounced when the solution of cobalt chloride is more concentrated, even though the same amount of cobalt is given.

21. When glucose and cobalt chloride solutions are injected simultaneously into the stomach of the rat by stomach tube, the normal decrease of glucose in the blood after 60 minutes or more is offset by the increased blood sugar formed by the effect of the cobalt salt, maintaining the blood sugar concentration at a constant elevated value for 60 to 120 minutes after the injection.

22. When 200 milligrams of glycine are injected with 20 milligrams of cobalt (as cobalt chloride) into the stomach, the glycine prevents the cobalt salt from increasing the blood sugar concentration. When 100 milligrams of glycine are injected with 20 milligrams of cobalt, the hyperglycemia is prevented in about half of the cases.

23. When glycine and cobalt chloride are added to the growing ration, the glycine does not prevent the added cobalt salt from inhibiting growth or reproduction of rats.

24. The increased hemoglobin concentration and erythrocyte count caused by adding cobalt chloride to the growing ration are not appreciably decreased toward the normal values by simultaneous feeding of glycine.

SUMMARY

A study was made of the effects of feeding cobalt chloride and cobalt nitrate on the growth, reproduction and lactation of rats. Concentrations of 25 parts per million or less of cobalt added to the diet were found to be without appreciable effect on the growth rate, success of lactation or reproduction of the first litter. When 50 parts per million of cobalt were added to the diet, growth of rats was decreased, primarily due to the decreased feed intake; the number of young per litter, the weight of each litter and the average weight of each young were all decreased and success in lactation was decreased to a small extent. With concentrations of 100 parts per million or more of cobalt added to the diet, reproduction ceased entirely. With 200 parts per million of cobalt in the diet growth was decreased both because of decreased feed intake and because of some other toxic effect of the cobalt salt within the organism. The decreased growth rate caused by 200 parts per million of cobalt in the diet was not due to any permanent injury to the rat (as far as growth was concerned) since growth was resumed when the cobalt salts were omitted from the diet. In all cases, the decreased growth caused by adding cobalt salts to the diet was approximately proportional to the concentration of cobalt salt fed.

When concentrations of 500 parts per million of cobalt were fed to young rats, they died within 3-5 weeks. Higher cobalt concentrations in the ration were lethal in a shorter time.

Cobalt analyses were made on tissues of rats fed cobalt salt and on tissues of rats which had not received added cobalt salt in their diet. In general, the tissues of rats not fed cobalt salts contained less than one part per million of cobalt. The tissues of rats fed cobalt salts showed marked increase in cobalt content in the liver and kidneys with lesser increases in the spleen, pancreas, heart, lungs and testes. The least increases in cobalt content occurred in the intestine and the stomach. Only in the liver was the concentration of deposited cobalt proportional to the concentration of cobalt fed in the diet. Deposition of cobalt in the kidneys was less than in liver but greater than in the other organs studied. When young rats were fed a diet containing 100 parts per million of added cobalt, the concentration of cobalt deposited in the liver reached its maximum value in 4 to 5 days after the feeding of such a ration was started.

When rats were fed 50 or 200 parts per million of added cobalt and control groups were fed the same weight of ration as consumed by the rats receiving cobalt, the amount of reducing sugar in the urine of the rats fed cobalt salt was markedly increased over that of the controls. Albumin was present in the urine of rats fed cobalt salt as well as in that of rats not fed cobalt salt. The concentration of non-protein nitrogen in

the blood was apparently not altered by feeding cobalt salt under these circumstances.

Ten parts per million of cobalt (as cobalt nitrate) in the diet seemed to be the lowest concentration that would stimulate hemoglobin formation and this stimulation was not lasting, the hemoglobin concentration decreasing after about 14 weeks to that of the controls not fed added cobalt salt.

When cobalt nitrate or cobalt chloride solutions were injected by stomach tube into the stomachs of anesthetized rats, the minimum lethal dose was found to be approximately 20 milligrams of cobalt per 100 grams of body weight. There was little difference in the toxicity of the two salts.

When less than the lethal amount of cobalt salt was injected by stomach tube, the blood sugar concentration increased markedly in 30 to 60 minutes after the injection and increased still more from 60 to 120 minutes after the injection. The effect was more pronounced when more concentrated solutions were injected, even though the same amount of cobalt was given. The hyperglycemia was not due to the slight acidity caused by hydrolysis of the cobalt chloride. When 200 milligrams of glycine were injected by stomach tube, along with 20 milligrams of cobalt, no such increase in blood sugar concentration occurred, the glycine apparently detoxifying the cobalt salt. When glucose solutions were injected into the stomach with cobalt salt in a similar manner, the normal decrease in blood sugar concentration in 60 to 120 minutes after injection (expected of

glucose injections alone) did not occur, the blood sugar concentration remaining at a constant, elevated value during the 60 to 120 minute period after injection. Apparently the normal blood sugar decrease expected from the glucose injection was just offset by the blood sugar increase caused by the toxic action of the cobalt salt.

When glycine and cobalt chloride were fed to rats in the growing ration, glycine failed completely to prevent the cobalt salt from decreasing growth and stopping reproduction. In addition, the added glycine failed to prevent the added cobalt salt from increasing the hemoglobin concentration and the erythrocyte count in the blood.

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