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Some changes produced in growth, reproduction, blood and urine of rats by salts of zinc with certain observations on the effects of cadmium and beryllium salts

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NOTE TO USERS

SOME CHANGES PRODUCED IN GROWTH, REPRODUCTION, BLOOD AND URINE OF RATS BY SALTS OF ZINC WITH CERTAIN OBSERVATIONS ON THE EFFECTS OF CADMIUM AND BERYLLIUM SALTS

by

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INTRODUCTION

A Brief Discussion of the Rat as an Experimental Animal for Studies on Growth, Reproduction, Blood and Urine.

Facts obtained by subjecting experimental animals to conditions of an extraordinary nature may yield information of physiological value. When the regime to which the animals are subjected is of a chemical nature, the information gained may be of value in clarifying our knowledge of metabolism.

The rat, Mus Norvegicus albinus, was selected as the most desirable experimental animal for this investigation. The reasons for such choice become apparent, when cognizance is taken of certain facts relating to the rat. The rat is a small and easily handled animal; it adapts itself readily to laboratory conditions, and its life span is relatively short. The food consumption of a rat is low, which makes it economical to keep. Not least in importance is the fact that the rat apparently possesses a poorly developed vomiting center. This allows a study of the rat under conditions tolerated by relatively few experimental animals. The emetic action of certain zinc salts makes this behavior of the animal highly desirable. No one is more conscious of the restrictions placed upon investigations employing the use of rats as experimental animals than is the experimenter. In general, such restrictions result from the size of the animals.

In this thesis growth of an animal is measured by the weight in-

crease. Interruption of growth is one of the earliest signs of malnutrition. Chronic intoxication in the living animal may be evidenced by the growth response. The rats of the Iowa State College colony grow well on a feed mixture composed of naturally occurring food substances; furthermore this mixture has served in this laboratory to promote good growth and reproduction for many years. Growth of rats on this ration is considered normal. In all cases the growth obtained during an experiment was referred for comparison to the growth of animals which received this stock ration.

One of the most fundamental of all the physiological properties of living organisms is reproduction. Without this property life would soon cease to exist. Sexual maturity in the female rat is evidenced soon after the opening of the vagina; this occurs at about the seventy-second day of life. A series of changes subsequently occurs in the mucous membrane of the vagina and uterus; this series of changes may be observed microscopically by making smears of the vaginal contents. The changes are of considerable importance in reproduction studies.

The first part of the sexual period has been identified as the proestrus or Stage One. This stage is marked by a slightly dry vaginal mucosa, and regular nucleated epithelial cells appear in the vaginal smear during this period. Stage Two is marked by a dry and lusterless vaginal mucosa. The lips of the vagina are slightly swollen, and the nucleated epithelial cells of Stage One have given way abruptly to a cornified type of epithelial cells. Stage Three shows an increasing amount of cornified material in the smear and is difficult to distinguish from Stage Two.

Stage Four is marked by a slightly moist vaginal mucosa, and leucocytes begin to appear with the cornified cells in the vaginal smear. The swelling of the lips of the vagina has disappeared at this stage. During Stage Five the vaginal mucosa appears moist and glistening. Leucocytes and epithelial cells with variable amounts of mucus appear in the vaginal smear. The female will not accept the male while in the proestrus stage. During the oestrus stage which occupies the latter part of Stage One and all of Stage Two, the female will accept the male. If conception takes place as a result of coition during oestrus, this period is followed by gestation. The earliest infallible sign of pregnancy detectable in the living animal is found in the "placental sign" or "erythrocyte sign", which appears during the thirteenth to the sixteenth days of the gestation period. Hemorrhage in the form of a small leakage from the vagina appears at this time, and erythrocytes are readily detectable in the smear.

The gestation period of the rat is normally twenty-one to twenty-two days in duration. A precipitous drop occurs in the weight of the female at parturition. Parturition is followed by the lactating period. The oestrus cycle ceases during the gestation period, but if the young are removed from the female following parturition, the oestrus cycle may begin again in three to twelve days.

If, on the other hand, conception does not occur during oestrus, this stage is succeeded by a short metoestrus during which the activity of the generative system subsides. In the rat this metoestrus period is followed by a short interval of quiescence. This short interval, which

lasts for only a few days, is called the dioestrus period. This, in turn, is followed by another pro-oestrus period and the cycle is repeated. Normally the reproductive cycle of the rat averages slightly less than five days in length. Stage One occupies approximately twelve hours, Stage Two and Stage Three together occupy approximately twenty-seven hours, Stage Four occupies approximately six hours, and Stage Five occupies approximately fifty-seven hours. The reader is referred to the excellent works of Marshall (26) and Long and Evans (25) for complete details on the physiology of reproduction.

In certain cases the gestation period may not be terminated by a normal parturition. An abnormal termination to a gestation may result from any influence which causes injury and death to the foeti. Death of the foeti before the end of term may result in resorption of the foeti by the female. Still born young may be cast when death occurs near the end of term. Death and resorption of the embryos is indicated by the arrest and gradual decline in the body weight of the female. Depending upon the nature of the causative agent which produced such behavior, the female may either become sterile or regain her reproductive ability when such cause is removed.

Blood has been defined as the circulating tissue of the body. It comprises a protein-rich fluid, plasma, in which are suspended various "formed elements" including red blood corpuscles and other cells. An important function of the blood, imparted by virtue of the hemoglobin contained within the red blood corpuscles, is the transfer of oxygen from the lungs to the body tissues. Another function of the blood is to aid

in maintaining the proper hydrogen ion concentration in the tissues. Another function of the blood is to carry food materials, among which is glucose, to the tissues, and to transport waste materials to the kidneys for excretion.

Hemoglobin, the red coloring matter of the blood, is a conjugate protein consisting of a protein part called globin and an iron pyrrol compound known as heme. Hemoglobin forms a loose combination with oxygen giving oxyhemoglobin. Oxyhemoglobin is the oxygen carrier of the blood and belongs to the class of bodies known as respiratory pigments. It is held within the stroma of the erythrocytes. When blood loses its oxygen there is a change in color due to the fact that the oxyhemoglobin has been transformed into reduced hemoglobin. Oxyhemoglobin is a stronger acid than hemoglobin and this fact is of considerable importance in the transportation of carbon dioxide by the blood. Furthermore, there is a reciprocal action between oxygen and carbon dioxide; the entrance of one into the blood facilitating the loss of the other and vice versa. It is also known that changes in pH affect the oxygen dissociation curves of hemoglobin; alkali increases the capacity of hemoglobin to transport oxygen and acid decreases the combination between hemoglobin and oxygen.

Factors which influence the chemical composition of the blood may be broadly classed as physical and metabolic. Alterations in the chemical composition of the blood resulting from either of these may be induced. Cases of chemical change in blood due to retention by virtue of alteration in the permeability of membranes are examples of the physical

factor. Cases of chemical change in blood due to increased or diminished formation or utilization of various constituents are examples of the metabolic factor. It may be said that changes in the blood chemistry need not be anticipated unless some condition affecting formation, utilization or elimination is present.

It is known that salts modify the water balance by their osmotic effects and may temporarily alter the blood and urine volume. This action is exerted by some salts when given by mouth. Blood alkalinizers, like sodium bicarbonate, favor hydremia, whereas acidifiers, like ammonium chloride, antagonize it and may even produce plasma concentration and diminished blood volume. Briefly summarized, the role of salts in the regulation of the blood volume may be referred to (1) the mechanism of intake, absorption, metabolic mobilization and elimination of water and (2) the osmotic shifts due to the "salt action" of the various dissolved substances.

Certain metallic salts administered by mouth give rise to a condition of hyperglycemia and glycosuria. In some cases glycosuria may appear without hyperglycemia. That all metallic ions do not stimulate the same physiologic response is a fact well known. The antagonistic action of the sodium and the potassium ions, as demonstrated by perfusion experiments on the heart, is a classic example. The calcium and the magnesium ions are also antagonistic. Magnesium sulfate, when injected into rats or rabbits in proper concentration, produces anesthesia. This anesthesia is accompanied by hyperglycemia. Calcium chloride in proper concentration injected during the anesthesia causes

immediate recovery from the anesthesia. If calcium chloride in proper concentration is injected into rats simultaneously with the injection of magnesium sulfate, anesthesia does not result, and the hyperglycemia is reduced. Reactions arising from a single large dose of a metallic salt are not necessarily provoked by a similar quantity of the material when given in smaller doses. Likewise the mode of administration may influence the reaction called forth. Magnesium sulfate administered by mouth may exert a marked purging action, but no anesthesia results; moreover, much smaller quantities of magnesium sulfate administered by intramuscular injection may produce anesthesia and a change in the blood sugar concentration, but exert no purging action.

A deficiency of blood or corpuscles, known as anemia, may result from several causative factors. A very general classification of anemias divides them into those whose etiology is unknown, primary anemias, and those whose etiology is known, secondary anemias. In this classification nutritional anemia is listed among the secondary anemias. Nutritional anemia is characterized by a low hemoglobin level and erythrocyte count. It is caused by a lack of copper and iron in the diet.

Aplastic anemias, which include examples of primary and secondary anemias, are produced by inadequate blood formation and are characterized by the appearance of abnormal cells within the blood stream. Bone marrow lesions can usually be demonstrated. Lead and radium poisoning may produce a pernicious anemia which is of the aplastic type.

Posthemorrhagic anemia is characterized by a blood picture showing

the regular number of red blood cells, but hemoglobin is not present in normal amount. In such cases a building of hemoglobin is essential, and this may be accomplished by iron administered in large doses.

Hemolytic anemias which result from mass destruction of blood cells within the body may be produced by toxins released into the blood stream by disease or pregnancy. The anemia of pregnancy is a well known example of this type.

Investigation of the urine may yield information on the condition of the excretory organs of the animal. In certain diseased conditions associated with metallic poisoning the kidneys function abnormally. In such cases the volume and composition of the urine may be different than that normally excreted.

Statement of the Problem

Nutritional studies have revealed various food substances essential to the growth and well-being of animals. Chemical studies are continually elucidating the composition of these food substances. More recent studies upon the nature of an adequate diet have shown that there exist many inorganic elements whose presence in minute quantities in the diet is essential. The field of nutritional study afforded by these so called "trace elements" is large and fascinating. Although the necessity of such elements as manganese, zinc, cobalt and copper has been shown, and the functions of some of these are known, in part, there are other elements such as silicon, fluorine, bromine, vanadium, arsenic and lead whose presence have been observed but whose nutritional importance is unknown.

It is believed that progress toward the solution of the problem of the physiological and nutritional role of these elements will be more complete when studies have been made upon some of the effects produced by large doses of the elements in question. This thesis is the result of studies on some changes in growth, reproduction, blood and urine of rats produced by salts of zinc with observations on some effects produced by salts of cadmium and beryllium.

Zinc, cadmium and beryllium are members of a family of elements which also contains the pharmacologically important and widely described elements, magnesium and mercury. Observation of the vast difference in physiological activity existing between salts of magnesium and mercury indicates that an investigation into the physiological role of salts of other members of this group may yield some interesting and unsuspected facts.

It was first proposed that the physiological role of zinc salts be investigated. Later it was found desirable to have data on other metals, and therefore, the lesser studied elements of the same group, cadmium and beryllium, were chosen. The results of experiments carried out during this investigation and described in this thesis came from attempts to answer the following specific questions:

1. What is the maximum level at which ingested zinc salt is tolerated by growing rats?

2. What are the evidences of intoxication by ingestion of a zinc salt?

3. What are some changes produced by levels of zinc which are

apparently tolerated by the animals?

4. Do these changes which are produced by ingestion of a zinc salt disappear when the added zinc is removed from the diet?

5. What is the maximum level at which ingested cadmium salt is tolerated by growing rats?

6. What are the evidences of intoxication by ingestion of a cadmium salt?

7. What are some changes produced by levels of cadmium which are apparently tolerated by the animals?

8. What is the relative toxicity of zinc, cadmium and beryllium salts when administered to rats by stomach tube?

9. Does the administration of soluble zinc, cadmium and beryllium salts by stomach tube cause any change in the hemoglobin concentration or in the number of erythrocytes in the blood of the rat?

10. What effect do salts of zinc and beryllium have on concentration of sugar in the blood?

REVIEW OF THE LITERATURE

There exists a disease associated with the smelting of zinc and other metals which is popularly known as "brass founders' ague" or more commonly "the shakes". This condition has apparently been known for many years, since the making of bronze and the smelting of zinc is an ancient art. The disease has been attributed to various causes by different authors. The question as to the probability of zinc being a causative factor in "brass founders' ague" was raised by several observers. Zinc in various forms has been used medicinally in considerable quantities. Many of its former uses are now not only considered of doubtful value, but some are positively known to be harmful. Much of our knowledge concerning the pharmacology and toxicology of zinc has come from observations of reactions produced in practice by medicinally used zinc compounds and from experiments conducted in the hope of answering the question directly.

The first report of an attempt to describe systemic effects from internal doses of zinc appeared more than one hundred years ago. P. Drinker (9) cites an investigation by Michaelis conducted in 1851 on the toxicity of certain zinc compounds. This investigator used rabbits, cats, dogs and horses as experimental animals. Postmortem findings were reported and Michaelis himself took various doses of zinc oxide and described his experiences. He concluded that, while harmless in small doses, zinc oxide in large doses produced definite effects. This he attributed to the solubility of the oxide in the acids of the stomach.

Harnack (14), in 1875, reported injecting dogs and rabbits intravenously with a weakly alkaline solution of sodium zinc pyrophosphate, which he had prepared. He observed that the animals became weak and were unable to stand. This paralytic action he attributed to the effect of the zinc salt upon the nervous system.

Amore, Falcone and Marmaldi (2), in 1892, reported studies on the toxic action and anatomical changes produced by the ingestion of zinc oxide. These workers used dogs and administered a huge dose of zinc oxide (1 gm.). This was followed a few days later by smaller doses of zinc oxide (0.5 gm.). Vomiting occurred and a progressive weakness attended the animals. The appetite was lost and the weight of the dogs was reduced. The urine contained casts and albumin. The color of the blood diminished, the red corpuscles of the blood decreased in number, and the white cells increased in number. The dogs died in ten to fourteen days.

Lehmann (23), in 1896, fed a dog for eleven months on a diet which consisted entirely of meat to which was added zinc carbonate. During this time 155.638 grams of zinc had been fed and presumably consumed by the dog. The daily dose of zinc was progressively increased up to 0.5 gram toward the end of the experiment. Since the dog weighed 12.5 kilograms at the end of the experiment, the daily consumption of zinc at this time was approximately forty milligrams per kilogram of body weight. Lehmann observed no ill effects in the dog at the end of the experiment. The dog had gained more than four kilograms while on the zinc diet. Lehmann concluded that zinc was not toxic, because he

could find no evidence of chronic poisoning. It is interesting to note the character of the diet used by Lehmann in this experiment. It is quite possible that the meat, a high protein foodstuff, may have decreased the toxicity of the zinc by making it more unavailable for absorption. It is well known that some zinc proteinates are very insoluble. In any case the highest dose given by Lehmann was not as great as the dose of zinc administered by Amore, Falcone and Marmaldi (2).

P. Drinker's article (9) carries a review of the literature up to 1922 on the toxicity of zinc as it is usually met in industry. Workers are often exposed to fumes and dusts laden with particles of zinc or its compounds. Solutions of soluble zinc salts are another form in which zinc is used in some industrial works. In these cases the portal of entry into the body is usually other than the mouth and gastro-intestinal tract. Investigators are not in complete agreement upon the question of the probability of zinc poisoning as an industrial menace. However, it may be concluded that there is agreement upon the question of the possibility of intoxication by zinc when entrance is gained into the body by way of the respiratory tract. Lehmann (24) came to this conclusion in 1910 after a long series of studies upon the hygienic importance of certain vapors and fumes. Lehmann described his own experiences with the ague in an experiment designed to test this possibility of intoxication. Merk's pure zinc was vaporized and burned in a room the air of which showed upon analysis a concentration of 0.1 to 0.4 of a milligram of zinc oxide per liter. Lehmann (24) was unable to produce characteristic symptoms of the disease in experimental

animals which were forced to breath the zinc oxide fumes, and he was unable to produce the symptoms in animals by tracheal injection of zinc oxide.

Batchelor, Fehnel, Thompson and Drinker (3) subjected the theory of zinc intoxication under actual working conditions to test. Working with the facilities of the New Jersey Zinc Company these investigators concluded that zinc offers no industrial hazard and that the reported ill health attributed to zinc must be due to certain more toxic impurities which contaminate zinc ores. These investigators explain their finding of eighty-one per cent as the average hemoglobin value of the workers examined as a value to be expected in any similar group of men of the same ages, of the same social status and doing approximately the same amount of physical work in an atmosphere with a moderate amount of dust but with no exposure to zinc.

There is no laboratory evidence that the entrance of zinc into the body through the skin is a factor in zinc intoxication. While this belief is generally accepted, there continues to appear from time to time reports (4) (31) of observations made on individuals apparently poisoned by contact with solutions or dusts of zinc compounds.

Apparently unconvinced that zinc was non-toxic or would not occasion chronic damage of any sort, K. Drinker and co-workers (7) set about to determine, by long-continued feeding experiments on cats and dogs, if some changes might be observed in these animals when zinc oxide was incorporated into their diets. The basal diet used in the experiments on cats was milk and canned salmon. The basal diet used

in the experiments on dogs was milk and a dog mash containing bread crumbs and ground-up meat. The dogs received in addition Hamburger steak and bones twice each week, during a large part of the experimental period. Although no figures are given, it can be seen that the protein content of the basal diet was very high. Dry zinc oxide was placed on the food and administered daily. Daily doses of the zinc oxide fed to the cats and dogs ranged from 175 to 1000 milligrams. The experimental period varied from three to fifty-three weeks. These workers reported that, as a result of their studies, they were unable to observe any significant clinical symptoms nor obtain any significant laboratory evidence of damage resulting from the daily ingestion of zinc oxide over long periods of time. The studies further revealed that a small fraction of absorbed zinc leaves the body in the urine, but the main bulk of it is excreted into the alimentary tract and leaves the body in the feces.

Thompson, Marsh and Drinker continued their investigation of zinc compounds and reported (36) a study of the effect of zinc administration upon reproduction and growth in the albino rat. For these experiments, parent rats were selected which had received previously for twenty-nine weeks (from the time they were six weeks old), solutions of organic zinc salts in the form of malate, acetate and citrate in daily doses of zinc which ranged from 4.4 to 12.4 milligrams. A few of the parent males selected had for twenty-nine weeks previous to these experiments (from the time they were twenty-six weeks old), received heavy suspensions of zinc oxide in gum acacia amounting to 34.4

milligrams of zinc per day. The rats had grown well and all appeared perfectly healthy, except that a few cases of so-called rat pneumonia developed and for which an explanation was given. The basal ration used in these experiments was a stock mixture of five parts rolled oats, three parts hominy, two and one-half parts beef scraps, one and one-half parts milk powder and a trace of salt. In addition to this, the animals received a molasses, corn, oat and alfalfa mixture, dog biscuits and lettuce twice weekly. These adult zinc fed rats were mated and their reproductive behavior observed. The authors concluded from their experiments that the feeding of zinc as malate, acetate, citrate and oxide, in doses of from two to thirty-eight milligrams of zinc daily, for many weeks previous to mating and during pregnancy and lactation, had no significant effect upon the health of the parents, upon their fertility, or upon the health of the offspring.

It will be observed from the data presented by Thompson, Marsh and Drinker (36) that 9.7 milligrams of zinc is the highest daily dose received by a female during the many weeks prior to mating. Four of the eight males used in the experiment on reproduction received only 12.4 milligrams of zinc per day during the many weeks prior to mating. One of the eight males received only two milligrams of zinc per day, and the remaining three received 34.4 milligrams of zinc per day as a suspension of zinc oxide in gum acacia for twenty-nine weeks prior to the experiment, after having attained the age of twenty-six weeks before the zinc regime began.

Drinker, Thompson and Marsh (8) reported results of an investigation of the effect upon rats of long-continued ingestion of zinc com-

pounds. In these experiments, rats placed on the diets containing added zinc were five to twenty-two weeks of age. Forms in which the zinc was administered and the composition of the basal ration used in these experiments were the same as were used in the previous investigation to which reference has been made (36). Daily doses of zinc varied from 0.5 to 34.4 milligrams and extended for a period varying from thirty-five to fifty-three weeks. A report of the very complete clinical and laboratory studies made upon these animals is given (8). As a result of the observations made by Drinker, Thompson and Marsh (8), these investigators concluded that they never obtained any significant clinical symptoms nor any significant laboratory evidence of damage resulting from the daily ingestion by rats, during long periods of time, of zinc oxide, zinc acetate, zinc citrate or zinc malate. These conclusions are in accord with previous findings reported on cats, dogs, rats and men.

Heller and Burke (18) reported an investigation designed to test the toxicity of zinc. These workers used rats as experimental animals and incorporated zinc to the extent of 0.25 per cent of the basal feed mixture in the form of the metal, zinc carbonate, zinc sulfate and zinc chloride. Each group of test rats received zinc in one of the forms mentioned. One group of rats studied received zinc in the form of the oxide to the extent of 0.5 per cent, and another group received zinc in the form of the chloride to the extent of 0.5 per cent of the basal ration. The basal ration used in these experiments was essentially the same as that used in the experiments to be

reported in this thesis. The age at which the young rats were started on the zinc diet was not given. They observed that two to seven litters of young were born to females in each group. Mortality of the young born to females on the various zinc diets was not abnormal in any group, except the one which received 0.5 per cent zinc as zinc chloride. In this group it may be observed (18) that seven litters were produced containing forty-six young of which nineteen died. The number of rats in each group receiving the zinc diets was four to nine. Heller and Burke observed that their zinc fed rats grew normally, reproduction was unimpaired, and third generation rats still maintained on the same diet were vigorous. These investigators could obtain no evidence of intoxication by zinc.

Meyers, Beard and Barnes (28) observed that when one per cent of zinc chloride was added to the diet the hemoglobin concentration of normal rats definitely decreased. There was no change in the number of red blood corpuscles.

Text-books of pharmacology (5) (19) (34) describe zinc sulfate as an astringent and zinc chloride as a corrosive substance. Sollmann (34) classes zinc salts with those of copper and describes zinc salts as having a rather specific action when given by mouth. The irritation affects first the nerve structure which forms the starting point of the vomiting reflex. In consequence of this, vomiting takes place before there is time for corrosion, and even very large doses present no danger to men. The ability of zinc salts to produce this local irritation has occasioned their use as emetics. Sollmann (34)

further states that there is no danger of chronic zinc poisoning. In a description of the pharmacological action of zinc salts, it is said (34) that if introduced into the circulation they cause death through paralysis of the cardiac muscle. Zinc and cadmium salts affect the central nervous system, and their action is probably direct. The effects are paralytic. The brain is first affected, consciousness is then lost, but the motor areas are not involved. The rapid fall in blood pressure is due mainly to cardiac depression.

Holland (19) says of zinc sulfate, "---This effect (emetic) is so constant that even after a dose of one ounce is taken recovery is the rule. When complete expulsion does not occur, it acts as a gastro-intestinal irritant causing vomiting, purging and secondarily, dangerous prostration." Holland (19) cites a case in which a large dose of zinc sulfate taken by mouth did not cause vomiting or purging, and death occurred in less than four hours.

The influence of magnesium salts upon the blood sugar content of rabbits was studied by Underhill (39) who found that hyperglycemia subsequent to magnesium injection was most evident during the period of anesthesia. Meltzer and Auer (27) had shown that an animal anesthetized by an injection of magnesium sulfate could be quickly restored to normal by an intravenous injection of calcium chloride. Underhill (39) showed that the hyperglycemia produced in rabbits anesthetized with magnesium salt was reduced to normal in two or three hours time by calcium chloride. Salant and Wise (30) observed the production of glycosuria in rabbits by zinc salts. Zinc malate when

introduced intravenously into rabbits produced glycosuria accompanied by hyperglycemia. Zinc acetate when administered by mouth and zinc malate when injected subcutaneously gave rise to glycosuria. Salant and Wise (30) did not mention observing hyperglycemia, after these salts were administered by mouth or subcutaneously injected. These workers report that intravenous injection of calcium chloride did not cause a decrease in the glycosuria resulting from the zinc malate injection. They did not report testing the effect of calcium chloride upon the hyperglycemia.

The acidosis and the hyperglycemia characteristic of diabetes mellitus are familiar. Dertil (6) observed that the introduction of acids into the blood produced hyperglycemia, which was believed to be due to a diminution of the alkaline reserve and not to any specific action of the acids introduced.

It is known that certain drugs and anesthetics produce changes in the blood sugar titer. In studying some factors influencing nembutal anesthesia, Hrubetz, Blackberg and Dotti (20) observed that the maximum depression in blood sugar occurred during the recovery period.

The rapidly growing use of cadmium in medicine and industry calls for a thorough investigation of the physiological and pathological changes produced by intoxication from this metal. The literature particularly upon this phase of study leaves much in doubt. Prodan (29) in an excellent review of the literature up to 1932 said in conclusion, "The pathologic changes reported, with the exception of changes in the kidneys, are only suppositions with no confirmatory evidence."

Cases of cadmium poisoning were observed and reported as early as the middle of the nineteenth century. Scientific studies of the effect upon the animal body were reported by Athanasiu and Langlois (1) in 1895. These workers observed that it was impossible to obtain a transparent blood serum from animals intoxicated by cadmium. Hemolysis of the red blood corpuscles occurred very rapidly in vitro and in vivo. Hematin and methemoglobin were produced, and an increase in serum globulin was noted.

Schwartze and Alsberg (32) studied the pharmacology of cadmium and zinc with particular reference to emesis. Comparing the chlorides of cadmium and zinc, these workers concluded that both substances were powerful emetics. It was noted that intoxication generally accompanied emesis. Schwartze and Alsberg found that, for rabbits and rats in which emesis could not occur, cadmium was five to seven times more toxic than zinc. They found no evidence of cumulative poisoning. These workers report that their analytical results showed cadmium and zinc to be stored principally in the liver and kidneys.

Johns, Finks and Alsberg (21) studied chronic intoxication by small quantities of cadmium chloride in the diet. In feeding experiments upon rats, using cadmium chloride in various concentrations ranging from 62.5 to 1000 parts cadmium per million, Alsberg and co-workers (21) concluded that in concentrations above 125 parts per million growth was definitely impaired. The higher levels proved extremely toxic and on the ration containing 125 parts cadmium per million the males died in about fifty days. Since food consumption was greatly

diminished upon the higher levels, the effects produced cannot be attributed to cadmium alone. Food consumption on the lower level was almost normal and the daily intake was calculated to be 0.56 milligram of cadmium. Initial growth was normal on the diet containing 125 parts cadmium per million. No blood or urinary changes or studies on reproduction were reported on any of the levels of cadmium.

Schwarz and Otto (33) using rabbits injected 0.6 to one milligram of cadmium subcutaneously every two days. The strong local reaction produced caused them to inject only 0.3 milligram per day at which concentration this reaction was not noted. All animals showed in from one to two and one-half months a considerable weight drop. Two of the four animals so treated died while on the experiment, and all of the animals were emaciated. The blood color of these animals shortly after investigation was begun showed a ten to twenty per cent reduction, and a corresponding drop in red blood corpuscles was observed. These workers did not believe that the mechanism of red cell formation was affected by cadmium.

The physiological and pharmacological action of beryllium has received little attention. Sollmann (35) cites the observations of Seaman, who, in 1912, found that when beryllium sulfate was administered in the food to dogs there resulted nutritional disturbances. Large doses produced vomiting without acute toxic symptoms. When administered hypodermically it caused local edema and necrosis. Intravenous injections were markedly toxic and resulted in disturbances of circulation, respiration and temperature.

There is a disease known as beryllium rickets, which results experimentally when beryllium carbonate is fed to rats (13). Fabroni (10) studied the effect of beryllium upon certain organisms. After intravenous injection of beryllium hydroxide into rabbits, Fabroni was unable to observe any toxic or harmful effects.

Gelman (12) has studied the possibility of beryllium intoxication from the industrial standpoint.

EXPERIMENTAL

Preparation of Materials

Stock ration

The basal ration used in the feeding experiments described in this thesis consisted of the following ingredients expressed in parts by volume: ground yellow corn 4 parts, ground hulled oats 4 parts, ground wheat 1 part, alfalfa meal 1 part, tankage 1/2 part, linseed meal 1/2 part, buttermilk powder 1/2 part, bone ash 0.35 lb. per 100 lbs. of ration and sodium chloride 0.5 lb. per 100 lbs. of ration. These ingredients were purchased from a dealer in the city of Ames, Iowa. The feed for the rat colony was mixed three times each week. It will be designated hereafter as the stock or basal ration.

Zinc carbonate

Mallinckrodt's analytical reagent quality basic zinc carbonate was used in the studies in which zinc was added to the basal ration. The lot numbers of the analyses of the two lots of zinc carbonate used in these experiments are 1128454 and 1175854. In both cases the listed maximum impurities are given as arsenic 0.000 per cent, chloride (Cl) 0.002 per cent, iron 0.01 per cent, lead 0.002 per cent, nitrate (N_2O_5) 0.000 per cent, and sulfate (SO_3) 0.01 per cent. An analysis for zinc was performed on this basic zinc carbonate. The potassium ferrocyanide method for zinc was used, and values of 58.05 and 57.81 per cent zinc were obtained. When incorporating zinc into the stock ration, calculations were based on these determinations.

Preparation of diets containing added zinc carbonate

The zinc carbonate powder was weighed to the nearest decigram and carefully placed on the dry stock ration. The combined weight of the stock ration and zinc carbonate was one kilogram. The zinc carbonate was thoroughly mixed with the stock ration by means of a rotating feed mixer. The feed mixtures thus prepared contained 0.1, 0.5 and one per cent by weight of added zinc in the form of zinc carbonate. The mixtures were bottled and labeled. The feed cups were kept filled from these preparations; furthermore new feed mixtures were prepared at intervals which did not exceed one week.

Cadmium carbonate

Baker and Adamson's chemically pure quality cadmium carbonate was used in the studies in which cadmium was added to the basal ration. All calculations involving cadmium were based on the formula $CdCO_3$.

Preparation of diets containing added cadmium carbonate

The actual weight of cadmium carbonate used in the preparation of a kilogram of feed mixture was small. This material was weighed on the analytical balance to the nearest milligram. The powdered cadmium carbonate was placed on the dry stock ration and thoroughly mixed by rotating in the closed mixer. The feed mixture thus prepared contained 0.025, 0.050 and 0.10 per cent by weight of added cadmium in the form of cadmium carbonate. The feed mixtures were bottled and labeled, and the feed cups were kept filled from these preparations. New feed mixtures were prepared at intervals which did not exceed one week.

Zinc sulfate, zinc chloride, cadmium chloride and beryllium sulfate

Baker and Adamson's chemically pure quality zinc sulfate, $ZnSO_4 \cdot 6H_2O$, zinc chloride, $ZnCl_2$ and cadmium chloride, $CdCl_2 \cdot 2H_2O$ were used in the study of the changes produced by the administration of these salts by stomach tube. Calculations involving zinc or cadmium were based on the formulas given. Beryllium sulfate, $BeSO_4 \cdot 4H_2O$, also used in these studies, was prepared from chemically pure beryllium oxide obtained from the E. H. Sargent Company. The beryllium oxide was dissolved in a slight excess of dilute 1:1 sulfuric acid. The cool solution was poured into three volumes of ninety-five per cent ethyl alcohol, and the crystalized beryllium sulfate was filtered and dried in the air. Calculations involving beryllium were based on the formula $BeSO_4 \cdot 4H_2O$.

Preparation of solutions for stomach tube administration

Solutions of the salts to be tested were prepared by dissolving the required amount of the salt in distilled water and diluting to volume in a volumetric flask. Solutions which were one molar with respect to zinc, cadmium and beryllium were thus prepared. These stock solutions also served as sources of these materials when more dilute solutions were required. In such cases the salt solutions were withdrawn by means of a pipette and diluted to volume with distilled water.

Preparation of calcium chloride solutions for injection

Pure crystals of Iceland spar, $CaCO_3$, were crushed and dissolved in dilute hydrochloric acid. The solution was evaporated to dryness on the steam plate and then placed in the oven at 120°C. The material

was then removed to a porcelain crucible and heated to dryness over a low flame. The dry calcium chloride was bottled. This material was used to prepare two stock solutions of calcium chloride. One stock solution of calcium chloride contained ten milligrams of calcium chloride per cubic centimeter, while the other contained fifteen milligrams of calcium chloride per cubic centimeter.

Dextrose solution for administration by stomach tube

A solution which contained 250 milligrams of dextrose per cubic centimeter was prepared by dissolving Baker and Adamson's anhydrous reagent quality dextrose in distilled water.

Sodium sulfate solution for administration by stomach tube

A one molar solution of sodium sulfate was prepared by dissolving Baker and Adamson's chemically pure sodium sulfate in distilled water and diluting to volume in a volumetric flask.

Ferric chloride and diets containing added iron and zinc

Baker and Adamson's reagent quality ferric chloride, $FeCl_3 \cdot 6H_2O$, was used in the study in which iron was added to the stock ration. Calculations involving iron were based on the formula $FeCl_3 \cdot 6H_2O$. The required weight of ferric chloride to make a feed mixture containing 0.5 per cent of added iron was weighed on the pan balance to the nearest decigram and dissolved in ether. This solution was sprayed on the dry stock ration by means of an atomizer; the mixture was stirred by hand during this process. The ether was allowed to evaporate spontaneously from the feed mixture. When the odor of ether was no longer detectable,

zinc to the extent of 0.5 per cent of the mixture was added in the form of zinc carbonate powder. This was thoroughly mixed by rotation in the closed mixer.

Sulfuric acid solution

A solution of sulfuric acid was used in the study in which this acid was administered by stomach tube. The concentration was made one molar by the addition of the required amount of Mallinckrodt's chemically pure quality sulfuric acid to distilled water and diluting to volume in a volumetric flask.

Nembutal

Nembutal, sodium pentobarbiturate, was obtained from Abbott Laboratories in Chicago, Illinois. A solution was prepared which contained three milligrams of nembutal per cubic centimeter. Solutions of this concentration, when injected intraperitoneally into rats at the rate of one cubic centimeter per 100 grams of body weight, will produce anesthesia in five to eight minutes. Complete anesthesia usually continues for two hours.

Care of Animals

The young rats were weaned from the stock mothers at twenty-five to twenty-eight days of age. Experimental animals were selected from the combined litters obtained at a weaning period. The young rats were weighed and identified by clipping the ears. Two males and three females were grouped together in a cage made of 1/4 inch galvanized wire screen. The cages measured twenty-four inches in length, twelve inches in width and eight inches in height. The cages were equipped with raised wire bottoms, which allowed feces to fall through to a galvanized pan below.

Food and water were supplied ad libitum. The food containers were made of tin and were in the form of a cup, which was soldered to a tin pan that served as a base. Each cup had a removable concave cap at the apex of which was a hole large enough to permit a rat to obtain food. Feed loss due to scattering was reduced to a minimum by this arrangement. Water was supplied from an inverted bottle fastened to the outside of the cage. This bottle was closed by a stopper carrying a short piece of glass tubing through which water could be procured.

The cages were set in a frame which formed a battery of cages. The conditions under which the experimental animals lived were the same as those of the stock colony. The laboratory which houses the stock rats is maintained at an approximately uniform temperature, 28°C. throughout the year.

Pregnant females in the experimental groups were removed to wood shavings when it became apparent that parturition would soon occur.

In the studies on the changes produced by certain metallic salts administered by stomach tube, the rats used were selected from stock animals without any previous experimental history. These normal rats were selected the day before they were to be used in an experiment, and during the fasting period they were placed in a cage, such as has been described above, with water but no feed. In these cases the weights were recorded just prior to the experiment.

Operative Methods

Administration of the anesthetic

The rats were anesthetized, in order to facilitate passage of the stomach tube, for the administration of the test solutions. Anesthesia was produced by an intraperitoneal injection of nembutal at the rate of three milligrams per 100 grams of body weight. The anesthetic was administered from a glass syringe using a 20-gauge needle. The syringe and needles were manufactured by Becton, Dickinson and Company of Rutherford, New Jersey.

The proper quantity of nembutal solution to produce anesthesia was drawn into the syringe. The rat to be anesthetized was clasped in the left hand, the palm of the hand resting on the rat's back and the fingers almost encircling the body with the little finger under the rat's throat. The rat may be firmly held in this fashion without injury. The rat thus held was lifted and turned belly up. The needle was inserted under the skin on the median line posterior to the umbilicus and directed posterially. By raising the barrel of the syringe and with slight downward pressure the needle entered the peritoneal cavity. The syringe was then emptied and quickly withdrawn. The operation may be executed quickly and there is usually no struggling.

Blood sampling

Blood samples for hemoglobin determinations and red cell counts were obtained from the tail. The rat was held firmly on the table, the palm of the left hand resting on the rat's back and the fingers of the left hand almost encircling the body with the little finger clasped under

the rat's throat. The tail was wiped clean and stroked toward the tip until there was evidence of a quantity of blood in the capillaries. The tip of the tail was then lanced and the blood sample was drawn into the pipette from the flowing drops. The pipettes used with the Newcomer hemoglobinometer were obtained from the Bausch and Lomb Optical Company of Rochester, New York. The blood sample for counting red blood cells was drawn into a diluting pipette of the American Standard Hemocytometer type obtained from the Arthur H. Thomas Company of Philadelphia.

Initial blood samples for glucose determinations were obtained from the tail. In a number of cases the blood pressure fell to such a low level in two to three hours after administration of test solutions that it was impossible to obtain blood from this source. In these cases final blood samples for glucose determinations were obtained from the external saphenous vein at a position between the knee and ankle, or an incision was made and the blood sample obtained from the abdominal vein.

Passage of stomach tube

The anesthetized rat was placed on its back, and the jaws were opened by means of a forceps handle. The tongue was pulled forward and to one side, and the tip of the stomach tube inserted into the throat. Entrance into the oesophagus was easily accomplished by slightly rolling the tube between the thumb and forefinger as it was gently pushed forward. A piece of insulating tubing, ten centimeters long and two millimeters in diameter, such as is used in radio work and called "radio spaghetti," served as the stomach tube. This stomach tube was marked to the proper length to be inserted.

Administration of test solutions by stomach tube

Solutions to be administered by stomach tube were pipetted from the containers; pipettes graduated at 0.02 of a cubic centimeter and delivering two cubic centimeters of solution were employed for this purpose. The capillary tip of a funnel, made by drawing out a test tube, was inserted into the exposed end of the stomach tube. The solution was drained from the pipette into the funnel and it gravitates or is aspirated into the stomach by the respiratory movements of the animal. When drainage was complete, the stomach tube was removed from the oesophagus.

Injection of test solutions

Injection of solutions to be tested was made subcutaneously or intramuscularly. The left hind limb was chosen as the site for these injections. In testing the effect of calcium and zinc together, the administration of zinc by stomach tube was followed immediately by an injection of the calcium chloride test solution into the subcutis of the flank. Care was taken to pinch the skin upon withdrawal of the needle, in order to insure closure of the wound. No loss of solution by leakage occurred when this was done. Subcutaneous and intramuscular injections were made using a Becton, Dickinson tuberculin syringe with 26-gauge hypodermic needles.

Collection of urine

Urine was collected in a wire metabolism cage designed to collect urine uncontaminated by feces. The cage was made of 1/4 inch galva-

nized screen wire. This cage measured twelve inches in diameter and was twelve inches in height. A small cylinderical compartment two inches long and one and one-half inches in diameter made of this screen wire joined the large cage. Into this small compartment the rat could thrust his head and obtain water. A twelve inch glass funnel was placed beneath the cage and directed the urine excreted by the rat into a graduated cylinder. A porcelain cone in the funnel prevented feces from falling into the container below. No waste water from the drinking bottle could fall into the funnel and contaminate the urine.

The rat was placed in this cage, which was previously cleaned and dried, at approximately five o'clock P. M. The urine was collected at eight o'clock the following morning. While in the cage the rat received water but no feed. Rats were allowed several days between experimental periods in the metabolism cage.

Vaginal smear technique

A spatula was made from glass rod, eighteen centimeters in length and two millimeters in diameter, by slightly flattening one end in a flame. This spatula was held between the thumb and forefinger of the right hand. The female rat to be observed was held in the left hand, without squeezing the animal, by firmly grasping well behind the shoulders. The palm rests on the back and the fingers partially encircle the body. The tail of the rat was grasped between the third and fourth fingers of the right hand; the rat thus held was lifted and turned over. The spatula was inserted into the

exposed vagina and withdrawn. The adhering material was collected in a drop of physiological saline solution on a clean microscope slide; microscopic observations were made at once.

Analytical Methods

Determination of zinc in biological materials

The method proposed by Todd and Elvehjem (37) for the determination of small quantities of zinc, such as are found in biological material, was used. Zinc values obtained by this method were in good agreement with the actual zinc content of known samples containing 0.1 to 2.0 milligrams of the metal.

Determination of zinc by the potassium ferrocyanide method

The potassium ferrocyanide method for zinc as described by Treadwell and Hall (38) was used for the analysis of the zinc carbonate powder.

Hemoglobin determination

Hemoglobin determinations were made by the acid hematin method, which is described by Hawk and Bergeim (16). A Newcomer hemoglobinometer, which was obtained from the Bausch and Lomb Optical Company of Rochester, New York, was used to record the concentration of hemoglobin.

Blood sugar determination

A method for determining blood sugar concentration, which was

originally described by Folin and Malmros (11) and modified by Keil and Nelson (22), was used in obtaining the blood sugar values presented in this thesis.

Determination of sugar in urine

Determination of the concentration of sugar in urine was made by Benedict's picrate method, which is described by Hawk and Bergeim (17).

Presentation of Data

Young rats weighing between forty and fifty grams were selected from the combined stock litters and placed in groups of five, three females and two males, in the raised screen bottom cages. Food and water were supplied ad libitum. Zinc carbonate was incorporated in the stock ration at three levels corresponding to 0.10, 0.50 and one per cent of zinc. The stock ration as it was fed to the rats of the stock colony was analyzed and found to contain 34.8 ±3.1 parts of zinc per million of dry feed.

The animals grew well on the rations containing 0.10 and 0.50 per cent of zinc. The rats were vigorous when the experiment was terminated, and there was very little difference in the weight of the rats which received zinc and those which received only the stock ration. The limit of tolerance of the animals to zinc carbonate lies between 0.50 and one per cent of zinc in the diet. The animals on the one per cent zinc diet failed to grow normally in all cases, although some individuals weighed 140 to 160 grams after ten to twelve

weeks on this level. Some of the animals began dying within four weeks after being placed on this diet. If not removed at once from the cage, they were usually partly eaten.

Reproduction on the lowest level of zinc intake was normal, and the young were healthy and grew normally. Several generations of these rats continued on the same zinc diet showed no abnormalities. Reproduction was markedly affected when the animals received 0.50 per cent of zinc in the diet. The females on this diet became pregnant between the fourteenth and seventeenth weeks of age. One female which had given birth to three young lost thirty grams at parturition. Another female had three young (one born dead) and lost forty grams in weight at parturition. Another female when observed had five dead young in the cage. The young were fully matured but appeared white and bloodless. The total weight of the five still born young was twenty-four grams. The females with the live young mothered them satisfactorily. The young weighed almost thirty grams at twenty-one days of age. The original females again mated when returned to their cages. The young that were born were dead in every case. No live young were born after the first pregnancy to any of these females. A total of twenty-three still born rats were counted and others were undoubtedly missed, having been consumed by the mothers. The females on the 0.50 per cent zinc diet ceased to become visibly pregnant after five months on the ration, although their weights and outward appearances were normal. No reproduction occurred on the one per cent zinc level.

Table I gives hemoglobin and red blood cell counts on the various levels of zinc intake. Normal hemoglobin values were found in the group of rats receiving 0.10 per cent of zinc after thirty-nine weeks on this diet. That there was something organically wrong with the animals on the 0.50 per cent zinc level was first made evident from observations on their reproductive behavior. At the age of initial reproduction of these females the hemoglobin values were only slightly lower than those of the control group. They were still within the limits given by some investigators as normal. As the observation of hemoglobin was continued, it became evident that the rats, as they were kept longer on the ration, became anemic. An average value of 10.2 grams per cent was found after thirty weeks. No change in the number of red blood corpuscles was observed. Both hemoglobin and red blood corpuscles were diminished on the one per cent zinc diet. Hemoglobin values in this lot usually started to decrease after three to five weeks on the ration. Low values of four grams per cent were sometimes observed. A striking thing in this study was the fact that no correlation between the hemoglobin value and the apparent state of well-being of the rat could be found. Rats often lived for five weeks with hemoglobin values below six grams per cent, while others succumbed with the hemoglobin only slightly below normal. The blood cell picture of the animals on one per cent of zinc was interesting. Although the red cells became irregular in size and shape within five weeks after being placed on this diet, the number was not appreciably diminished until after eight to ten weeks on this regime. There was apparently an excess of white and immature red corpuscles in every case.

Table II shows the change in hemoglobin that occurs in normal animals during pregnancy. Studies were made on females receiving the stock ration, which was used as the basal diet in all of the experiments. The observations were made in a study of the anemia that develops during pregnancy.

The symptoms of zinc carbonate poisoning disappeared when the salt was removed from the diet. Hemoglobin and reproduction became normal in the animals which had received 0.50 per cent of zinc when the salt was removed from the ration. The same was true of the hemoglobin of the rats which received one per cent of zinc.

It was evident that young rats could be reared on an adequate basal ration into which was incorporated zinc carbonate to the extent of 0.50 per cent of zinc. The animals grew well as indicated by the weight increase. It was observed that the hemoglobin values declined in the rats which received 0.50 per cent of zinc in their diet, and reproduction in this group was very unsatisfactory. It was proposed that the investigation of the effect of zinc, when ingested at the 0.50 per cent level, be continued. The primary objective of this study was to collect data on weight, food consumption, hemoglobin, blood sugar and urinary sugar of rats which received 0.50 per cent of zinc as zinc carbonate in the diet. These data were compared with similar data obtained simultaneously from rats of the same age and kept under the same conditions but which received only the stock ration without added zinc. The rats selected for use in this experiment were twenty-five days of age and weighed approximately thirty to forty grams.

These rats were placed in raised screen bottom cages in groups of five, two males and three females, and supplied with food and water ad libitum. The food consisted of the stock ration to which was added zinc to the extent of 0.50 per cent as zinc carbonate.

Table III shows the data obtained on the weights of the rats which received the diet containing 0.50 per cent of zinc and the weights of the rats which received only the stock ration with no added zinc. This table shows that after four months on the 0.50 per cent zinc diet the average weight of the animals was eighty per cent of the average weight of the control group. The appearance of the zinc fed rats gave no evidence that they had suffered from this diet. None of the animals died during the experiment.

Table IV shows the data obtained on the food consumption of rats which received 0.50 per cent of zinc in the diet and the food consumption of rats which received the stock ration with no added zinc. This table shows that food consumption in the group receiving 0.50 per cent of zinc in the diet was ninety-three per cent of the food consumption in the group which received the basal ration with no added zinc. The comparison of the two groups was made simultaneously, and the age of the rats in each group was the same.

Table V shows the data obtained on the hemoglobin values of rats receiving 0.50 per cent of zinc in the diet and the values obtained from rats receiving the basal ration with no added zinc. This table shows that the hemoglobin values of the rats which received the added zinc in their diet were far below those of the group which received no added

zinc in the stock ration. An interesting observation was that the minimum values of the hemoglobin concentration, recorded after fifty days on the zinc diet, were not further diminished after one hundred days of this regime.

Table VI shows the data obtained from determinations of blood sugar and urinary sugar concentrations of rats which received 0.50 per cent of zinc in their diet and of rats which received only the stock ration with no added zinc. This table shows that the non-fasting blood sugar level of rats which received added zinc was slightly higher than that of the non-fasting control group, which received no added zinc in their diet. Determinations made on rats which had been fasted for twenty hours showed blood sugar values in the two groups which were not significantly different. The volume of urine voided by the males of the zinc fed group was higher than that voided by the males of the control group. The volume of urine excreted by the zinc fed males was abnormally high. The females of the zinc fed group did not show a high excretion of urine. There was no evidence of greater urinary sugar excretion in the group which received 0.50 per cent of zinc than in the control group.

The feeding of zinc carbonate in the stock ration at a level of 0.50 per cent of zinc was found to affect growth and food consumption slightly, but reproduction and hemoglobin were markedly changed by this diet. In the first experiment it was observed that a few live young were born, following the first pregnancy, to females reared on this 0.50 per cent zinc diet. A large number of still born young were ob-

served during the first and second pregnancies, and the females in all cases failed to show signs of becoming pregnant after the second pregnancy. When removed to the basal ration without added zinc, these females became pregnant again and cast normal litters of young, which they mothered satisfactorily.

The rats selected for use in the second experiment, designed to study some of the changes produced in reproduction by a diet which contained 0.50 per cent of zinc, were one week younger when started on the zinc ration than the rats used in the first experiment. The rats used in this second experiment were twenty-five days of age and weighed thirty to forty grams when placed on the zinc diet. These rats were placed in groups of five, two males and three females, in raised screen bottom cages and supplied with food and water ad libitum.

The reproductive behavior of the female rats after ninety days on the zinc diet was studied by the vaginal smear technique. The rats at this time were 115 days of age. Four females were selected for study, and three of these showed signs of the oestrus cycle when observed. The fourth is believed to have been pregnant when this study was started (see protocol of Case I). Upon observation of the pro-oestrus stage, as evidenced by the appearance of regular small round epithelial cells in the vaginal smear, the female was placed in a cage with a male of known fertility selected from the stock colony. With the one exception mentioned, sperm cells were observed in the vaginal smears in every case. This offered proof of the mating. The weights of the rats were observed regularly, and the "erythrocyte sign", which appears

on the thirteenth to the fifteenth day of gestation, offered proof of pregnancy. This blood sign was observed in all cases except one (see protocol of Case I). The case history of each of the females studied was recorded, and the protocols are shown. No young were observed to have been born to any of the females. Daily record of the weights of the females show that each pregnancy resulted in resorption of the foeti. Hemoglobin studies show that a marked drop in hemoglobin occurs at parturition in normal pregnancies. Hemoglobin studies made during gestations of females receiving 0.50 per cent of zinc in the diet show that a drop in hemoglobin occurred toward the end of the period, and at the same time the weight of the female underwent a gradual decline. This indicates death and resorption of the foeti. This fall in hemoglobin may not be so marked in females showing abnormally low initial hemoglobin values which result from the zinc diet. It was strikingly shown by a female whose initial hemoglobin value was only slightly below normal at the onset of pregnancy. Proof of pregnancy in the females receiving the 0.50 per cent zinc diet was established still further by sacrificing two of the females, which were known to have mated with a normal male and had shown evidence of pregnancy by the "erythrocyte sign" and weight increase. These females were observed to undergo a gradual weight decline toward the end of what should have been a normal gestation period. At this time each was sacrificed, and the uterus containing the embryos removed. Macroscopically and microscopically the tissues were definitely abnormal. Photographic record of these uteri were made and are shown (see Photographs I and II).

The fertility of a male rat on the 0.50 per cent zinc diet was tested. This animal had been on the zinc diet for 125 days, and for ninety days preceding this test he had shown hemoglobin values of 7.3 grams per 100 cubic centimeters of blood or lower. This male weighed 270 grams at this time and appeared none the worse for his zinc diet. Females of tested fertility were selected from the stock colony and observed by the vaginal smear technique for the oestrus cycle. With the appearance of the pro-oestrus stage, they were placed in the cage with the male to be tested and observed the next day for sperm cells. The male proved his fertility by mating with the two females selected. The females were observed through their gestation periods, and each cast litters of ten young. The average weight of the young in each litter was 4.3 and 4.5 grams, respectively. The litters were reduced to seven young each, and the females mothered them satisfactorily. None of the young died, and the average weight of the young in each litter was 20.0 and 20.4 grams, respectively, at twelve days of age.

The first experiment had shown that rats became anemic when reared on an adequate basal ration into which was incorporated zinc carbonate to the extent of 0.50 per cent of zinc. It was observed that this anemia was not accompanied by a decrease in the number of red blood corpuscles. It was further observed that the hemoglobin value returned to normal when the zinc carbonate was removed from the diet. In the initial studies on hemoglobin changes in rats receiving 0.50 per cent of zinc, it was observed that for some unknown reason the anemia produced in rats of a given group was not always of the same

degree. It was also observed that, while receiving the same diet, the hemoglobin value of an anemic rat might improve when the rat was removed from the screen to wood shavings. The importance of iron and copper in nutritional anemia had been demonstrated before (15). With these observations in mind, it appeared pertinent to investigate the effect of an iron supplement to the anemia producing zinc diet.

Adult rats which had been made anemic by the 0.50 per cent zinc diet were used in these experiments. All the rats used were started on the 0.50 per cent zinc diet at the same time (twenty-five days of age) and had remained on this diet up to the time they were placed on the iron supplemented diet. Iron chloride was dissolved in ether and sprayed over the dry feed mixture. The mixture when fed contained 0.50 per cent added iron and 0.50 per cent added zinc. Three separate feeding experiments were made over a period of three months. The rats used had been on the 0.50 per cent zinc diet for various lengths of time and showed various degrees of anemia. The rats were housed in the galvanized wire cages with raised screen bottoms. Food and water were supplied ad libitum, and weekly record of weights and hemoglobin values were kept for each rat.

The data obtained in these experiments are presented in tabular form. Table VII shows that the initial hemoglobin value of 6.9 grams per 100 cc. of blood, which was observed in a male rat, was increased to 13.5 grams per 100 cc. in twenty-five days on the iron supplemented zinc diet. At the time of this experiment this male rat was 100 days of age and had lived for seventy-five days prior to this experiment on

the 0.50 per cent zinc diet. The initial weight at the start of the iron feeding was 216 grams, and the final weight after twenty-five days on the iron supplement was 220 grams.

Table VIII shows that the initial hemoglobin value of 9.0 grams per 100 cc. of blood observed in a female rat was increased to 14.5 grams per 100 cc. in twenty-five days on the iron supplemented zinc diet. At the time of this experiment this rat was 100 days of age and had lived for seventy-five days prior to this experiment on the 0.50 per cent zinc diet. The initial weight at the start of the feeding experiment was 132 grams, and the final weight after twenty-five days on the iron supplement was 150 grams.

Table IX shows that the initial hemoglobin value of 5.0 grams per 100 cc. of blood observed in a male rat was increased to 9.2 grams per 100 cc. of blood by the iron supplement. At the time of this experiment this rat was 127 days of age and had lived for 102 days prior to this experiment on the 0.50 per cent zinc diet. This case was complicated by pneumonia, which was observed after the rat was started on the iron supplement. The hemoglobin showed a slow improvement, but the weight continually fell during the experiment. The initial weight of 215 grams at the start of the feeding experiment fell to 184 grams at the end of the experimental period.

Table X shows that the initial hemoglobin value of 6.3 grams per 100 cc. of blood observed in a male rat was increased to 13.5 grams per 100 cc. in forty days by the iron supplemented zinc diet. At the time of this experiment this rat was 127 days of age and had lived for

102 days prior to this experiment on the 0.50 per cent zinc diet. The weight at the start of the feeding experiment was 212 grams, and at the end of the iron feeding it was 198 grams.

Tables XI and XII show the initial hemoglobin values of 6.5 and 7.2 grams per 100 cc. of blood observed in a female and a male rat, each 160 days of age, and each of which had been 145 days prior to the experiment on the 0.50 per cent zinc diet. These tables show that the hemoglobin values increased to 14.8 and 13.2 grams per 100 cc. of blood in twenty-one days by the iron supplemented zinc diet. The weights of the rats while on the iron supplement showed slight declines.

It was believed desirable to have other data for comparison with the results obtained from the feeding of zinc. It was proposed that a study be made of some changes produced by the feeding of cadmium. The primary interest in the investigation of cadmium feeding was to obtain a level at which the cadmium would be tolerated without being too toxic to the rats. Cadmium carbonate was incorporated into the basal stock ration to the extent of 0.10, 0.05 and 0.025 per cent of cadmium in the feed mixture.

Young rats weighing between fifty and sixty grams were selected from the combined stock litters and placed in groups of five, two males and three females, in the raised screen bottom cages. Food and water were supplied ad libitum. Weekly record of weights and hemoglobin values were made for each rat.

The average weight of the rats in every cadmium fed group remained constant or dropped during the first week on the cadmium diet. The

average hemoglobin values for the group were higher than normal at the end of the first week. Food consumption was observed to be poor in the groups which received 0.10 and 0.05 per cent of cadmium and below normal in the group receiving 0.025 per cent of the metal. The rats in the group receiving 0.10 per cent of cadmium started to die during the second week on the diet. The body weights of the rats in the groups which received 0.10 and 0.05 per cent cadmium continued to fall during the second week on these diets. The hemoglobin values of the rats on the two higher levels of the metal continued above the average for the control group, which received no added cadmium. The animals on the higher levels were very emaciated, and the one remaining rat which received 0.10 per cent of cadmium in the diet died during the fourth week after the investigation was started. The previously high hemoglobin values in this group had fallen below normal after the first two weeks on this diet.

The rats in the group which received 0.05 per cent of cadmium showed abnormally high hemoglobin values during the first three weeks on this diet. The body weights of the rats in the group which received 0.05 per cent cadmium slowly declined, and food consumption was only slightly better than in the group which received 0.10 per cent of cadmium. The rats in the group which received 0.05 per cent of cadmium in the diet became very emaciated; the rats in this group which survived the second week lived for eighty days on this diet. At this time they were all killed. They had become so emaciated that all were on the point of death. Final hemoglobin values recorded from the rats

which received the 0.05 per cent cadmium diet averaged 9.0 grams per 100 cc. of blood. This represents a decrease in the hemoglobin of thirty-six per cent from the average value of 14.0 grams per 100 cc. recorded in the control group.

The average weight of the rats in the group which received 0.025 per cent cadmium in their diet showed neither gain nor loss during the first week on this regime. The average hemoglobin values were slightly above normal at the end of the first week. It was noted that those individuals which lost the most weight during the first week also showed the highest hemoglobin values. One of the rats in the 0.025 per cent cadmium group died during the second week on the diet. The remaining rats of this group lived for 100 days on this 0.025 per cent cadmium diet. At this time the experiment was terminated. The rats at the end of the experiment were under weight as compared to rats of the same age which received the stock ration with no added cadmium. Otherwise, these rats appeared to be in fair condition. The food consumption in this group was below normal. The average hemoglobin values at the end of the experiment were below normal in the group which received 0.025 per cent cadmium. The average value recorded for the group at this time was 9.8 grams per 100 cc. of blood.

Several groups of rats were investigated which received 0.05 per cent of cadmium in their diet. It was always observed that food consumption was diminished and that the hemoglobin values rose to a marked degree during the first week on this diet. The rise in hemoglobin was accompanied by a loss in weight. As the rats were continued on the

0.05 per cent cadmium diet, the hemoglobin values declined rapidly to levels below normal. Within the group receiving 0.05 per cent of cadmium, it was noted that deaths nearly always occurred during the second week on the diet. Those rats which survived this period lived for eight to ten weeks on this diet. No correlation between deaths and sex of the rats could be found.

Rats reared on the feed mixture containing 0.025 per cent added cadmium were studied further. The preliminary study had indicated that rats reared on a diet containing 0.025 per cent of cadmium were smaller, consumed less food, and showed lower hemoglobin values than control animals of the same age which received no added cadmium in the diet. An experiment was designed to collect data on the weight, hemoglobin, food consumption, blood sugar, urinary sugar and reproductive behavior of rats reared from the weaning age (twenty-five days) on a diet composed of the stock ration into which was incorporated cadmium carbonate to the extent of 0.025 per cent of cadmium. These observations were compared with similar data obtained from a group of rats which received only the basal ration without added cadmium and kept under the same conditions as those which received the cadmium diet. The feeding experiment extended over a period of four months. At the end of this time the animals had reached the age of 168 days and had been on the cadmium diet for 143 days. Observations were made on two groups of cadmium fed rats.

Young rats were selected at twenty-five days of age from the com-

bined stock litters and placed in groups of five, two males and three females, in raised screen bottom cages. Food and water were supplied ad libitum. Two female rats died during the experiment. The data obtained in this experiment are recorded in tabular form.

Table XIII shows a record of the weights obtained from rats which received 0.025 per cent of cadmium in their diet and the weights obtained from the control group, which received only the basal ration with no added cadmium.

Table XIV shows a record of food consumption by the rats which received 0.025 per cent of cadmium in their diet and the food consumption of the control group, which received only the stock ration with no added cadmium.

Table XV shows a record of the hemoglobin values obtained at various periods during the experiment from rats which received 0.025 per cent of cadmium in their diet and the hemoglobin values obtained at the same time from the rats of the control group, which received only the stock ration with no added cadmium.

Table XVI shows the blood sugar values and the volume and sugar concentration of the urine of rats which received 0.025 per cent of cadmium. Similar data are presented in this table which were collected from the control group, which received only the stock ration with no added cadmium. The urine was collected in the metabolism cage.

Female rats receiving 0.025 per cent of cadmium in the diet were observed at various intervals, after reaching the age of 100 days, for signs of the oestrus cycle by the vaginal smear technique. The vagi-

nas opened normally, but at no time was there evidence of oestrus rhythm or pregnancy. These observations were continued until the experiment was terminated. The fertility of one male from the cadmium fed group was tested by allowing him to mate with a female selected from the stock colony. At the time this experiment was performed, the male had received 0.025 per cent of cadmium in the diet for the previous 130 days and was 155 days of age. This male weighed 180 grams. The oestrus cycle of the normal female rat was followed by the vaginal smear technique, and sperm cells were observed in the smear following copulation. The weight of the female was observed every other day during her gestation period. The litter was cast at the expected time, but only three young rats were observed to have been born. These rats were of normal size and appeared to be vigorous. Another female from the stock colony became pregnant by this same male rat as evidenced by her weight increase and outward appearance, but the experiment was terminated before the end of her gestation period.

A study was made of some blood changes which accompany acute intoxication by zinc, cadmium and beryllium. Soluble salts of these elements in the form of the chlorides or sulfates were administered by stomach tube to rats. Rats for use in these experiments were selected from the stock colony and fasted for twenty hours prior to the experiment. In order to facilitate passage of the stomach tube with a minimum of struggling and discomfort, the rats were anesthetized by an intraperitoneal injection of nembutal. The use of this anesthetic has been found very satisfactory. Checks were made upon the influence of the

anesthetic on the results obtained. Neither the hemoglobin value nor the number of red blood corpuscles changed during nembutal anesthesia. Initial values for hemoglobin and red blood cells recorded in the tables refer to blood drawn from the rat just before administration of the anesthetic.

The relative toxicity of zinc and cadmium as evidenced by changes produced in hemoglobin values and red blood cell counts increases with the atomic weight of these elements. Beryllium was found to be more toxic, when compared to zinc, than was expected. Magnesium shows physiological as well as chemical properties which are different from the other members of this group of elements. Doses of seventy milligrams of zinc as the sulfate, fifteen milligrams of cadmium as the chloride and eighteen milligrams of beryllium as the sulfate, when administered by stomach tube in one cubic centimeter of solution, proved to be of equal toxicity to rats of 150 to 200 grams in weight as measured by the number surviving. Gram for gram beryllium was found to be approximately four times as toxic as zinc and equal to cadmium. When taken mole for mole, beryllium was found to be one-half as toxic as zinc and only one-sixteenth as toxic as cadmium.

Table XVII shows that hemoglobin values and the number of red blood corpuscles increased following administration of the salts of zinc, cadmium and beryllium by stomach tube. In some cases this apparent increase in these blood constituents may be as much as forty per cent of the initial values. The rise in the hemoglobin values and the increase in the number of red blood corpuscles reached a maximum within

three hours after the salt was administered. A marked lowering of the blood pressure was evident during the intoxication, as evidenced by the difficulty in securing blood samples from the peripheral blood vessels. Postmortem examination of animals which died from the effects of acute intoxication revealed a gross enteritis with much mucus in the stomach and intestines. No ill effects were apparently visible in surviving animals several days after a single dose of any of the salts used.

An investigation into the blood sugar changes produced by salts of beryllium and zinc, when administered to rats by stomach tube, revealed that a rise in blood sugar accompanied intoxication by these salts. Rats weighing between 130 and 160 grams which had been reared on the stock ration were used in these experiments. The initial blood sugar values were obtained from the unanesthetized animals after a twenty hour fast. All animals at the time they received the test solutions were anesthetized by an intraperitoneal injection of nembutal at the rate of three milligrams of nembutal in one cubic centimeter of solution per 100 grams of body weight. Various concentrations of the beryllium and zinc salts were administered and the concentrations of the salts used may be found in the tables.

Table XVIII shows the rise in blood sugar caused by zinc in the form of the chloride; furthermore, Table I also shows that the rise in blood sugar which accompanies the administration of glucose is increased by zinc chloride. Nembutal anesthesia causes a lowering of the blood sugar. Table XIX shows the effect of beryllium sulfate upon the blood sugar up to two hours after administration of the salt. Table XX shows that

subcutaneous injection of calcium chloride may depress the rise in blood sugar caused by zinc sulfate, without diminishing the accompanying rise in hemoglobin and red blood corpuscles. Calcium chloride does not inhibit the blood sugar rise caused by administration of glucose. Calcium chloride injected subcutaneously one hour before or one hour after administration of zinc sulfate did not lower the blood sugar, but when the two salts were administered simultaneously, the hyperglycemia was markedly reduced. The absorption of zinc sulfate is slower than the absorption of zinc chloride, as evidenced by the rate of blood sugar increase. Tables XXI and XXII show that sulfuric acid also causes hyperglycemia and an increase in hemoglobin and red blood corpuscles when administered by stomach tube to rats fasted for twenty hours.

During the investigation on blood sugar changes accompanying acute intoxication by zinc salts, it was observed that under certain conditions calcium chloride solution, if injected subcutaneously and simultaneously with zinc sulfate administration, would inhibit to a marked degree the usual blood sugar rise. It was observed that the time of injection of the calcium chloride solution relative to the administration of zinc sulfate was very important to the results obtained. It was also observed in this study that under nembutal anesthesia a fall in the blood sugar level of normal fasted rats occurred.

The study was continued to obtain more data upon the observation that calcium chloride solution would inhibit the rise in the blood sugar caused by zinc sulfate solution. In the study herein described, certain conditions of the previous experiment were varied and found to in-

fluence the results obtained. These conditions are (1) the time of the final blood sugar determination after the zinc sulfate administration, (2) the concentration of calcium chloride used in the injection, (3) the effect of injected distilled water upon the blood sugar concentration following zinc sulfate administration, (4) the effect of the depth of nembutal anesthesia upon the final blood sugar values and (5) the actual number of milligrams of calcium chloride which were most effective in inhibiting the rise in blood sugar. The importance of some of the above conditions was not recognized in the preliminary study. From the data obtained in the more detailed study,it became apparent that the difference of a few milligrams of calcium chloride, such as might be lost in a few drops of leakage from an injection site, an improper measurement of volume when a large bore syringe was used, the matter of as little as fifteen minutes difference in taking a blood sample or a difference in the degree of anesthesia produced may all work to cause variation in the blood sugar values obtained.

Rats weighing 150 to 190 grams were selected from the stock colony for use in these experiments. Those to be used were isolated and fasted eighteen to twenty hours before the experiment. Initial blood sugar determinations were made on samples of blood obtained from the fasted and unanesthetized animals. Except when otherwise stated in the tables, the animals were anesthetized with an intraperitoneal injection of nembutal at the rate of three milligrams per 100 grams of body weight. This enables passage of the stomach tube without struggle. One cubic centimeter of zinc sulfate solution containing sixty-five milligrams of zinc

was pipetted into this tube. This was followed immediately by an injection of the calcium chloride solution into the subcutis of the flank. Care was taken to pinch the skin upon withdrawal of the needle, in order to insure closure of the wound. No loss of solution by leakage occurred when this was done. Animals anesthetized at the rate of three milligrams of nembutal per 100 grams of body weight will begin to show signs of coming from under the anesthesia in two hours. Those anesthetized more deeply than this will not. It was found impossible to use more than four milligrams of nembutal per 100 grams of body weight, as the animals died when the zinc sulfate was administered.

Table XXIII shows the rise in blood sugar which accompanies intoxication by zinc sulfate. The final determinations were taken two hours after the administration of the zinc sulfate by stomach tube to fasting rats under nembutal anesthesia.

Table XXIV shows that two hours after the dose of zinc sulfate, the blood sugar rose fifty to seventy per cent of the initial value even though calcium chloride was injected in quantities ranging from two to fifteen milligrams in 1.5 cc. of solution. The blood sugar also rose when 1.5 cc. of distilled water was injected.

Table XXV shows that the anesthesia induced by intraperitoneal injection of three to four milligrams of nembutal per 100 grams of body weight does not depress the blood sugar rise one and one-half hours after the dose of zinc sulfate.

Table XXVI shows that for rats anesthetized at the usual rate of three milligrams of nembutal per 100 grams of body weight, subcutaneous

injection of calcium chloride in concentrations of six and eight milligrams in 1.5 cc. exerted its maximum action in suppressing the blood sugar rise one and one-half hours after administration of the zinc sulfate. Calcium chloride in greater or lesser concentrations than this acts less effectively. Any concentration of calcium chloride up to ten milligrams in 1.5 cc. of solution is better than distilled water alone in depressing the blood sugar rise caused by the zinc sulfate.

Table XXVII shows that maximum depression of the rise in blood sugar occurred when the rat was anesthetized with four milligrams of nembutal per 100 grams of body weight and eight milligrams of calcium chloride in 1.5 cc. of solution were injected subcutaneously. The final determination of blood sugar in this case was made one and one-half hours after the dose of zinc sulfate. When slightly less nembutal (3.5 milligrams per 100 grams of body weight) and seven milligrams of calcium chloride in 1.5 cc. of solution were used, the blood sugar rise one and one-half hours after the dose was no lower than that obtained with anesthesia at the usual rate of three milligrams per 100 grams of body weight. Table XXVII shows that under deep nembutal anesthesia (four milligrams per 100 grams of body weight) the rise was delayed by subcutaneous injection of distilled water. The inhibition was much less an hour and three-quarters after the dose than it was an hour and a half after the dose. This indicates that the inhibition was of short duration. Table XXVII also shows that a matter of fifteen minutes difference in taking a final blood sample may make a difference of considerable magnitude in the blood sugar value obtained.

The amount of calcium chloride in solutions of the same concentration most effective in diminishing the blood sugar rise, which accompanies zinc sulfate intoxication, was found to be six to eight milligrams of the salt. The volume of solution containing this amount of calcium chloride was 0.6 to 0.8 cc., respectively. The final blood sugar determinations were made on samples taken two hours after the administration of the zinc sulfate.

Table I

The effect of zinc carbonate on hemoglobin and red cells of rat blood

Lot no.	Zinc intake per cent	Initial Hb. gms. per 100 cc.	Final Hb. gms. per 100 cc.	Initial R.B.C. millions per cu. mm.	Final R.B.C. millions per cu. mm.	Weeks on ration
1	0.10	14.5	14.7	8.4	9.1	39
2	0.50	13.9	10.2	8.6	8.5	39
3	1.00	14.2	6.1	8.5	6.6	12-16
4	1.00	13.6	6.5	7.8	6.5	10-16
5	1.00	13.7	6.8	8.0	6.8	12-16
6	1.00	13.5	7.8	8.1	5.8	10
7	0.00	14.1	14.1	7.8	8.9	34
8	0.00	14.6	15.1	---	---	30

Table II

Hemoglobin of normal adult female rats

Rat no.	Hemoglobin one week before birth of young, gms.per 100 cc.	Hemoglobin day before birth of young, gms.per 100 cc.	Hemoglobin at birth of young, gms.per 100 cc.	Hemoglobin one week after birth of young, gms.per 100 cc.
1	14.0	12.6	12.0	14.4
2	14.7	13.0	13.0	14.5
3	14.6	11.0	10.0	12.0
4	14.4	12.0	12.5	13.2
5	15.4	12.0	11.4	13.5
6	14.0	13.5	13.6	17.0
7	14.2	12.5	12.5	13.9
8	15.4	14.4	12.8	15.0
Av.	14.6	12.6	12.2	14.2

Table III

Comparison of weights of rats which received 0.50 per cent of zinc with the weights of rats which received the basal ration with no added zinc

Rat	Initial weight, grams	Weight after 1 mo. on diet, grams	Weight after 2 mo. on diet, grams	Weight after 3 mo. on diet, grams	Weight after 4 mo. on diet, grams
			Zinc Group		
W♂$_1$	38	104	196	235	265
W♂R$_1$	36	94	172	215	204
W♀$_1$	30	70	115	131	141
W♀R$_1$	25	72	130	158	171
G♀$_1$	35	92	130	159	166*
G♂$_2$	42	125	212	220	310
W♀$_2$	36	90	140	130	158
W♀R$_2$	37	84	125	148	159
W♀L$_2$	36	86	123	129	140
W♂$_2$	41	114	170	220	206
			Control Group		
W♀	40	106	168*	155	170
W♂	44	106	184	213	240
W♂R	35	100	190	250	302
B♂	40	120	224	297	332
W♀R	41	102	144	202	182

* = pregnant

Table IV

Food consumption of rats which received 0.50 per cent of zinc in the diet and rats which received the basal ration with no added zinc

No. rats	Diet	Length of experiment	Kgs. feed consumed	Av. no. grams of feed per rat per day
12	Stock ration	100 days	12 Kgs.	10 grams
10	Zinc diet	97 days	9 Kgs.	9.3 grams

Table V

Hemoglobin values of rats which received 0.50 per cent of zinc in the diet and rats which received the basal ration with no added zinc

Rat	Hemoglobin after 50 days on diet gms. per 100 cc.	Hemoglobin after 100 days on diet gms. per 100 cc.
	Zinc Diet	
$W\sigma_1$	6.5	7.0
$W\sigma R_1$	7.3	6.5
$W♀_1$	8.5	8.5
$W♀R_1$	6.3	7.0
$G♀_1$	10.2	12.5
$W\sigma_2$	8.5	5.0
$W♀L_2$	8.8	10.2
$W♀R_2$	8.5	7.8
$W♀_2$	12.5	**
$G\sigma_2$	6.3	**
	Control Group	
$G♀_2$	14.5	14.1
$W\sigma_2$	15.8	15.0
$B♀_2$	14.1	14.5
$W♀R_2$	15.0	15.0
$W\sigma NL_2$	15.5	14.7

** = on iron-zinc diet at this time

Table VI

Comparison of blood sugar and urinary sugar values of rats which received 0.50 per cent of zinc in the diet with values obtained from rats which received the basal ration with no added zinc

Rat	Blood sugar after 50 days on Zn diet (animals unfasted and unanesthetized) mgs. per 100 cc.	Blood sugar after 62 days on Zn diet (animals fasted but unanesthetized) mgs. per 100 cc.	Volume urine collected 5P.M.-8A.M. cc.	Urinary sugar in sample collected 5P.M.-8A.M.
		Zinc Diet		
W♂$_1$	133	89	25.0	less than 0.1%
W♂R$_1$	124	80	18.0	less than 0.1%
W♀$_1$	151	82	1.0	0.1%
W♀R$_1$	145	—	2.5	0.2%
G♀$_1$	138	—	1.0	0.1%
G♂$_2$	127	91	12.0	less than 0.1%
W♀$_2$	116	100	—	—
W♀R$_2$	133	95	2.4	0.15%
W♂$_2$	124	108	23.0	less than 0.1%
W♀L$_2$	120	105	—	—
		Control Group		
B♂	108	96	4.5	less than 0.1%
W♀	115	92	5.0	less than 0.1%
W♂	93	80	6.5	0.1%
W♀R	—	—	3.3	0.1%

Table VII

Changes in hemoglobin value and weight which followed the supplementing of the 0.50 per cent zinc diet with 0.50 per cent of iron

Gso was 100 days of age and had been 75 days on 0.50 per cent zinc diet at the time the iron supplement was started.

Date	Hemoglobin grams per 100 cc.	Weight, grams
5/ 3/38	6.9	216
5/14/38	9.5	223
5/21/38	12.0	218
5/28/38	13.5	220

Table VIII

Changes in hemoglobin value and weight which followed the supplementing of the 0.50 per cent zinc diet with 0.50 per cent of iron

W_2 was 100 days of age and had been 75 days on the 0.50 per cent zinc diet at the time the iron supplement was started.

Date	Hemoglobin grams per 100 cc.	Weight, grams
5/ 3/38	9.0	132
5/14/38	12.5	126
5/21/38	14.0	124
5/28/38	14.5	130

Table IX

Changes in hemoglobin value and weight which followed the supplementing of the 0.50 per cent zinc diet with 0.50 per cent of iron

$W\acute{6}_2$ was 127 days of age and had been 102 days on the 0.50 per cent zinc diet at the time this iron supplement was started.

Date	Hemoglobin grams per 100 cc.	Weight, grams
5/30/38	5.0	215
6/ 6/38	6.2	208
6/12/38	6.2	206
6/19/38*	7.4	203
6/26/38	8.3	196
7/ 3/38	8.5	193
7/10/38	9.2	184

* It was observed that this rat had a lung infection.

Table X

Changes in hemoglobin value and weight which followed the supplementing of the 0.50 per cent zinc diet with 0.50 per cent of iron

$W\acute{o}R_1$ was 127 days of age and had been 102 days on the 0.50 per cent zinc diet at the time the iron supplement was started.

Date	Hemoglobin grams per 100 cc.	Weight, grams
5/30/38	6.3	212
6/ 6/38	6.7	209
6/12/38	7.3	207
6/19/38	9.0	204
6/26/38	10.8	196
7/ 3/38	12.5	202
7/10/38	13.5	198

Table XI

Changes in hemoglobin value and weight which followed the supplementing of the 0.50 per cent zinc diet with 0.50 per cent of iron

W‡R$_1$ was 160 days of age and had been 145 days on the 0.50 per cent zinc diet at the time the iron supplement was started.

Date	Hemoglobin grams per 100 cc.	Weight, grams
7/ 3/38	6.5	173
7/17/38	12.2	174
7/24/38	14.8	177

Table XII

Changes in hemoglobin value and weight which followed the supplementing of the 0.50 per cent zinc diet with 0.50 per cent of iron

Wo_1 was 160 days of age and had been 145 days on the 0.50 per cent zinc diet at the time the iron supplement was started.

Date	Hemoglobin grams per 100 cc.	Weight, grams
7/ 3/38	7.2	275
7/17/38	10.5	255
7/24/38	13.2	265

Table XIII

Comparison of weights of rats which received 0.025 per cent of cadmium with the weights of rats which received the basal ration with no added cadmium

Rat	Initial weight, grams	Weight after 1 mo. on diet, grams	Weight after 2 mo. on diet, grams	Weight after 3 mo. on diet, grams	Weight after 4 mo. on diet, grams
			Cadmium Group		
$W\delta_1$	26	50	80	134	150
$W\delta_1R$	38	74	120	145	175
$W♀_1$	28	58	66	65	Died
$W♀_1R$	32	62	85	106	131
$B♀_1$	37	52	80	82	104
$W\delta_2$	37	74	92	110	142
$W\delta_2R$	43	72	112	153	180
$W♀_2$	34	66	82	121	136
$W♀_2R$	37	68	100	124	134
$Gs♀_2$	30	Died	--	--	--
			Control Group		
W♀	40	106	168*	155	170
W♂	44	106	184	215	240
W♂R	35	100	190	250	302
B♂	40	120	224	297	332
W♀R	41	102	144	202*	182

* = pregnant

Table XIV

Food consumption of rats which received 0.025 per cent of cadmium in the diet and rats which received the basal ration without added cadmium

No. rats	Diet	Length of experiment	Kgs. feed consumed	Av. no. grams per rat per day
12	Stock ration	100 days	12 Kgs.	10 grams
9	Cadmium diet	113 days	7 Kgs.	7 grams

Table XV

Hemoglobin values of rats which received 0.025 per cent of cadmium in the diet and rats which received the basal ration without added cadmium

Rat	Hemoglobin after 50 days on diet gms. per 100 cc.	Hemoglobin after 110 days on diet gms. per 100 cc.	Hemoglobin after 135 days on diet gms. per 100 cc.
		Cadmium Group	
$W\delta_2$	12.5	7.5	8.9
$W\delta_2R$	12.0	11.0	10.5
$W♀_2$	12.8	17.5	14.8
$W♀_2R$	12.5	12.5	11.7
$W\delta_1$	12.1	14.0	---
$W\delta_1R$	12.7	12.5	---
$W♀_1$	11.0	6.7	---
$W♀_1R$	12.0	8.7	---
$B♀_1$	12.7	8.8	---
		Control Group	
$Gs♀_2$	14.5	14.5	12.5
$W\delta_2$	15.8	15.7	14.0
$B♀_2$	14.1	14.4	14.5
$W♀_2R$	15.0	12.0	14.0
$W\delta RL$	15.5	15.6	15.5

Table XVI

Comparison of blood sugar and urinary sugar values of rats which received 0.025 per cent of cadmium in the diet with values obtained from rats which received the basal ration with no added cadmium

Rat	Blood sugar after 50 days on Cd diet (animals unfasted and unanesthetized) mgs. per 100 cc.	Blood sugar after 62 days on Cd diet (animals fasted but unanesthetized) mgs. per 100 cc.	Volume urine collected 5P.M.-8A.M. cc.	Urinary sugar in sample collected 5P.M.-8A.M.
		Cadmium Group		
$W\sigma^{\prime}_1R$	114	89	1.5	0.3%
$W\varphi_1$	107	99	1.5	0.2%
$B\varphi_1$	111	93	1.0	0.3%
$W\sigma^{\prime}_1$	108	83	2.4	less than 0.1%
$W\varphi_1R$	105	95	---	---
$W\sigma^{\prime}_2R$	116	109	2.0	0.1%
$W\varphi_2$	105	86	2.0	0.2%
$W\varphi_2R$	117	95	1.5	0.2%
$W\sigma^{\prime}_2$	118	---	4.0	less than 0.1%
		Control Group		
$B\sigma^{\prime}$	108	95	4.5	less than 0.1%
$W\varphi$	115	92	5.0	less than 0.1%
$W\sigma^{\prime}$	93	80	6.5	0.1%
$W\varphi R$	---	---	3.3	0.1%

Table XVII

The effect of various inorganic salts administered by stomach tube upon hemoglobin and red blood corpuscles of the white rat

Rat no.	Sex and weight grams	Concentration of salt administered	Hemoglobin initial gms. per 100 cc.	R.B.C. M. per cu. mm. initial	Hemoglobin after 2 hrs. gms. per 100 cc.	R.B.C. M. per cu. mm. after 2 hrs.
1	♀ 200	1cc. $ZnCl_2$ (70mgs. Zn)	15.5	10.64	23.0	14.22
2	♀ 200	1cc. $ZnCl_2$ (70mgs. Zn)	16.5	10.14	23.0	13.50
3	♀ 147	2cc. $ZnSO_4$ (63mgs. Zn)	10.0	6.24	16.6	9.66
4	♀ 150	2cc. $ZnSO_4$ (63mgs. Zn)	16.4	9.40	22.3	11.92
5	♀ 150	1cc. $ZnSO_4$ (63mgs. Zn)	12.3	7.82	21.0	11.28
6	♂ 240	2cc. $CdCl_2$ (20mgs. Cd)	11.8	10.82	15.9	12.69
7	♀ 130	1cc. $CdCl_2$ (10mgs. Cd)	12.7	8.88	15.2	10.03
8	♂ 125	1cc. $CdCl_2$ (10mgs. Cd)	15.5	8.99	16.8	12.10
9	♂ 125	1.5cc. $CdCl_2$ (15mgs. Cd)	16.5	10.31	19.2	13.17
10	♂ 120	1cc. $CdCl_2$ (10mgs. Cd)	16.0	10.08	18.2	12.23
11	♀ 135	0.8cc. $CdCl_2$ (8mgs. Cd)	13.1	9.68	18.0	12.70
12	♂ 120	1cc. $BeSO_4$ (9mgs. Be)	15.0	9.68	19.5	12.02
13	♂ 110	2cc. $BeSO_4$ (9mgs. Be)	14.8	9.98	18.0	10.64
14	♀ 150	2cc. $BeSO_4$ (9mgs. Be)	13.2	7.72	18.5	10.18
15	♀ 155	1cc. $BeSO_4$ (9mgs. Be)	14.5	9.76	17.5	12.08
16	♀ 150	2cc. $BeSO_4$ (18mgs. Be)	14.6	8.66	23.0	13.28
17	♀ 150	2cc. $BeSO_4$ (18mgs. Be)	14.8	8.28	23.0	13.34
18	♀ 150	1cc. $BeSO_4$ (9mgs. Be)	16.2	9.80	17.1	10.80
19	♀ 130	1 cc. water only	14.2	10.39	14.5	10.50
20	♀ 108	1 cc. water only	16.5	-- --	16.8	-- --

Table XVIII

Blood sugar changes produced by zinc chloride, zinc chloride and glucose and glucose administered by stomach tube to the fasting white rat under nembutal anesthesia

Rat no.	Substance administered	Volume of solution and mgs. of dissolved substance administered	Glucose mgs. per 100 cc. after 20-hour fast (animals not anesthetized)	Glucose mgs. per 100 cc. 1 hour after dose (animals anesthetized)	Glucose mgs. per 100 cc. 2 hours after dose (animals anesthetized)	Glucose mgs. per 100 cc. 3 hours after dose (animals anesthetized)
1	$ZnCl_2$	2cc.(30 mgs.Zn)	94	125	180	167
2	$ZnCl_2$	2cc.(30 mgs.Zn)	80	114	171	190
3	$ZnCl_2$	1cc.(50 mgs.Zn)	90	130	153	210
4	$ZnCl_2$ and Glucose	2cc.(30 mgs.Zn-250 mgs. Glucose)	102	140	227	263
5	$ZnCl_2$ and Glucose	2cc.(30 mgs.Zn-250 mgs. Glucose)	109	137	208	N.D.
6	$ZnCl_2$ and Glucose	1cc.(50 mgs.Zn-250 mgs. Glucose)	89	125	200	250
7	$ZnCl_2$ and Glucose	1cc.(50 mgs.Zn-250 mgs. Glucose	95	137	Died	N.D.
8	Glucose	2cc.(250 mgs. Glucose)	94	113	104	96
9	Glucose	1cc.(250 mgs. Glucose)	83	95	116	140
10	Nembutal	1.5 cc.(4.5 mgs. Nembutal) injected	97	90	83	78
11	Nembutal	1.5 cc.(4.5 mgs. Nembutal) injected	102	90	88	N.D.
12	Nembutal	1.5 cc.(4.5 mgs. Nembutal) injected	97	83	75	N.D.

N.D. = no determination

Table XIX

Changes in blood sugar in various intervals of time after the administration of beryllium sulfate by stomach tube to the fasting white rat under nembutal anesthesia

Rat no.	Substance administered	Volume of solution and mgs. of dissolved substance administered	Glucose mgs. per 100 cc. after 20-hour fast (animals not anesthetized)	Glucose mgs. per 100 cc. 1/2 hour after dose (animals anesthetized)	Glucose mgs. per 100 cc. 1 hour after dose (animals anesthetized)	Glucose mgs. per 100 cc. 2 hours after dose (animals anesthetized)
1	$BeSO_4$	2cc.(9 mgs. Be)	94	94	--	--
2	$BeSO_4$	1cc.(9 mgs. Be)	90	85	--	--
3	$BeSO_4$	1cc.(9 mgs. Be)	83	83	113	--
4	$BeSO_4$	1cc.(9 mgs. Be)	92	90	98	--
5	$BeSO_4$	1cc.(9 mgs. Be)	109	--	--	136
6	$BeSO_4$	1cc.(9 mgs. Be)	89	--	--	141
7	$BeSO_4$	1cc.(9 mgs. Be)	95	--	--	144
8	$BeSO_4$	1cc.(9 mgs. Be)	83	--	113	150
9	$BeSO_4$	1cc.(9 mgs. Be)	82	--	121	160
10	$BeSO_4$	1cc.(9 mgs. Be)	83	--	131	145
11	$BeSO_4$	1cc.(6 mgs. Be)	90	--	100	84
12	$BeSO_4$	1cc.(6 mgs. Be)	100	--	106	110
13	$BeSO_4$	1cc.(6 mgs. Be)	92	--	100	89

Table XX

The effect of calcium chloride upon blood sugar, hemoglobin and red blood corpuscle changes produced by administration of zinc sulfate by stomach tube to the fasting white rat under nembutal anesthesia

Rat no.	Substance administered	Volume of solution and mgs. of dissolved substance administered	Volume of solution and mgs. dissolved substance injected subcutaneously	Glucose mgs. per 100 cc. after 2 hours (animals anesthetized)	Hemoglobin gms. per 100 cc. after 2 hours (animals anesthetized)	R.B.C. million per cu. mm. after 2 hours (animals anesthetized)
1	$ZnSO_4$	1cc.(65 mgs. Zn)	0	126	21	13.64
2	$ZnSO_4$	1cc.(65 mgs. Zn)	0	125	23	13.82
3	$ZnSO_4$	1cc.(65 mgs. Zn)	0	129	--	-- --
4	$ZnSO_4$	1cc.(65 mgs. Zn)	1.5 cc.(7.5 mgs. $CaCl_2$)	75	23	13.60
5	$ZnSO_4$	1cc.(65 mgs. Zn)	1.5 cc.(7.5 mgs. $CaCl_2$)	70	21	11.50
6	$ZnSO_4$	1cc.(65 mgs. Zn)	1 cc. (5 mgs. $CaCl_2$)	98	23	13.60
7	$ZnSO_4$	1cc.(65 mgs. Zn)	1 cc. (5 mgs. $CaCl_2$)	103	--	-- --
8	Glucose	1cc.(250 mgs. Glucose)	0	113	15.5	9.65
9	Glucose	1cc.(250 mgs. Glucose	1 cc.(5 mgs. $CaCl_2$)	139	14.6	8.80
10	Na_2SO_4	1cc.(96 mgs. SO_4= 142 mgs. Na_2SO_4)	0	84	14.4	8.55

$CaCl_2$ was injected simultaneously with the zinc sulfate.

Table XXI

The effect of sulfuric acid administered by stomach tube on the blood sugar of the fasting white rat under nembutal anesthesia

Rat no.	Volume of solution and concentration of acid given	Initial blood sugar (mgs. per 100 cc.) after 20-hour fast (animals not anesthetized)	Blood sugar (mgs. per 100 cc.) 1 hr. after giving acid (animals anesthetized)	Blood sugar (mgs. per 100 cc.) 2 hrs. after giving acid (animals anesthetized)
1	1 cc. 2N H_2SO_4	102	102	126
2	1 cc. 2N H_2SO_4	96	111	121
3	1 cc. 2N H_2SO_4	83	105	125
4	1 cc. 2N H_2SO_4	89	117	125
5	1 cc. 2N H_2SO_4	82	88	143
6	1 cc. 2N H_2SO_4	102	123	125

Table XXII

The effect of sulfuric acid administered by stomach tube on the hemoglobin and number of red blood corpuscles of the fasting white rat under nembutal anesthesia

Rat no.	Volume of solution and concentration of acid given	Hemoglobin (gms. per 100 cc.)		Red blood corpuscles (M. per cu. mm.)	
		initial after 20-hour fast (animals not anesthetized)	final 2 hours later (animals anesthetized)	initial after 20-hr. fast (animals not anesthetized)	final 2 hours later (animals anesthetized)
1	1 cc. 2N H_2SO_4	17.0	24+	10.48	14.54
2	1 cc. 2N H_2SO_4	16.4	22.5	10.86	11.48
3	1 cc. 2N H_2SO_4	17.5	24+	9.88	12.94
4	1 cc. 2N H_2SO_4	14.8	24+	9.74	15.28

Table XXIII

Blood sugar changes which accompanied administration of 1 cc. $ZnSO_4$ solution containing 65 gms. Zn to fasting rats under nembutal anesthesia

Rat no.	Weight grams	Initial blood sugar (unanesthetized) mgs. per 100 cc.	Final blood sugar (anesthetized) mgs. per 100 cc.	Hours after dose $ZnSO_4$	Rise in blood sugar mgs. per 100 cc.
1	160	95	208	2	113
2	160	88	180	2	92
3	170	109	200	2	91
4	170	98	126	2	28
5	170	92	156	2	64
					Av.= 77.6

Table XXIV

Blood sugar changes which accompanied administration of 1 cc. $ZnSO_4$ containing 65 mgs. Zn with simultaneous injection of 1.5 cc. of solution containing varying amounts of $CaCl_2$ to fasting rats under nembutal anesthesia

Rat no.	Weight grams	Initial blood sugar (unanesthetized) mgs. per 100 cc.	Final blood sugar (anesthetized) mgs. per 100 cc.	Hours after dose $ZnSO_4$	Rise in blood sugar mgs. per 100 cc.	Mgs. $CaCl_2$ in 1.5 cc.
1	180	71	127	2	56	0
2	175	75	111	2	36	2
3	180	67	125	2	58	7.5
4	180	71	108	2	37	8
5	180	95	129	2	34	8
6	160	80	114	2	34	12
7	150	84	125	2	41	15
8	150	87	117	2	30	15
9	150	89	150	2	61	15
10	175	87	133	2	46	15*

* deep intramuscular injection of $CaCl_2$

Table XXV

Blood sugar changes which accompanied administration of 1 cc. $ZnSO_4$ solution containing 65 mgs. Zn to fasting rats under nembutal anesthesia 1.5 hours after administration of dose $ZnSO_4$

Rat no.	Weight grams	Initial blood sugar (unanesthetized) mgs. per 100 cc.	Final blood sugar (anesthetized) mgs. per 100 cc.	Rise in blood sugar mgs. per 100 cc.	Mgs. nembutal injected per 100 gms. body weight
1	140	103	161	58	3
2	180	84	118	34	3
3	190	82	144	62	3
4	180	84	129	46	3.5
5	180	83	101	18	3.5
6	180	77	154	77	4

Table XXVI

Blood sugar changes which accompanied administration of 1 cc. $ZnSO_4$ solution containing 65 mgs. Zn with simultaneous injection of 1.5 cc. of solution containing varying amounts of $CaCl_2$ to fasting rats under nembutal anesthesia

Rat no.	Weight grams	Initial blood sugar (unanesthetized) mgs. per 100 cc.	Final blood sugar (anesthetized) mgs. per 100 cc.	Rise in blood sugar mgs. per 100 cc.	Mgs. $CaCl_2$ in 1.5 cc.
1	150	84	143	59	0
2	150	99	182	83	0
3	150	82	124	42	2
4	150	81	121	40	2
5	150	82	143	61	3
6	150	83	118	35	4
7	150	80	109	29	5
8	150	86	129	43	5
9	150	91	102	11	6
10	150	100	118	18	8
11	150	93	138	45	10

All final determinations were taken 1.5 hours after administration of dose $ZnSO_4$ and all animals were anesthetized at the rate of 3 mgs. nembutal per 100 grams.

Table XXVII

The effect of time of the final determination, the extent of anesthesia and the amount of $CaCl_2$ upon the changes produced in the blood sugar by 1 cc. $ZnSO_4$ solution containing 65 mgs. Zn when administered to fasting rats

Rat no.	Weight grams	Initial blood sugar	Final blood sugar	Hours after dose	Rise in blood sugar mgs. per 100 cc.	Mgs. $CaCl_2$ in 1.5 cc.	Mgs. nembutal per 100 gms. body weight
1	190	97	116	1.5	19	0	4
2	190	85	129	1.75	44	0	4
3	180	87	110	1.5	23	7	3.5
4	190	83	110	1.75	27	6	4
5	165	100	158	1.75	58	6	3
6	180	90	149	1.75	59	7.5	3
7	190	103	112	1.5	9	8	4

Case I

Protocol of the reproductive behavior of a female rat which received a diet containing 0.50 per cent of zinc

$G\mathfrak{P}_1$ was 115 days of age and had been ninety days on the 0.50 per cent zinc diet.

Date	Notes of observation
5/16/38	weight 142 grams Vaginal smear showed dioestrus stage.
5/17/38	dioestrus stage
5/18/38	dioestrus stage
5/19/38	dioestrus stage
5/20/38	dioestrus stage This female showed no oestrus rhythm.
5/21/38	dioestrus stage; weight 146 grams
5/22/38	dioestrus stage
5/23/38	dioestrus stage
5/24/38	dioestrus stage
5/25/38	dioestrus stage
5/26/38	dioestrus stage; weight 159 grams
5/28/38	hemoglobin 12.5 grams per 100 cc. of blood
5/29/38	weight 165 grams It was grossly apparent that this female was pregnant.
5/31/38	weight 165 grams
6/ 1/38	weight 153 grams; hemoglobin 9.5 grams per 100 cc. of blood (Note - There was no sign of parturition to account for the weight drop.)
6/ 2/38	weight 146 grams; hemoglobin 10.5 grams per 100 cc. of blood
6/ 3/38	weight 143 grams
6/ 4/38	weight 148 grams; hemoglobin 11.5 grams per 100 cc. of blood
6/ 5/38	weight 147 grams Vaginal smear showed dioestrus stage.
6/ 6/38	dioestrus stage
6/ 7/38	dioestrus stage
6/10/38	dioestrus stage; weight 154 grams
6/12/38	hemoglobin 14.2 grams per 100 cc. of blood
6/13/38	weight 164 grams This female looked to be pregnant.
6/17/38	weight 166 grams Hemorrhage from the vagina was noted. (Note - This was a typical erythrocyte sign of pregnancy.)
6/18/38	weight 170 grams
6/19/38	weight 163 grams; hemoglobin 8.3 grams per 100 cc. of blood This female was sacrificed this same day, 6/19/38, as it was apparent that she was under-going resorption of her young. The uterus and its contents was removed. Examination showed the tissues to be in a pathologic condition. The embryos were in various stages of development, and apparently death and resorption had taken place at various periods. (See Photograph I)

Case II

Protocol of the reproductive behavior of a female rat which received a diet containing 0.50 per cent of zinc

W♯1 was 115 days of age and had been ninety days on the 0.50 per cent zinc diet.

Date	Notes of observations
5/16/38	weight 125 grams Vaginal smear showed dioestrus stage.
5/17/38	Pro-oestrus stage The female was placed in a cage with a fertile male from the stock colony.
5/18/38	Vaginal smear showed early oestrus stage, but there was no sign of mating.
5/19/38	Vaginal smear showed large numbers of spermatozoa.
5/20/38	weight 126 grams
5/26/38	weight 131 grams
5/28/38	hemoglobin 8.5 grams per 100 cc. of blood
5/29/38	weight 133 grams
5/31/38	weight 137 grams
6/ 2/38	weight 140 grams
6/ 3/38	hemorrhage from vagina (erythrocyte sign of pregnancy)
6/ 4/38	weight 142 grams; hemoglobin 7.8 grams per 100 cc. of blood
6/ 6/38	weight 142 grams
6/ 7/38	weight 139 grams; hemorrhage from the vagina
6/ 9/38	weight 136 grams
6/10/38	weight 136 grams
6/11/38	weight 136 grams
6/12/38	hemoglobin 7.7 grams per 100 cc. of blood (Note - It was evident that this female had undergone a resorption, and she was watched by the vaginal smear technique for earliest resumption of the oestrus rhythm.)
6/16/38	dioestrus stage
6/17/38	pro-oestrus stage The female was placed in a cage with a fertile male of the stock colony.
6/18/38	Sperm cells were observed in the vaginal smear.
6/19/38	weight 142 grams; hemoglobin 6.5 grams per 100 cc. of blood
6/20/38	One-half cubic centimeter of freshly prepared wheat germ oil was given to this female to insure sufficiency of vitamin E.
6/22/38	weight 145 grams
6/25/38	weight 148 grams
6/26/38	weight 150 grams; hemoglobin 7.5 grams per 100 cc. of blood
6/27/38	One-half cubic centimeter of freshly prepared wheat germ oil was given.
6/29/38	weight 150 grams
7/ 1/38	weight 153 grams

Case II (Con'd)

7/ 3/38	weight 163 grams Hemorrhage from the vagina indicated this female was pregnant (erythrocyte sign).
7/ 4/38	hemoglobin 8.8 grams per 100 cc. of blood This female was removed to shavings in hope she would cast a litter.
7/ 5/38	Hemorrhage continued from the vagina. weight 163 grams
7/ 6/38	weight 164 grams; hemorrhage from vagina
7/ 7/38	weight 164 grams; hemorrhage from vagina
7/ 8/38	weight 164 grams; hemoglobin 7.5 grams per 100 cc. of blood; hemorrhage from vagina
7/ 9/38	weight 156 grams There was no evidence of parturition.
7/10/38	hemoglobin 8.2 grams per 100 cc. of blood
7/11/38	weight 154 grams (Note - This female remained on the shavings, and it was noticed that the hemoglobin values improved. Hemoglobin on 7/17/38 was 10.8 and on 7/24/38 it was 12.4 grams per 100 cc. of blood.)

Case III

Protocol of the reproductive behavior of a female rat which received a diet containing 0.50 per cent of zinc

W#R$_1$ was 115 days of age and had been ninety days on the 0.50 per cent zinc diet.

Date	Notes of observations
5/16/38	weight 152 grams Vaginal smear showed dioestrus stage.
5/17/38	dioestrus stage
5/18/38	dioestrus stage
5/19/38	dioestrus stage
5/20/38	pro-oestrus stage This female was placed in a cage with a fertile male from the stock colony.
5/21/38	Sperm cells were observed in the vaginal smear.
5/26/38	weight 158 grams
5/28/38	hemoglobin 7.0 grams per 100 cc. of blood
5/29/38	weight 165 grams
5/31/38	weight 170 grams
6/ 3/38	weight 175 grams; hemorrhage from the vagina (erythrocyte sign)
6/ 4/38	hemoglobin 5.3 grams per 100 cc. of blood
6/ 5/38	weight 169 grams
6/ 6/38	weight 168 grams
6/ 7/38	weight 166 grams
6/12/38	weight 170 grams; hemoglobin 6.4 grams per 100 cc. of blood (Note - This female had apparently undergone a resorption and she was watched by the vaginal smear technique for resumption of her oestrus cycle.)
6/16/38	Vaginal smear showed the pro-oestrus stage. (Note - This female was placed in a cage with a fertile male from the stock colony, but evidence of mating was never found. The oestrus activity ceased and her weight remained at 170 grams with slight variation during the following month. The hemoglobin values were very low. Hemoglobin on 6/26/38 was 5.5 grams and on 7/4/38 it was 6.5 grams per 100 cc. of blood. At this time the female was removed to the iron-zinc diet for improvement of the hemoglobin. Reproduction during this experiment was not followed.)

Case IV

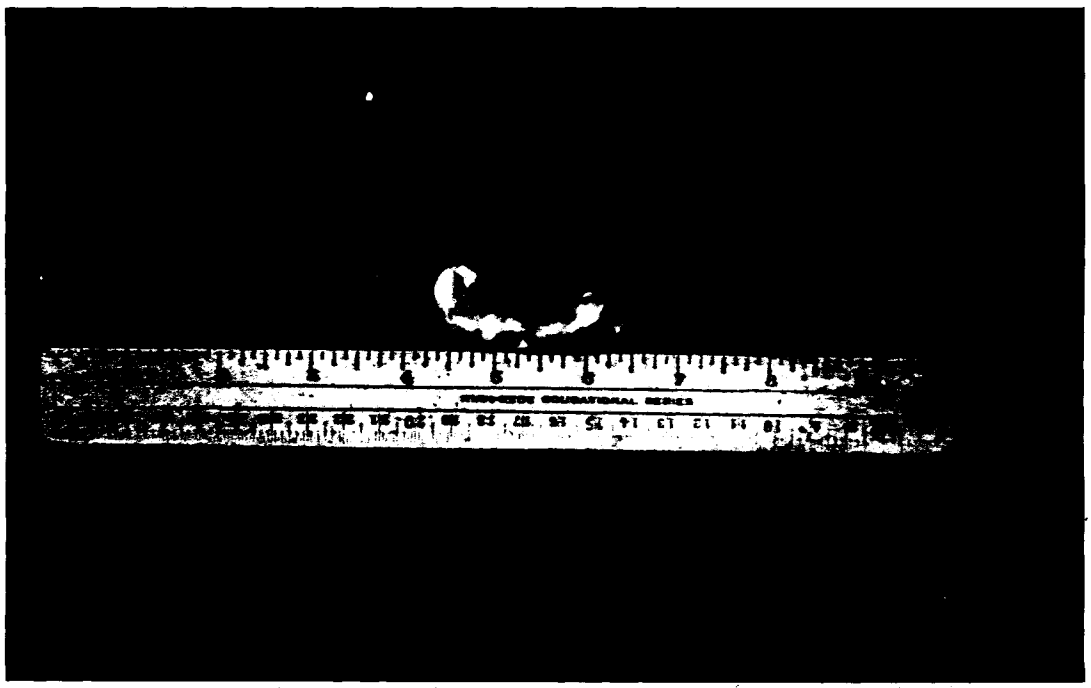
Protocol of the reproductive behavior of a female rat which received a diet containing 0.50 per cent of zinc

W$\frac{1}{2}$R$_2$ was 115 days of age and had been ninety days on the 0.50 per cent zinc diet.

Date	Notes of observations
5/16/38	weight 137 grams Vaginal smear showed dioestrus stage.
5/17/38	pro-oestrus stage This female was placed in a cage with a fertile male from the stock colony.
5/18/38	Vaginal smear showed the early oestrus stage but no evidence of mating was seen.
5/19/38	Sperm cells were observed in the vaginal smear.
5/20/38	weight 148 grams
5/28/38	hemoglobin 7.8 grams per 100 cc. of blood
5/29/38	weight 154 grams
5/31/38	weight 161 grams
6/ 3/38	weight 164 grams Hemorrhage from the vagina indicated that this female was pregnant (erythrocyte sign).
6/ 4/38	hemoglobin 6.4 grams per 100 cc. of blood; weight 161 grams
6/ 6/38	weight 156 grams
6/ 7/38	weight 156 grams
6/11/38	weight 157 grams
6/12/38	hemoglobin 6.0 grams per 100 cc. of blood (Note - This female had apparently undergone a resorption, and she was watched by the vaginal smear technique for earliest resumption of her oestrus cycle.)
6/17/38	pro-oestrus stage This female was placed in a cage with a fertile male from the stock colony.
6/18/38	Sperm cells were observed and this gave evidence of mating.
6/19/38	weight 159 grams; hemoglobin 5.9 grams per 100 cc. of blood
6/21/38	weight 163 grams
6/23/38	weight 166 grams
6/25/38	weight 170 grams
6/26/38	hemoglobin 6.5 grams per 100 cc. of blood
6/27/38	weight 174 grams
6/29/38	weight 178 grams
7/ 1/38	weight 182 grams Hemorrhage from the vagina indicated pregnancy (erythrocyte sign).
7/ 2/38	weight 187 grams; hemorrhage from the vagina

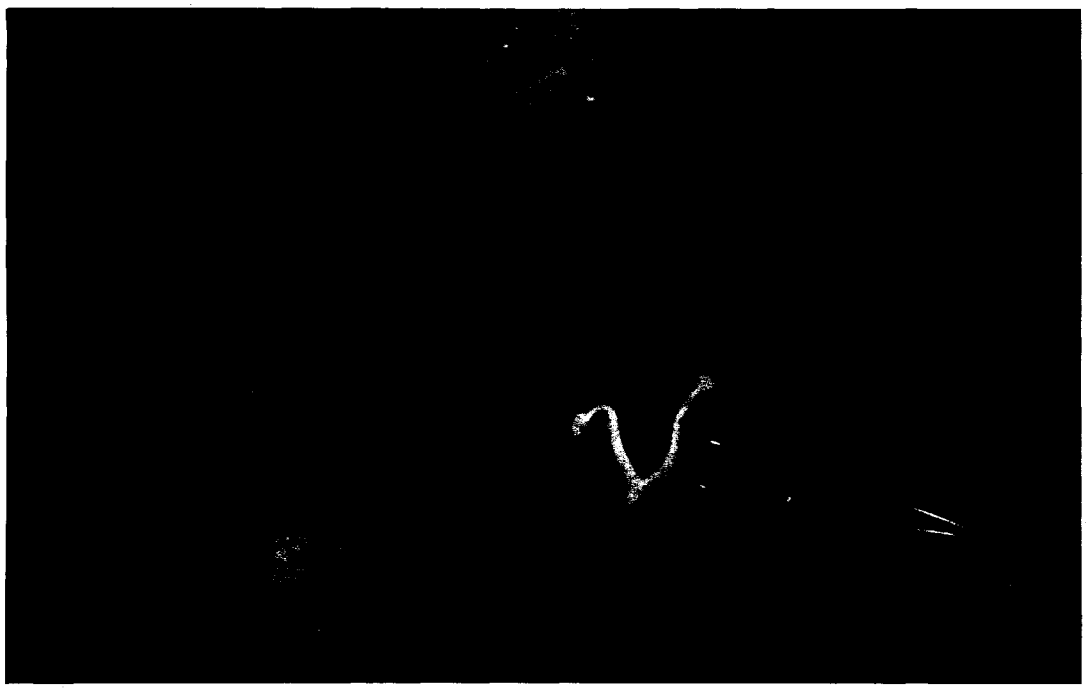
Case IV (Con'd)

7/ 3/38	hemoglobin 7.7 grams per 100 cc. of blood
7/ 4/38	weight 186 grams
7/ 6/38	weight 194 grams This female was removed to wood shavings in the hope she would cast a litter.
7/ 7/38	weight 191 grams
7/ 8/38	weight 188 grams; hemoglobin 8.0 grams per 100 cc. of blood
7/ 9/38	weight 184 grams
7/10/38	weight 180 grams; hemoglobin 7.5 grams per 100 cc. of blood
7/11/38	weight 176 grams (Note - This female was sacrificed on this day, 7/11/38, and the uterus and its contents removed. The implantation sites in the uterus offered additional proof of the pregnancy of this female. The condition of the uterus offered evidence that the embryos were undergoing resorption. (See Photograph II)



Photograph I

Uterus containing embryos undergoing resorption
(see protocol of Case I)



Photograph II

Uterus containing embryos undergoing resorption
(see protocol of Case IV)

DISCUSSION OF RESULTS

The results obtained and shown in Table I indicate that zinc in the form of the carbonate is toxic to rats when ingested at levels of 0.50 and one per cent of the ration. Intoxication by zinc at these levels is indicated by the development of anemia and loss of normal reproductive function. The anemia was marked by diminished hemoglobin concentration in rats intoxicated with zinc at the 0.50 per cent level, but diminished hemoglobin concentration and decreased numbers of red blood corpuscles were also evident in the rats which received zinc at the level of one per cent of their diet. Zinc at 0.10 per cent of the diet did not seem to influence materially the growth, reproduction or the normal blood picture of the rats.

Although rats were fed 0.50 per cent of zinc in their ration for thirty-nine weeks and had shown no indication of reproduction during the last seventeen weeks on this diet, they resumed normal reproduction when the added zinc carbonate was removed from the ration. Hemoglobin values became normal again in the rats which received 0.50 and one per cent of zinc in their diets when the added zinc was removed from the ration.

Table II shows results obtained in hemoglobin studies on normal female rats during pregnancy and post-parturition. An anemia occurs normally prior to and during parturition. This anemic condition is not evident one week before or one week following parturition. It may be possible that this normal anemic condition is so increased by zinc when ingested at a 0.50 per cent level that normal reproduction is impaired. The observation of diminished hemoglobin concentration, the limited num-

ber of pregnancies and the number and appearance of the still born young of females which received 0.50 per cent of zinc lend evidence to this interpretation.

More detailed studies on rats which received 0.50 per cent of zinc in their diet revealed that the weights of the zinc fed animals were below the weights of the rats which received no added zinc in their diet. The respective weights of the animals in the two groups showed this difference soon after the zinc feeding was inaugurated. The difference in the weight of rats in the zinc fed and the control groups could be only partially accounted for by the difference in food consumption in the two groups. The observation that the minimum values of the hemoglobin concentration recorded in the zinc fed group, after fifty days on this diet, were not further diminished after 100 days on this zinc diet may indicate an adaptation of the rats to the ingested zinc.

Table VI shows that the volume of urine excreted by male rats which had received 0.50 per cent zinc for fifty to sixty days was abnormally high. This urine was transparent and almost colorless. The large volume of urine which was excreted by the male rats may have been indicative of a pathological condition of the kidneys caused by the zinc diet. This condition of large urine volume was not observed in the female rats of this group at this time. The females were smaller by forty to eighty grams than the males when this experiment was performed. The ingestion of food and the zinc intake were probably lower in the female than in the male rats. The resulting damage

to the kidneys may not have advanced to the same degree in the females as in the males on this zinc diet. The validity of this assumption is not known and is merely given as a possible explanation for the differences observed between animals of different sex which received the same diet.

From observation of the protocol of Case I, it is evident from the weight change which occurred between 5/16/38 and 5/29/38 and the appearance of the vaginal smear during this period that the female rat of Case I was pregnant at the time these observations were started. The appearance of the vaginal smear was recorded in the notes as dioestrus, since the condition of the female was unknown at that time. The appearance of the late dioestrus smear and the smear observed during pregnancy, except during the hemorrhagic period, were not readily distinguishable. The drop in weight which occurred during the twenty-four hour period, 5/31/38 to 6/1/38 (165 to 153 grams), followed a period of similar length during which the weight remained constant. This weight drop is more abrupt than is usually experienced during a typical resorption. However, the continued decline in weight during the succeeding forty-eight hour period, 6/1/38 to 6/3/38, indicated that a resorption was in progress. It is possible, however, that one or two young were born either dead or alive to this female and that they were destroyed by the mother. This is highly improbable, since close observation of the female was made during this period. The variations in hemoglobin values between 5/28/38 and 6/4/38 are interesting. It is within this period that this female is believed to have been under-

going resorption of her young.

The female rat of Case I became pregnant again following this behavior. As nearly as can be estimated from the time of the appearance of the "erythrocyte sign" and the condition of the embryos which were later removed, this mating took place about 6/4/38. The weight of this female increased to a maximum of 170 grams on 6/18/38 and dropped to 163 grams during the following twenty-four hours. At this time the female was killed, and the uterus and its embryos were removed. It was believed that she was undergoing a resorption, and it was desirable to have more evidence of her pregnancy. Observation of Photograph I will show the appearance of the uterus and the embryonic sites. Histological examination of these sites was made, and the pathological condition of the tissues was confirmed.

The female rat whose case history is shown in Case II showed a typical oestrus rhythm, and the date of mating was identified by the observation of sperm cells in the vaginal smear. The hemoglobin values during the gestation period remained constantly at the low value of 8.0±0.5 grams per 100 cc. of blood. Proof of pregnancy was obtained in the "erythrocyte sign" and the weight change. The weight decline started on the eighteenth day of gestation following a period of twenty-four hours during which the weight remained constant. The weight changes observed are indicative of a typical resorption. The oestrus cycle was resumed, and again this female mated as indicated by the observation of sperm cells in the vaginal smear. The results obtained after administration of freshly prepared wheat germ oil at two periods

during this gestation proved that these resorptions were not due to a deficiency of vitamin E in the diet. The "erythrocyte sign" and the weight change again indicated the pregnancy of this female. Hemorrhage from the vagina continued in the form of a small leakage during the thirteenth to the eighteenth days of gestation. The weight became constant at a maximum of 164 grams on the sixteenth day of gestation; however, there followed a period of three days during which the gain in weight was only one gram. The weight decline which followed this period indicated a resorption. The hemoglobin values remained unchanged during this decline. The values recorded were slightly above 8.0 grams per 100 cc. of blood. Further study of the reproductive activity of this rat was not made. An improvement of the hemoglobin values was noted when this rat remained on shavings instead of the screen.

The female whose case history is shown in Case III showed an oestrus activity by the vaginal smear technique and was observed to have mated with the male. The date of this mating was indicated by the observation of sperm cells in the vaginal smear. The hemoglobin value of 7.0 grams per 100 cc. of blood dropped to 5.3 grams at one period during this gestation. The "erythrocyte sign" and the weight increase gave evidence of the pregnancy of this female. The weight reached a maximum of 175 grams on the thirteenth day of gestation. The "erythrocyte sign" was observed this same day. The decline in weight during the following days was typical of a resorption. The hemoglobin showed no significant variation during this period. With the hemoglobin concentration as low as was recorded in this case, it is reasonable to expect early death and resorption of the foeti. The already low hemoglobin values

probably account for the slight change observed in hemoglobin. As was mentioned in the notes on this case, the oestrus activity of this female apparently ceased following this pregnancy.

The female rat whose case history is shown in Case IV showed oestrus activity and mated with the male as indicated by the observation of sperm cells in the vaginal smear. The weight of this female increased from 148 grams to 164 grams, and the hemoglobin value decreased from 7.8 to 6.4 grams per 100 cc. of blood during the first thirteen days of pregnancy. Proof of pregnancy was offered by observation of the "erythrocyte sign" on the thirteenth day of the gestation period. The weight decline from the thirteenth to the seventeenth days of the gestation period was typical of early death and resorption of the embryos. The hemoglobin value recorded twenty-four days after the onset of pregnancy was 6.0 grams per 100 cc. of blood. This female resumed her oestrus activity following this initial pregnancy and resorption and mated with the male as evidenced by the observation of sperm cells in the vaginal smear. The hemoglobin value at this time was 5.9 grams per 100 cc. of blood, and the weight was 159 grams. The weight increased progressively to the eighteenth day of pregnancy. The maximum value recorded was 194 grams. The weights on the nineteenth, twentieth, twenty-first, twenty-second and twenty-third days following the observation of sperm cells in the vaginal smear were 191, 188, 184, 180 and 176 grams, respectively. The weights and the observation of the "erythrocyte sign" offered evidence of pregnancy of the female and death and resorption of the young. It is interesting to note that

during this second pregnancy the hemoglobin values of this female showed a gain (5.9 to 8.0 grams per 100 cc. of blood), whereas, during the first gestation period the hemoglobin values fell (7.8 to 6.0 grams per 100 cc. of blood). From comparison of the weights obtained in the two pregnancies, one may conclude that death and resorption of the young occurred earlier in the first gestation (thirteenth day) than in the second gestation (eighteenth day). The variation in the hemoglobin values during these respective pregnancies may have contributed to the differences observed.

The female of Case IV was killed on the twenty-third day following the observation of sperm cells in the vaginal smear. The uterus and its embryos were removed. Photograph II shows the condition of the uterus. Close observation will reveal the embryonic sites. Resorption had been rapid, since the embryos at death were undoubtedly in a high state of development. The conclusions drawn from the data collected during the second pregnancy of this female were confirmed by these findings.

The differences observed in the reproductive behavior of the female rats in the first feeding experiment on zinc and the females of the second experiment may be accounted for by the difference in the ages of the rats selected for use in the two experiments. Those rats of the first experiment were five weeks of age when placed on the zinc diet. The rats of the second experiment were only twenty-five days of age when placed on the zinc diet.

It may be seen from the data presented in Tables VII to XII inclusive that the hemoglobin values improved when the 0.50 per cent zinc diet was supplemented with 0.50 per cent of iron. The hemoglobin values showed a steady improvement from the time the iron was added to the diet. The initial low values which were forty to sixty per cent of normal -- 14.5 grams of hemoglobin per 100 cc. of blood is considered normal -- improved to ninety per cent of normal or became normal in one month after iron supplemented the zinc feeding, except in one case which was complicated by a pathological condition.

It was observed during the first part of the experiment that the iron supplemented diet was consumed less readily than the ration without added iron. There was a tendency to scatter the feed. The weights observed indicate that the diet may be found less palatable, and the food consumption may be lower than on the 0.50 per cent zinc diet alone. It will be noted from the tables given that the majority of the rats tend to lose weight while on the iron supplement. In only one case, however, exclusive of the case complicated by the infection, was the difference between the initial and final weight greater than ten grams. All the rats which received the iron supplemented diet, except the one case mentioned, appeared to be in good condition at the close of the experiment.

Interpretation of the results obtained by feeding cadmium carbonate in the basal ration was complicated by the decreased food consumption of the animals. This was particularly true of the higher levels of cadmium. The initial feeding experiment in which cadmium carbonate

was employed showed that cadmium at levels of 0.10 and 0.05 per cent of the diet was very toxic for the rats. The rise in the hemoglobin which was observed to occur during the first weeks on the higher levels of cadmium is believed to be the result of the diminished food consumption and loss of weight, which was also observed to occur. Emaciation and concentration of the body fluids may account for the initial rise in hemoglobin values. As the rats succumbed to the effects of the cadmium feeding the hemoglobin values fell.

Table XIII shows that rats which were raised from weaning age on a diet containing 0.025 per cent of cadmium as cadmium carbonate survived at least four months on this regime. Growth was impaired, as evidenced by the weight of the animals. When compared with rats receiving the basal ration without added cadmium, the weight of the cadmium fed rats was approximately sixty-one per cent of the weight of the rats of the control group. The general unthrifty condition of the cadmium fed rats was conducive to diseases to which the rats were susceptible. This fact must be considered when studying the effect of chronic intoxication by means of cadmium.

Data shown in Table XIV indicate that food consumption in the group which received 0.025 per cent of cadmium was only seventy per cent of the food consumption in the group which received no added cadmium in the diet. Hemoglobin values in the group which received 0.025 per cent of cadmium were generally below those of the control group. In this case the female rats which were most unthrifty showed the greatest deviation in hemoglobin values. The cadmium fed rats

did not show blood sugar concentrations significantly different from the group which received no added cadmium in the diet. The cadmium fed rats excreted a smaller volume of urine, and the amount of reducing material per unit volume estimated as sugar was generally higher than in the group which received only the stock ration. An investigation of the physiological changes produced by cadmium in quantities too small to reduce the food consumption significantly may yield valuable information in long-term feeding experiments.

The maximum increase in red blood cells and hemoglobin, following administration of sublethal doses of salts of zinc, cadmium and beryllium, occurred two to three hours after the dose. The data presented in Table XVII show that, as the limit of tolerance of the animal to the salt is approached, the hemoglobin values may be increased fifty per cent above the initial values, and the number of red blood corpuscles may be increased forty per cent above the initial number. The concentration of the salt solution employed is important in toxicity determinations. An adult rat may tolerate eighteen milligrams of cadmium as cadmium chloride when given by stomach tube in two cubic centimeters of solution. This same weight of cadmium would probably be fatal to the rat if given in one cubic centimeter of solution.

It was shown in Table XVII that the blood sugar of rats was increased 100 per cent above the normal fasting level by stomach tube administration of zinc salt in proper concentration. When the same amount of zinc was given with glucose, the blood sugar was increased 150 per cent above the normal fasting level in three hours. The ab-

sorption of glucose from the gastro-intestinal tract was delayed during nembutal anesthesia as was evidenced by the rate of rise in the blood sugar values, when comparison was made with the data of Keil and Nelson (22). Nembutal anesthesia caused a lowering of the blood sugar of fasting rats.

Beryllium sulfate, when administered by stomach tube in proper concentration, caused an increase in the blood sugar content of fasting rats. The rise was not as rapid nor as marked as with a correspondingly toxic dose of zinc sulfate. The toxicity of these two salts was measured by the number of rats surviving the doses given.

Subcutaneously injected calcium chloride may inhibit the rise in the blood sugar caused by zinc sulfate. This inhibiting action exerted by calcium chloride is dependent upon several conditions. In depressing the rise in blood sugar caused by zinc sulfate, calcium chloride has no effect upon the rise in hemoglobin and red blood corpuscles caused by zinc sulfate. Calcium chloride alone does not influence the number of red blood cells or hemoglobin values, nor does it have any effect upon blood sugar during the normal rise accompanying glucose absorption. The sulfate ion as sodium sulfate has no effect upon the blood sugar, red blood cells or hemoglobin values.

Tables XXI and XXII show that some of the changes produced by sulfuric acid administered by stomach tube are similar to the reactions produced by zinc sulfate solution administered in a similar way. It may be that some of the physiological changes produced by zinc sulfate are due to the tendency of this salt to change the acidity of the body

fluids.

A study of the conditions under which the rise in blood sugar, following stomach tube administration of zinc sulfate, could be depressed by subcutaneously injected calcium chloride revealed several interesting facts. The maximum depressing action of subcutaneously injected calcium chloride upon the rise in blood sugar, which accompanies zinc sulfate intoxication, occurred one and one-half hours after the administration of zinc sulfate. The most effective concentration of calcium chloride found was that which contained six to eight milligrams of calcium chloride in one and one-half cubic centimeters of solution. Two hours after the dose of zinc sulfate, the depressing action exerted by calcium chloride was much less apparent than at one and one-half hours after the dose was given. Calcium chloride was only effective in depressing the rise in blood sugar caused by the zinc sulfate when it was injected simultaneously with the administration of the zinc salt. All concentrations of calcium chloride studied were not equally effective in depressing the blood sugar rise of rats intoxicated by zinc sulfate. The antagonistic action of the subcutaneously injected solution must be due to the dissolved salt. It was found that subcutaneous injection of distilled water did not depress the blood sugar rise accompanying zinc sulfate intoxication. It is not known how calcium chloride may function in this role. Whether or not this action is specific for calcium chloride is not known.

CONCLUSIONS

As a result of the observations made in the study upon some of the changes produced in rats by salts of zinc, cadmium, and beryllium, the following conclusions were made:

1. Zinc, as zinc carbonate, was tolerated by growing rats at a level of 0.50 per cent of the ingested ration to a fair degree.

2. Intoxication by zinc when ingested at levels of 0.50 and one per cent of the ration was evidenced by changes in growth, reproduction, hemoglobin and urine volume. More drastic changes occurred in the rats which received one per cent of zinc in the ration.

3. Reproduction, hemoglobin concentration, volume of urine excreted by the males, growth and food consumption, in the order mentioned, were the most outstanding changes noted in the rats which received zinc at a level of 0.50 per cent of the ration.

4. Reproduction and hemoglobin became normal in the rats which had received 0.50 per cent of zinc when the added zinc salt was removed from the ration.

5. The level at which cadmium was tolerated by growing rats was below the lowest level investigated in this work.

6. Diminished food consumption and inanition which occurred in the groups receiving 0.05 and 0.10 per cent of cadmium complicated the changes produced in the rats by cadmium intoxication.

7. Rats survived at least 150 days, and some attained weights as high as 180 grams on a ration which contained 0.025 per cent of cadmium.

8. On the 0.025 per cent cadmium level, diminished food consump-

tion was apparent, but the food was tolerated much better than on the higher levels.

9. Growth and hemoglobin values of rats which received 0.025 per cent of cadmium in the ration were diminished from the normal.

10. Adult female rats on the 0.025 per cent cadmium diet showed no evidence of reproduction, but a male rat on this diet was potent after 130 days on the cadmium diet.

11. As the maximum limit of tolerance of rats to doses of zinc, cadmium and beryllium salts was approached, it was found that cadmium as the chloride and beryllium as the sulfate were of approximately equal toxicity, but they were nearly five times as toxic as zinc in the form of the sulfate.

12. The administration of sublethal doses of zinc sulfate, cadmium chloride, and beryllium sulfate by stomach tube to rats caused an increase in the hemoglobin concentration and an increase in the number of red blood corpuscles.

13. The blood sugar concentration of rats was increased by stomach tube administration of sublethal doses of zinc sulfate, zinc chloride, and beryllium sulfate.

14. The rise in blood sugar concentration which accompanied intoxication by zinc sulfate was antagonized by subcutaneously injected calcium chloride.

15. The depressing action of the calcium chloride upon the blood sugar rise was most evident one and one-half hours after the calcium chloride had been injected simultaneously with the administration of

the zinc salt.

16. The low hemoglobin values observed in rats maintained on a diet containing 0.50 per cent of zinc as zinc carbonate were increased to normal by feeding an iron supplement.

SUMMARY

Some changes produced in rats by salts of zinc, cadmium and beryllium have been investigated.

Zinc carbonate was incorporated into an adequate basal ration at levels of 0.10, 0.50 and one per cent of zinc and fed to young rats. Feeding experiments were continued through several generations on the lowest level of zinc with no apparent changes being produced in the rats. The 0.50 per cent zinc diet was fed to groups of rats for various lengths of time up to thirty-nine weeks.

Growth, reproduction, hemoglobin, food consumption and the volume of urine excreted were affected by the 0.50 per cent zinc diet.

Reproduction and hemoglobin improved when the added zinc carbonate was removed from the ration.

An iron supplement to the 0.50 per cent zinc diet, which produced anemia in rats, caused marked improvement of the low hemoglobin values.

Studies on the blood sugar content of zinc fed rats showed no significant variation from the normal. There was no indication of glycosuria in the zinc fed rats.

Cadmium carbonate was incorporated into an adequate basal ration at levels of 0.10, 0.05 and 0.025 per cent of cadmium and fed to young rats for periods of four to twenty-one weeks.

The higher levels of cadmium were not tolerated well, and the diminished food consumption and emaciated condition of the surviving rats made an interpretation of the results in terms of cadmium intoxication impossible.

The 0.025 per cent cadmium level was tolerated better than the higher levels of this metal, and studies made on rats fed cadmium at this level showed diminished growth, decreased hemoglobin values and lowered food consumption. There was no evidence of reproduction in the females in the group which received 0.025 per cent of cadmium, but a male proved to be fertile after 130 days on this cadmium diet.

Studies on the blood sugar content of cadmium fed rats showed no significant variation from the normal. The volume of urine excreted by the cadmium fed rats was diminished, and there was an indication of excess sugar excretion in the urine.

Acute intoxication by salts of zinc, cadmium and beryllium administered by stomach tube to rats caused an increase in the hemoglobin concentration and an increase in the number of red blood corpuscles.

Acute intoxication by salts of zinc, cadmium and beryllium administered by stomach tube to rats produced a fall in blood pressure.

Fifteen milligrams of cadmium as the chloride, eighteen milligrams of beryllium as the sulfate and seventy milligrams of zinc as the sulfate were equally toxic when administered in one cubic centimeter of solution by stomach tube to rats.

The blood sugar concentration of rats was increased during acute intoxication produced by zinc sulfate and beryllium sulfate.

It was found that suboutaneously injected calcium chloride depressed the rise in blood sugar of rats intoxicated by zinc sulfate. The calcium chloride was most effective in depressing the blood sugar

rise which accompanied acute intoxication by zinc sulfate when the optimum concentration of calcium chloride was injected simultaneously with the administration of the zinc salt.

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