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I hereby recommend that the thesis prepared under my supervision by Alta Ashley

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Approved by:

Albert P. Mathews

George M. Guest

Glenn E Cullen

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A STUDY OF CERTAIN CHEMICAL CHANGES WHICH OCCUR IN RABBITS WITH EXPERIMENTAL DIPHTHERIA INTOXICATION

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by

Alta Ashley
B.A. Mount Holyoke College, 1927
M.Sc. University of Cincinnati, 1930

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

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A STUDY OF CERTAIN CHEMICAL CHANGES WHICH OCCUR IN RABBITS WITH EXPERIMENTAL DIPHTHERIA INTOXICATION

Alta Ashley

INTRODUCTION

Diphtheria is a disease characterized by both local and generalized manifestations. Though it is not always fatal it often leaves permanent organic disorders. For this reason the disease has been the subject of many investigations, which, for the most part, have been limited to the bacteriological, the anatomic pathological, and the immunological aspects of the disease. Certain metabolic disturbances occurring in the course of the disease have been studied as well. But much remains to be learned of the functional disturbances recognized clinically in diphtheria patients, and of the chemical changes induced by the direct or indirect action of diphtheria toxin on various tissues of the body. A study of certain of these changes has been made the subject of the present thesis.

Rabbits were injected with a standardized preparation of toxin. In the course of the intoxication thus induced studies were made of:

1. The effect of the intoxication on the distribution of phosphorus in the blood and on the chloride content of the cells and plasma.
2. The effect of the intoxication upon the glucose tolerance.
3. The effect of the intoxication upon the action of insulin.

4. The effect of the intoxication upon the erythrocyte count, relative cell volume, and blood solids.

Because of the suggestion of the interrelationship between the organic acid-soluble phosphorus and the chloride of the cells which Guest and Andrus found in their investigations on intestinal obstruction, studies of the chloride content of the cells were made in parallel with the studies of phosphorus distribution.

The effect of diphtheria intoxication on the curve of the inorganic phosphorus of the blood following the administration of glucose was studied in order to see if in diphtheria, as in diabetes mellitus, there might be found an abnormal phosphorus curve. Because it has been claimed by some that diphtheria toxin has an inhibitory effect upon the action of insulin, the curves of the inorganic phosphorus and the sugar of the blood were followed in two rabbits after the administration of insulin.

Studies of the effect of repeated bleeding and of fasting in normal rabbits were made because, during the course of the intoxication, large samples of blood were taken from each rabbit for chemical analysis, and because a few days before death the animals refused food.

Since in diphtheria intoxication nephritis may play a principle rôle in the development of the observed chemical changes in the blood a few experiments were made on the effect of bilateral ureter ligation on the phosphorus distribution and the chloride content of the blood.

REVIEW

I. CHEMICAL AND PHYSIOLOGICAL STUDIES OF DIPHTHERIA INTOXICATION

Diphtheria has been recognized as a clinical entity since Bretonneau of Tours published his observations in 1821. The first description of the Bacillus diphtheriae was made by Klebs in 1883, who found the bacilli in the pseudomembranes in the throats of diphtheria patients. A year later Loeffler successfully cultivated the organism on the injured mucous membranes of animals. In clinical observation it had been long recognized that the disease was not one of localized infection alone, but that it had profound systemic effects as well. The relation of the bacillus found in the localized infection to the constitutional effects observed in the disease was somewhat clarified in 1888 when Roux and Yersin identified the exotoxin which is produced by the diphtheria bacillus. Welch and Flexner (1891) found fatty degeneration of the liver, scarring of the kidneys, and congestion and hemorrhages in the adrenals following the injection of pure cultures of Klebs-Loeffler bacilli (Bacillus diphtheriae) into laboratory animals. A year later they found similar lesions when the exotoxin was injected in place of the cultures of the living organism, (Welch and Flexner, 1892).

Lyon (1904) found changes in the kidneys, such as were described by Welch and Flexner, in rabbits dying after lethal doses of diphtheria toxin; but in those animals which recovered from the intoxication there could be demonstrated no evidence of chronic lesions caused

by the toxin. Pearse and Eisenbrey (1911) studied the lesions produced in the kidneys by diphtheria toxin and concluded that the injury was mainly of the tubules in the early stages of the intoxication, while vascular changes were found in the later stages. Karsner and Denis (1914) demonstrated a retention of nitrogen in cats after diphtheria toxin injections. In a study of the effect of infectious diseases upon renal function Frothingham (1918) found an increase of urea excretion, but a decrease in phenolsulfonphthalein excretion during the febrile stage of diphtheria, but no evidence of acute nephritis. Reduced renal function, as measured by the rate of excretion of phenolsulfonphthalein, persisted even after the acute stages of the disease had passed.

Josephs (1927) studied the effect of diphtheria intoxication on the nitrogen metabolism of dogs. He found that total nitrogen and creatine excretion were greatly increased in the afebrile period of the disease, indicating a destruction of protein, presumably mainly muscle. By feeding a diet high in carbohydrate the increase in total nitrogen was prevented, but there was no decrease in the creatine excretion. Because of this finding Josephs felt that the action of the carbohydrate was not a tissue sparing one, but he was unable to explain its effect in decreasing the total nitrogen excretion. Diphtheria intoxication also produced an initial rise in basal metabolic rate and body temperature. This was shortly followed by a fall, and in the last stages of the intoxication the dogs were unable to maintain their normal temperatures when placed in a cold environment. This inability to regulate body temper-

ature was interpreted as evidence of diminished function of the adrenal glands.

A decrease in serum calcium was found by Peola (1930) in cases of diphtheria which showed toxemia and paralysis. Markowa (1930) found a decrease in the chloride content of the blood in early stages of clinical diphtheria and scarlet fever. However, her measurements were done exclusively upon whole blood and were not corrected for deviations from the normal relative cell volume. Since relatively more chloride is contained in the plasma than in the cells any increase in the relative cell volume will decrease the whole blood chloride content. According to Schwentker and Noel (1930) a concentration of the blood does occur in early and acute stages of diphtheria. Such changes might account for Markowa's findings. Wlademirowa (1930) observed an increase in the organic acids of the blood in scarlet fever and diphtheria patients. This increase of organic acids varied with the severity of the disease, and was accompanied by a decrease in alkali reserve and a decreased excretion of phosphorus in the urine, (Martinson, Markowa, Pocinkowa, and Slakina, 1931). These findings may be dependent upon the decreased renal function observed in diphtheria by other investigators.

Aside from the above citations little work has been reported concerning chemical changes and physiological disturbances in diphtheria intoxication except for studies of carbohydrate metabolism. The effect of diphtheria intoxication upon carbohydrate metabolism has, however, received much attention both from the clinicians and from

laboratory investigators. In clinical obserations Hibbard and Morrissey (1899) observed a transitory glycosuria in most of their fatal cases and in about one-fourth of their mild cases of diphtheria. Mikami (1925) found a rise in blood sugar following intravenous injections of large amounts of diphtheria toxin in rabbits. This rise was highest in from three to five hours after the injection and then gradually disappeared. A marked hypoglycemia was present before death. Rabbits injected with smaller doses (such as to cause death only after twenty-four hours or longer) developed no hyperglycemia, but hypoglycemia was present before death. Lereboullet and Pierrot (1928) reported hypoglycemia in several clinical cases of severe diphtheria, but they found no change from the normal blood sugar level in their mild cases. Signs of adrenaline insufficiency,-loss of muscle tone, low blood pressure, etc. - accompanied the hypoglycemia, and they believed that the degree of hypoglycemia varied directly with the degree of adrenalin insufficiency.

Rosenthall (1914) found that after large doses of toxin there was an increase in blood sugar, followed by a marked decrease together with reduction of the glycogen content of the liver and muscles to a mere trace or less. There was also an initial rise and a terminal fall in the adrenalin content of the blood. At autopsy the adrenals were found to be markedly affected. There was a reduction in the amount of chromaffin material and hemorrhages into the medulla. According to Rosenthall and many others diphtheria toxin is a specific poison for the adrenals. Rosenthall believed that the disturbances in

carbohydrate metabolism which occur in diphtheria intoxication are brought about principally by an overstimulation of the adrenals, resulting in an oversecretion of adrenalin which continues to a point of exhaustion of the glands and ending in a cessation of adrenalin production. The increased adrenalin secretion causes an increased glycogenolysis in the liver and muscle, thus depleting the glycogen stores of the body. The reason he felt that the effect of diphtheria intoxication on the liver was secondary to that upon the adrenals was the fact that in conditions of primary liver damage, such as acute phosphorus poisoning, where the liver cells are actually destroyed, there is no initial hyperglycemia. On the other hand Darrow and Yannet (1932) recently stated in a preliminary report of their work on carbohydrate metabolism in diphtheria that the disturbances in carbohydrate metabolism in diphtheria intoxication is largely due to damage of the liver cells. Normal rabbits and rabbits which had received injections of diphtheria toxin were starved for four or five days, after which time they were given intravenous injections of glucose and their livers were analyzed for glycogen content. The starved normal rabbits synthesized considerable amounts of glycogen in their livers while the starved diphtheria rabbits failed to synthesize any liver glycogen.

Hector (1926) found a lowered glucose tolerance in diphtheria patients and attributed it to a slowed absorption of sugar from the intestines, to liver damage caused directly by the toxin (the degree of damage measured by levulose tolerance tests), and to a deleterious effect of the toxin on the endocrine glands and on the

nervous system, the combined effect being a lessened ability of the tissues to utilize glucose. A lowered tolerance for glucose in diphtheria was also found by Elkels and Heimann (1928). Tisdall, Drake, and Brown (1926) found that the ability to remove injected glucose from the blood was impaired in puppies with diphtheria intoxication. They stated that the intoxication brought about an increased glycogenolytic function in the body, or an inability of the organism to store carbohydrate. They did not feel that there was an impairment in the function or production of insulin, because the administration of insulin in cases of intoxication (diphtheria and other bacterial intoxications) did not increase the rate at which injected glucose was removed from the blood. However, Sweeney (1928) was able to increase the tolerance for glucose in rabbits which had received injections of diphtheria toxin by the administration of insulin.

It is well known that diabetics require an increased amount of insulin during various infections, notably in diphtheria. It has been thought by many that the decreased tolerance for glucose which occurs during the course of diphtheria intoxication in non-diabetics is the result of the action of the toxin on the production or the function of insulin. Harris, Ringer, and Lasker (1927) mixed diphtheria toxin with insulin and injected it into mice whose reaction to insulin had been previously tested, and they found no appreciable change in the potency of the insulin. Their conclusions were based entirely upon the physical appearance of the mice. No blood sugar determinations were made. Netzley (1929) repeated this experiment in rabbits and

found that the hypoglycemic action of insulin was impaired after insulin and diphtheria toxin had been incubated together for eighteen hours. It was also impaired in rabbits previously injected with diphtheria toxin. From these observations Metzley stated that diphtheria toxin not only affects the body cells in such a way that the full action of insulin is prevented, but that it reacts chemically with the insulin, reducing its potency. Lawrence and Buckeley (1927) considered the effect of diphtheria toxin on insulin action to be secondary to a stimulation of the thyroid-adrenal mechanism. In fatal diphtheria these glands exhibit pathological changes which indicate that they have been stimulated to exhaustion. Sweeney and Lackey (1928) however, felt that the lowered tolerance for glucose occurring in diphtheria is primarily the result of a disturbance in the action or the production of insulin. They found, contrary to Hector, no change in the rate at which glucose is absorbed from the intestines in diphtheria.

II. THE DISTRIBUTION OF PHOSPHORUS IN THE BLOOD

A. IN NORMAL CONDITIONS

According to Bloor (1918) phosphorus compounds in the blood can be divided into two distinct groups, easily separated from each other by reason of their solubilities:

1. Acid-soluble - soluble in dilute acids, and precipitated with the proteins by alcohol-ether solution.
2. Alcohol-ether-soluble - soluble in alcohol-ether solution, and precipitated with the proteins by dilute acids.

In general the sum of these two fractions equals the determined total phosphorus; hence the presence of appreciable amounts of other forms of phosphorus not included in these two groups is doubtful.

Kay and Byrom (1927) have listed the forms in which phosphorus occurs in the blood as follows:

Acid-soluble Phosphorus	1. Inorganic phosphate	
	2. Nuceotide, adenine- and possibly a pyrimidine-	Ester Phosphorus
	3. Phosphoglyceric acid	
	4. Reducing phosphoric acid ester	
	5. At least one other ester	
Alcohol-ether-soluble or Lipin Phosphorus	6. Lecithin	
	7. Cephalin	
	8. Sphingomyelin	
Residual Phosphorus	9. Nucleic acid phosphorus	

In Table I are given the values for the various phosphorus compounds in normal human blood (Kay and Eyrom, 1927) and in normal dog blood (Guest and Andrus, 1932). Values for normal rabbit blood may be found on page 33 of this thesis.

The residual or nucleic acid phosphorus is found chiefly in the leucocytes and platelets, which are extremely rich in phosphorus. Under normal conditions the relative volume of the leucocytes is so small that the amount of phosphorus appearing as nucleic acid phosphorus is almost negligible.

The acid-insoluble phosphorus, the value for which is obtained by subtracting the Total Acid-soluble P from the Total P, consists of lipin and "residual" phosphorus in the whole blood, and of practically only lipin phosphorus in the plasma. The cells contain 60 - 70% of the total lipin phosphorus of the whole blood. Lipin phosphorus is not hydrolyzed to any appreciable extent by bone or muscle phosphatases, (Kay and Robison, 1924).

The inorganic phosphorus of the blood is usually considered to consist of a mixture of B_2HPO_4 and BH_2PO_4, and to be present in nearly equal concentrations in the cells and plasma.

The organic acid-soluble phosphorus of the blood is confined almost exclusively to the cells, although there is a small amount, less than 0.5 milligrams per hundred cubic centimeters, occurring in the plasma, (Martland and Robin, 1926). Kay and Robison (1924)

TABLE I

DISTRIBUTION OF PHOSPHORUS IN NORMAL HUMAN ADULT BLOOD

(According to Kay and Byrom)

Average values for eight men and eight women

		Men	Women
Inorganic	mg.%	3.1	3.2
Total acid-soluble		26.6	24.9
Ester (organic acid-soluble)		23.5	21.7
Enzyme hydrolyzable		6.9	7.2
Enzyme non-hydrolyzable		16.9	14.5
Lipin (alcohol-ether-soluble)		12.0	11.6
Total (by addition)		38.6	36.6
(by combustion)			36.9
Phosphorus-Index		52.7	52.8
Relative Cell Vol.	%	44.6	41.2

DISTRIBUTION OF PHOSPHORUS IN THE BLOOD OF THIRTY NORMAL DOGS

(According to Guest and Andrus)

		Whole Blood	Plasma	Cells
Inorganic	mg.%	2.85	3.52	2.16
Total acid-soluble		28.2	3.77	53.2
Organic acid-soluble		25.4	0.25	51.0
Total		43.4	15.7	71.7
Acid-insoluble		15.2	11.9	18.5
Lipin (alcohol-ether-soluble)		14.2	12.2	16.7
Residual		1.0	- 0.3	1.5
Ester-P:RBC count ratio		3.46		
Relative cell volume	%	49.5		
Erythrocyte count	miillions/c.mm.	7.325		

Note:- The Phosphorus-Index of Kay and Byrom is the quotient of the whole blood organic acid-soluble phosphorus expressed in milligrams percent divided by the relative cell volume percent. This value is identical to the Organic Acid-soluble Phosphorus of the Cells (this laboratory). The Ester-P:RBC count ratio is the quotient of the organic acid-soluble phosphorus of the whole blood expressed in milligrams percent divided by the erythrocyte count expressed in millions per cubic millimeter, and represents the ester phosphorus in milligrams X 10^{-5} per million erythrocytes.

found that the organic acid-soluble phosphorus of the blood consists of two fractions - one hydrolyzed by, the other resistant to the action of the phosphatase contained in the watery extract of ossifying cartilage. The hydrolyzable fraction comprises 14 - 36% of the total acid-soluble phosphorus of the whole blood. Probably none of the esters of the plasma are hydrolyzed by bone phosphatase.

Kay and Byrom (1927) found the ratio of the organic acid-soluble phosphorus of the whole blood to the relative volume of the erythrocytes to be remarkably constant in health in individuals of the same species, but to differ widely between species. This ratio, called by Kay and Byrom the Phosphorus-Index, was 53 in human blood, 86 in rabbit blood. Kay (1928) suggested that the quantity of organic acid-soluble phosphorus in the cells may be one of the factors that give the cell its individual characteristics. That is, in conditions which affect the quantity of organic acid-soluble phosphorus of the cells, the character of the erythrocytes themselves is altered.

The ester phosphorus of the cell is evidently an integral part of the cell structure. The compounds determined in this fraction are freely diffusible through a semi-permeable membrane which holds back the blood proteins, but in the whole blood they are almost completely confined to the intact cell. Kay and Byrom (1927) calculated the possible osmotic pressure which these compounds might exert, and found it to be one-sixteenth of the total osmotic pressure of all the cell constituents.

Peters and Van Slyke (1931) have suggested that the organic acid-soluble phosphorus of the cells plays an important part in acid-base equilibrium of the blood. It acts as an acid radical, probably binding one equivalent of alkali for each mol of phosphorus. Further discussion of the participation of the phosphate esters of the blood in the acid-base regulation of the body will be taken up in greater detail in the following section.

II. THE DISTRIBUTION OF PHOSPHORUS IN THE BLOOD (CONTINUED)

B. IN ABNORMAL CONDITIONS

In acute nephritis and in the terminal stages of chronic nephritis Byrom and Kay (1927) found an increased inorganic phosphorus and an apparent decrease in the total acid-soluble phosphorus of the whole blood. The lipin phosphorus of the whole blood was practically unchanged. However, the relative cell volume of the blood of these cases of nephritis was decreased, so that when the values for the phosphorus content of the cells was calculated it was found that there was an actual increase in the organic acid-soluble and lipin phosphorus of the cells. The increase in the organic acid-soluble phosphorus of the cells was entirely in the enzyme-hydrolyzable fraction, the non-hydrolyzable fraction was slightly decreased. There was also a slight increase in the ester phosphorus of the plasma which, too, consisted entirely of the hydrolyzable esters. These changes were progressive, and varied directly with the severity of the disease.

In untreated diabetic coma Byrom (1929) found an increase of inorganic phosphorus, a decrease of ester phosphorus, and practically no change in the lipin phosphorus of the whole blood. The lipin phosphorus of the plasma was high (confirming an observation made by Bloor in 1916). Byrom interpreted this increase in plasma phospholipins as the result of a faulty fat metabolism. The increased inorganic phosphorus was thought to arise from a retention of phosphates due to kidney damage, which in some cases lead to complete anuria. Byrom thought that it might also be the result of an increased hydrolysis of phosphorus esters because of a shift of the reaction of the blood to the acid side. Kay (1924) had found that in a normal individual the acidosis produced by the ingestion of large amounts of ammonium chloride lead to a marked decrease of the ester phosphorus in the blood cells. Martland (1925) found that the phosphoric esterase of the blood is extremely sensitive to changes in reaction; the rate of synthesis of phosphate esters is more rapid than the rate of hydrolysis at a pH or 7.3 or above, while the rate of hydrolysis of the esters is greater than the rate of synthesis below pH 7.3.

Byrom and Kay (1928) found an abnormal distribution of phosphorus in the blood in anemia, leukemia, and polycythemia. The changes observed seemed to be accounted for in large part by the changes in the relative volumes of the erythrocytes, leukocytes, and plasma which occur in these conditions. The Phosphorus-Index and the ratio of the hydrolyzable to the non-hydrolyzable esters was normal in

the blood of patients with secondary anemia. But in so-called primary anemia both the Phosphorus-Index and the ratio of the hyrolyzable to the non-hydrolyzable esters were altered. This change in the character and quantity of the phosphorus esters of the blood in primary anemia was considered by Kay and Byrom to be indicative of a change in the character of the erythrocyte.

Youngburg and Youngburg (1930) studied the distribution of phosphorus in the blood of twenty-one normal and fifteen cancer patients and found a low relative cell volume, low cell inorganic phosphorus, low lipin phosphorus of the plasma, and high ester-and lipin phosphorus of the cells in the blood from cancer patients in comparison with the values found in the blood from the normal individuals.

In rickets, according to Zucker and Gutman (1923) only the inorganic phosphorus of the blood is reduced unless there is present a secondary anemia. In hypervitaminosis-D Ashford (1930) found an increased inorganic phosphorus, but no change in the organic phosphorus of the cells, nor in the ratio of the hydrolyzable to the non-hydrolyzable esters. On the other hand Guest and Warkany (1932) found, after toxic doses (single or repeated) of irradiated ergosterol, a large increase in the ester phosphorus of the blood cells as well as an increase in the inorganic phosphorus. The increases in the cell ester phosphorus were greater and of longer duration than the increases in the inorganic phosphorus of the plasma.

Following high intestinal obstruction in dogs marked alterations in the phosphorus distribution in the blood were found

by Guest and Andrus (1932); and by Andrus, Guest, Gates, and Ashley (1932). Dogs with simple pyloric or mid-duodenal obstruction live from two to five days. Blood samples taken at intervals after the operation up to the time of death showed marked increases in phosphorus content, the increase being mainly in the ester phosphorus of the cells and the inorganic phosphorus of the plasma. Many investigative studies in the past few years have demonstrated the changes in electrolytes of the blood serum of animals with simple high obstruction, the more important of these changes being low chloride and increased bicarbonate values. The low chloride is due to losses of chloride by secretion into the stomach or obstructed gut, whence it is vomited or fails to be reabsorbed; the high bicarbonate content is a compensation for the loss of chloride. It was first pointed out by Gamble and Ross (1925) that a significant factor in the dehydration of the body which follows pyloric obstruction is the reduction of the ionic content of the body fluids by the losses of electrolytes (bases as well as chloride). The effeciency of the administration of NaCl solution in prolonging the life of animals with experimental high obstruction is one of the strongest indications that the loss of water and electrolytes is the principal lethal factor. In the studies of Guest and Andrus the changes in chloride and bicarbonate content of the blood were compared with concomitant changes in the distribution of phosphorus. In all the experiments there was a close correlation between the progressive losses of chloride from the blood cells and the increases of organic acid-soluble phosphorus. It seemed likely that as the organic phos-

phorus compounds increased in the blood they bound the alkali in the cells from which chloride had been lost.

Further evidence of the relationship of chloride to organic acid-soluble phosphorus in the blood is brought forth in some studies carried out in this laboratory on a dog. This dog was fed large amounts of ammonium chloride for five days, which provoked a marked acidosis. Two weeks later, when the dog had apparently recovered, a bilateral ureter ligation was performed. Blood samples were taken on the fifth day of ammonium chloride administration, and on the third and fourth day following the ureter ligation. The dog died on the fifth day. Data on this dog are given graphically in Figure VI. Feeding ammonium chloride produced a marked rise in cell and plasma chloride and a decrease in cell organic acid-soluble phosphorus. Arter bilateral ureter ligation, on the contrary, there was a tremendous increase in cell organic acid-soluble phosphorus which was accompanied by a fall in cell chloride. It seems that in these two conditions, as in simple pyloric and high intestinal obstruction, chloride and organic acid-soluble phosphorus bind at least a part of the alkali of the blood when one or the other acid factor is lost from the system.

III CARBOHYDRATE AND PHOSPHORUS METABOLISM

Many investigations have been carried out concerning the importance of phosphates in the intermediary metabolism of carbohydrates. Harden and Young found that phosphates were necessary for the alcohol-

ic fermentation of fructose by yeast. Furthermore, a hexose-diphosphoric acid ester was isolated from fermenting mixtures of yeast and fructose by Harden (1923). Barrenscheen and Albers (1928) found an increase in carbohydrate-phosphoric acid esters in green plants during the assimilation and utilization of carbohydrates. Most of the evidence that phosphorus plays an essential part in the metabolism of carbohydrate in the animal body is based on the effect of the administration of glucose and insulin on the inorganic phosphorus of the blood and on the extretion of phosphorus in the urine. Only a few investigations have been made of the changes in the phosphoric acid esters of the body during the storage or utilization of carbohydrates in animal metabolism.

Fiske (1920, 1921) observed that after meals or after the ingestion of glucose there was a temporary decrease in urinary phosphorus excretion. Wigglesworth, Woodrow, Smith, and Winter (1923) found a lowering of the sugar and inorganic phosphorus of the plasma and a decrease in the phosphorus excreted in the urine in rabbits following the injection of insulin. Blatherwick, Bell, and Hill (1924); and Sohkey and Allen (1924) found similar changes in human adults and in dogs, and suggested that during the assimilation of glucose a hexose-phosphate similar to that formed in the alcoholic fermentation of fructose by yeast is formed before glucose can be stored as glycogen or burned. This organic phosphate, according to Blatherwick, Bell, and Hill, is formed only temporarily, and is broken down to glucose and inorganic phosphorus when it is stored as glycogen or burned

as glucose.

Kay and Robison (1924) found an increase in the phosphoric acid esters of the blood of rabbits after insulin injections. The esters were formed at the expense of inorganic phosphorus and sugar in the blood. There was also an increase in the "lactacidogen" content of the muscles. "Lactacidogen" was thought to be a hexose-phosphoric acid ester similar to that found in fermenting mixtures of yeast, fructose, and inorganic phosphorus. It was thought to be synthesized in the presence of insulin from free sugar and inorganic phosphorus of the blood.

Kerr (1928) was unable to corroberate Kay and Robison, and stated that insulin and pancreatectomy had no significant influence upon the phosphate esters of the blood corpuscles.

In a previous thesis submitted to this department (Ashley, 1930) there were described the changes which were observed in the distribution of phosphorus of the blood of human adults during twenty-two glucose tolerance tests. Following the ingestion of glucose there was an increase in the organic acid-soluble (ester) phosphorus of the cells in eight cases, seven of whom had disturbed carbohydrate metabolism. In most normal cases there was a decrease rather than an increase in the ester phosphorus of the cells. It was thought that the decreases may have been the result of the secretion of phosphates into the intestinal tract by way of the digestive juices, (Bergeim, 1926). One of the most interesting cases in this series was that of a woman admitted to the hospital in coma, resembling diabetic coma, with glyco-

suria, acetonuria, and hyperglycemia. She had also an advanced osteomalacia, manifested in fragility of the bones and bony changes demonstrable by Roentgenogram. From her case history it was found that she was a vegetarian and had lived for many years on a diet quite deficient in phosphorus. It was thought that this prolonged phosphorus starvation was the basis of the disturbances found not only in the bones but also in carbohydrate metabolism. At the time of her admission to the hospital the inorganic phosphorus ofthe blood was less than 1.6 milligrams percent. After an adequate diet, irradiated ergosterol, and ultra violet light treatments had been given the inorganic phosphorus approached the normal level, and the "diabeteic" condition gradually disappeared, entirely without insulin therapy. Thus it is evident that the abnormal carbohydrate metabolism in this patient was the result of a phosphorus rather than an insulin insufficiency.

Bolliger and Hartman (1925), and Chang (1928) found that the rate of the disappearance of inorganic phosphorus from the blood following glucose administration depended upon the activity of insulin. In the normal individual the rate of disappearance was more rapid and recovery occurred sooner than in the diabetic. In completely diabetic organisms (depancreatized animals) the administration of glucose had no effect upon the inorganic phosphorus ofthe blood.

Harrop and Benedict (1924) found that following the administration of insulin there was a decrease of blood sugar and inorganic phosphorus and an increase in lactacidogen in the muscles. In completely depancreatized dogs sugar and inorganic phosphorus did

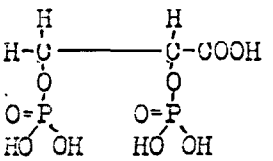
not disappear from the blood after the administration of glucose, nor was there an increase in the lactacidogen content of the muscles.

Bolliger and Hartman (1925) found an increase in the total phosphorus of the muscles of rabbits following the intravenous injection of insulin and inorganic phosphates. This was accompanied by a decrease in blood sugar, and was interpreted as due to a storage in the muscles of a sugar-phosphorus compound formed from the free sugar and inorganic phosphorus in the blood.

Recently it has been found that "lactacidogen" as determined by Embden's method (the method used by May and Robison, and by Harrop and Benedict) is composed not only of hexose phosphoric acid esters, but also of phosphocreatine (Fiske and Subbarow, 1929) and adenyl-pyrophosphoric acid (Lohmann, 1930). Using a modification of a method developed by Embden and Jost for the determination of only the hexose-phosphates in the muscles Cori (1931) measured the hexose-phosphate content of the gastocnemii of rats after injections of insulin and epinephrine. He found that epinephrine caused an increase in the hexose-phosphate content of the muscle, while insulin did so only when epinephrine secretion was stimulated by the onset of hypoglycemic convulsions. In adrenalectomized rats or in rats which had received sufficient glucose to counteract hypoglycemia no increase in hexose-phosphate of the muscles occurred after insulin injections. To explain the fall in the inorganic phosphorus of the blood following the administration of both insulin and glucose Cori suggesta that the glycogen stored under these conditions may contain phosphorus.

Goodwin and Robison (1924) isolated two barium salts of phosphoric acid esters from the organic acid-soluble fraction of the whole blood. One was practically insoluble in water, sparingly soluble in cold dilute acids, but readily soluble in warm HCl. It did not reduce Fehling's solution. It constituted the greater part of the organic acid-soluble phosphorus and contained the esters not hydrolyzed by bone phosphatase. The other salt was readily soluble in water. It reduced Fehling's solution. It was levorotatory. It was practically completely hydrolyzed by bone phosphatase. The ratio $\frac{\text{reducing power as glucose}}{\text{phosphorus}}$ indicated that it was probably a hexosemonophosphate ester.

Greenwald (1925) isolated from pig blood the barium salt of a levo-glyceric acid of the emperical formula $C_3H_5O_8P_2$ and structural formula:



This acid represented from 12.2 - 19.3 mg.% of phosphorus, and from 30.0 - 41.9% of the total acid-soluble phosphorus of the whole blood. This substance was isolated from dog and human blood as well as from pig blood, but quantitative measurements could not be done because of the difficulty in obtaining sufficiently large samples for analysis. The structural formula was established as the above, and two barium salts of the acid were obtained: (1) a pentabarium salt precipitated from aqueous solution by barium hydroxide; (2) a tribarium salt precipitated from acid solution by alcohol. In (1) the H-ion of each hydoxyl group was substituted, while in (2) only one hydroxyl group

in each phosphoric acid radical was substituted. Greenwald proposed the name diphospho-l-glyceric acid for this new compound.

EXPERIMENTAL

I. METHODS

Blood for phosphorus distribtuion studies was obtained by cardiac puncture with sterile technique and without anesthesia. The blood was drawn into air-tight syringes and discharged as follows:

2 - 5 c.c. into a vial containing heparin (0.5 milligrams per cubic centimeter of blood) for relative cell volume, erythrocyte count, hemoglobin, and blood solids.

6 c.c. into a graduated 15 c.c. conical centrifuge tube containing about 2 c.c. of paraffin oil for serum chlorides. The blood was introduced under the oil by means of a glass adapter in such a way that no air was admitted under the oil. When clotting was complete the clot was loosened by means of a fine glass rod, the oil drawn off, and the tube sealed with paraffin. When the paraffin had hardened the tube was centrifugalized, and the serum removed from the clot as soon as possible.

12 - 15 c.c. into a tube containing potassium oxalate

(2 - 3 millegrams per cubic centimeter of blood) for phosphorus distribution, sugar, non-protein nitrogen, whole blood chloride, and plasma protein.

After a sample of normal blood had been obtained from a rabbit the animal was allowed to rest a week or more before injections of diphtheria toxin were begun. When the animal showed signs of marked intoxication he was watched carefully and a second sample of blood was taken from the heart as near as possible to the expected time of death. In a few instances small samples of blood were taken from the ear or from the heart to follow the chemical changes in the blood in the progress of the intoxication. If the rabbit outlived the removal of the second large sample of blood for more than forty-eight hours a third sample was taken. But in the interpretation of the changes observed in such last samples it is recognized that the losses of blood may influence the results. Small samples of blood (2 - 6 c.c.) were usually obtained from the ear. The veins of the ear were dilated by rubbing the ear with xylol. A nick was made in the marginal vein in such a way that the blood flowed freely, but could be easily stopped by pressure. The blood was allowed to drop directly into a tube containing potassium oxalate crystals (2 - 3 millegrams per cubic centimeter of blood).

The following analytical methods were employed:

Relative cell volume and erythrocyte count according to Guest and Siler (unpublished).

Hemoglobin and CO capacity according to Van Slyke and Hiller (1928).

Blood and urine phosphates according to Fiske and Subbarow (1925) with slight modifications.

Blood chlorides according to Fiske (unpublished).

Blood solids according to the following manner:- 0.5 c.c. of blood or plasma were pipetted into weighed bottles containing filter paper dried to constant weight; the bottles and their contents were then dried to constant weight at 110° C., and the percentage solids by weight was computed.

Plasma protein according to Neuhausen and Rioch (1923), using the refractive index obtained with the Abbé refractometer.

Sugar and non-protein nitrogen on the filtrate from unlaked blood prepared according to Folin (1930); sugar according to Folin (1929); non-protein nitrogen according to Folin and Wu (1919).

Determinations of the chemical constituents of the whole blood and plasma were made, and the values for the constituents of the cells were calculated by means of the formula:

$$\text{mg.}_{\text{cells}} = \frac{\text{mg.}_{\text{whole blood}} - (\text{mg.}_{\text{plasma}} \times \text{volumes percent}_{\text{plasma}})}{\text{volumes percent}_{\text{cells}}} \times 100$$

Direct measurements of the cell constituents were not employed because of the difficulty in obtaining well-packed cells, the danger of the occurance of chemical changes in the blood during the necessarily long centrifugalization, and the difficulty in accurately measuring the packed cells.

Three ratios have been used to measure absolute changes within the cells which are indpendent of total blood volume, cell size, water loss and so forth. The ratio, grams of hemoglobin in the whole blood divided by the erythrocyte count in millions per c.mm., has been designated Hb:RBC count ratio and represents the hemoglobin in grams $X\ 10^{-5}$ per million erythrocytes. The ratio, mg. of organic acid-soluble phosphorus in the whole blood divided by the erythrocyte count, has been designated Ester-P:RBC count ratio and represents this phosphorus fraction in milligrams $X\ 10^{-5}$ per million erythrocytes. The ratio milliequivalents of chloride in the cells of 1 c.c. of whole blood divided by the erythrocyte count in millions, has been designated Cl:RBC count ratio and represents the chloride in milliequivalents $X\ 10^{-5}$ per million erythrocytes.

The diphtheria toxin used was supplied throught the courtesy of Dr. Benjamin White of the Antitoxin and Vaccine Laboratory of the Massachusetts Department of Health in Boston. Two samples of toxin were used. In the early experiments toxin #390, MLD 0.015 was used. In later experiments the toxin used was Schick Toxin 58M(51), MLD 0.015, 3-26-29. The action of the second toxin differed from that of #390 in that it produced no paralysis. The toxin was diluted for used 1:100 with

0.85% NaCl solution

The insulin used was Eli Lilly 10-U Iletin. The glucose which was used for glucose tolerance tests was C.P. Dextrose. This was made up in water solution, autoclaved, and administered with sterile precautions.

The rabbits used in this investigation were of both sexes and several breeds, chiefly New Zealand reds and New Zealand whites. Large animals were selected in order to minimize the effect of bleeding, since large blood samples were needed for analysis.

In the early part of the study the rabbits were segregated according to sex and kept in runs, each run accomodating about a dozen rabbits. Later each rabbit was kept in a separate cage, which, together with the food and water dishes, was sterilized once a week. The metabolism cages which were used for the collection of urine were fitted with a truncated cone-shaped Monel metal bottom and wire screen of wide mesh which allowed the urine and feces to drop though. The excreta passed through a hole in the bottom of the cage into a funnel which was fitted with a bulb to separated the feces from the urine, and the urine was collected in a bottle containing toluene. The urine bottle was changed daily and the cage was rinsed at this time with water to wash out the feces and hair which adhered to the bottom of the cage.

The diet consisted of Pratt's Rabbit Feed, a food mixture pressed into pellet form, and consisting of the following ingredients: dried buttermilk, rolled oats, ground wheat, ground barley, cod liver oil, corn gluten meal, corn meal, molasses, alfalfa, bran, oats,

bone meal, 0.75% calcium carbonate, 0.25% calcium phosphate, and 0.5% iodized salt. The manufacturers' analysis is as follows:

Crude Protein	15%
Crude Fat	3%
Crude Fiber	12%
Carbohydrate	63%
N-free Extract	51%

Water was furnished freely to all rabbits.

Toxin was injected into the ear veins in diluted form in doses calculated to cause death in from four to ten days. In early experiments the entire dose was given in one injection. The rabbits died suddenly without developing signs of toxemia, so the calculated dose was given in three injections every other day for three injections. Usually a dose of 1 c.c. of toxin diluted 1:100 was sufficient to cause death in the desired time. However, if the rabbit was resistant to the amount of toxin given larger doses were injected at intervals until the rabbit developed signs of increasing intoxication. The rate of weight loss, the levels of the inorganic phosphorus and of the non-protein nitrogen of the blood, and the physical appearance of the rabbits were used as indications of the progress of intoxication.

Erythrocyte count, relative cell volume, hemoglobin, non-protein nitrogen, sugar, and plasma protein were determined in all cases where the distribution of phosphorus in the blood was determined. In the rabbits in which glucose tolerance tests and tests for the hypoglycemic action of insulin were made plasma protein and non-protein

nitrogen were determined in every case; cell count, relative cell volume, and hemoglobin were determined in only a few cases.

II. RESULTS

The course of the intoxication in rabbits which received injections of toxin 58M(51) was fairly constant. For three or four days the rabbits remained apparently normal, they ate normally and maintained or even increased their weight. Then followed a period of a few days when they began to lose their appetites, and their weights began to decrease. Their activity, however, was not markedly reduced. After this came the period of severe intoxication. This period lasted two to three days, during which time the rabbits refused food and water; they became quite inactive, scarcely moving from one position; their eyes became dull; they developed diarrhea and became anuric. Shortly before death they became lethargic and were unable to support themselves. They died quietly, without convulsions or dyspnoea.

Toxin #390 produced a similar train of symptoms except that a few days before death a progressive paralysis developed, involving first the lower extremities, then mounting to the upper extremities, and terminating in a diaphragmatic paralysis. Death in these cases was accompanied by dyspnoea and convulsions.

The gross pathologic changes found in the rabbits which succumbed to diphtheria intoxication were similar to those described by Welch and Flexner (1892), Lyon (1904), and MacNider (1924). They were the following:

liver - fatty degeneration, fragility, somtimes congestion, sometimes anemia

pancreas - degeneration

heart - fatty degeneration

lungs - generally normal, sometimes bronchopneumonia

kidneys - scarring of the cortex, paranchymatous degeneration, fragility

urinary bladder - usually empty, sometimes partially filled with cloudy urine

stomach and duodenum - in some cases reduction of fat in the stomach wall, multiple gastric and duodenal ulcers, in other cases no apparent abnormalities.

All autopsy findings were based on the gross examination, no microscopic sections were made. Autopsies were performed as soon after the rabbit died as possible. In most cases the rabbits were killed by quick ether anesthesia after blood samples had been obtained and the animals were in extremis.

A. PROGRESSIVE CHANGES IN DIPHTHERIA INTOXICATION

Progressive changes in the blood during diphtheria intoxication were followed in six rabbits. Small samples of blood (5c.c.) were taken from the ear at frequent intervals and determinations of inorganic phosphorus and non-protein nitrogen were made. As a general rule there was a sharp rise in the inorganic phosphorus the day follow-

ing an injection of toxin. This rise did not, however, become marked until the animal began to lose weight. With the onset of a sudden fall in weight the inorganic phosphorus rose rapidly until death or until the animal recovered from the acute effects of the intoxications. If a sublethal dose had been given and the animal began to recover the inorganic phosphorus dropped, reaching well below the normal level, and the rabbit began to gain weight. If at this time a lethal dose of toxin was given the weight again began to drop and the inorganic phosphorus to rise, continuing so until death. The non-protein nitrogen of the blood followed a course similar to that of the inorganic phosphorus.

Urine secretion in most of the rabbits studied was quite irregular, and as a consequence the daily phosphorus excretion as measured was also irregular. However, in all the rabbits the excretion of phosphorus dropped practically to nothing in the late stages of the intoxication. Urine volume also decreased markedly. Rabbit #289 was anuric for seventy-two hours before death, and at autopsy the urinary bladder was found to be empty.

Since the marked increases of inorganic phosphorus and non-protein nitrogen are accompanied by marked losses in weight and a decreased excretion of phosphorus in the urine it is apparent that they can be accounted for by destruction of body tissue and a failing urine secretion.

Changes in weight, inorganic phosphorus and non-protein nitrogen of the blood, and the phosphorus content of the urine of three rabbits with diphtheria intoxication are given in Figures Ia and b.

FIGURE Ia

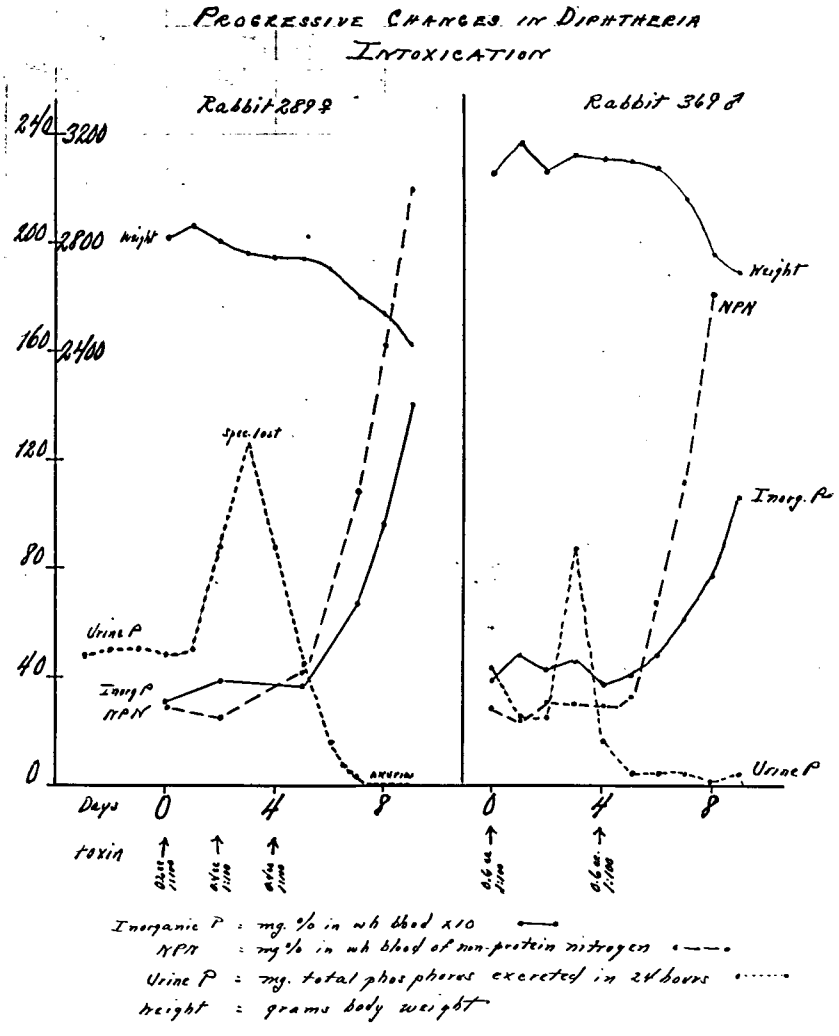
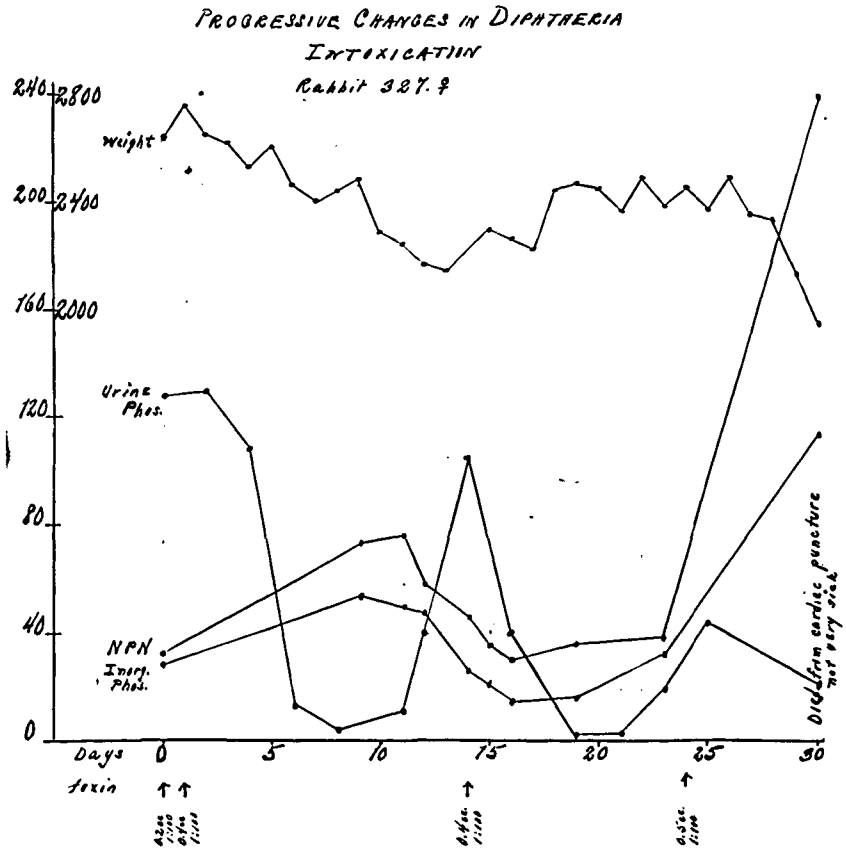


FIGURE Ib



Inorg, P	mg.% inorganic phosphorus in whole blood x 10
NPN	mg.% non-protein nitrogen in whole blood
Urine P	mg. phosphorus excreted in 24 hours

B. THE DISTRIBUTION OF PHOSPHORUS AND OF CHLORIDES IN THE BLOOD OF NORMAL RABBITS

The following are the average values for the distribution of phosphorus and chlorides in the blood of twenty-one normal adult rabbits, eleven males and ten females, used in the present study. The average values for the males seemed to differ slightly from those of the females, but since the variations within the sexes were sometimes greater than the variations between the sexes the values for all the rabbits were averaged regardless of sex.

		Whole Blood	Plasma	Cells
Inorganic P	mg.%	3.55	3.90	2.87
Total acid-soluble P		35.2		85.3
Organic acid-soluble P		31.6		82.4
Total P		43.9	9.5	99.2
Acid-insoluble P		8.7	5.6*	15.9
Ester P: RBC count ratio		5.75		
P-Index (Kay and Byrom)		83.3		
Chloride	mEq./L.	75	101	50
Cl: RBC count ratio		3.36		
Non-protein nitrogen	mg.%	30		
Relative cell volume	%	38.4		
RBC millions/cu.mm.		5.52		

* Total organic phosphorus

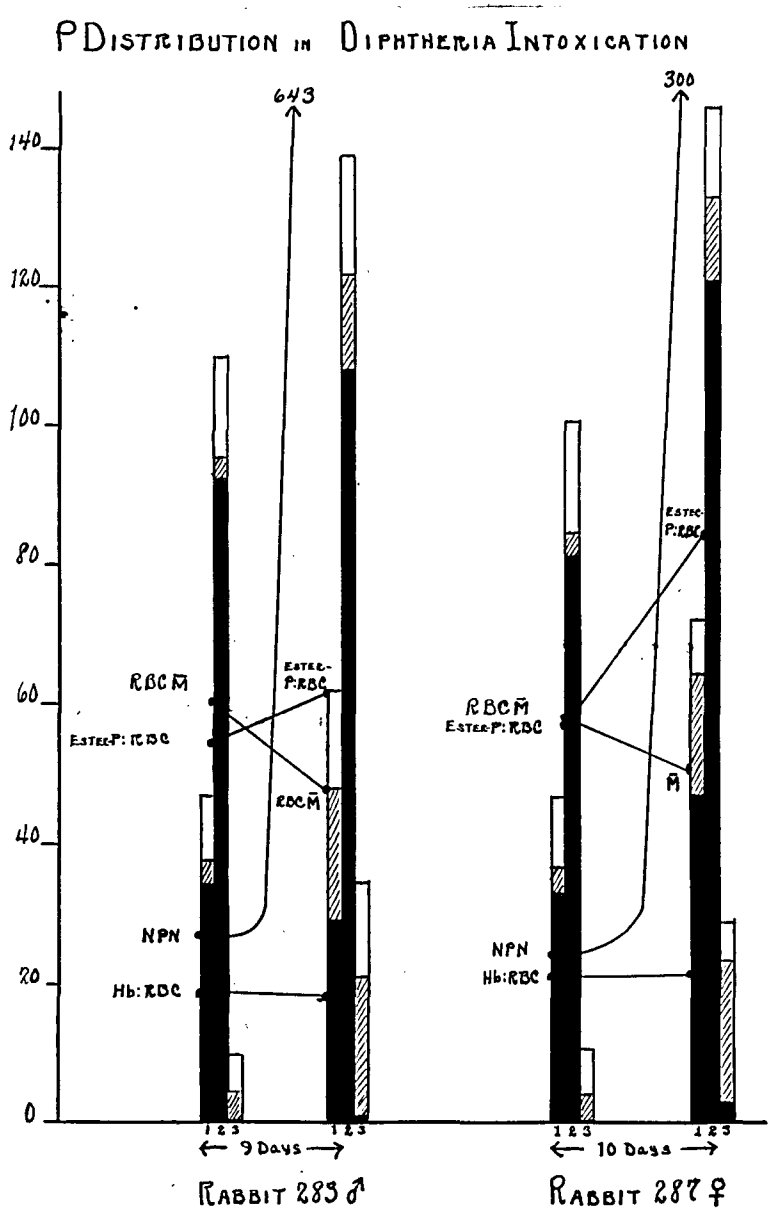
C. THE EFFECT OF DIPHTHERIA INTOXICATION ON THE DISTRIBUTION OF PHOSPHORUS AND OF CHLORIDES IN THE BLOOD

Studies of the ditribution of phosphorus in the blood during diphtheria intoxication were made in seven rabbits, three males and four females. The same general changes were produced in all the rabbits, but these changes varied greatly in magnitude in different rabbits. For this reason average values for the group cannot be used, and values obtained from individual rabbits will form the basis for discussion. Phosphorus distribution in the whole blood will be omitted from the discussion, because of the great fluctuations which occurred in the relative cell volume during diphtheria intoxication.

In all cases there was an increase in the inorganic, organic acid-soluble, and total phosphorus of the cells and plasma, the greatest increases being in the organic acid-soluble phosphorus of the cells, and in the inorganic phosphorus of the plasma. The changes in the acid-insoluble fraction of both the cells and plasma varied according to sex: in the male rabbits it increased in both the cells and plasma, while in the females it remained practically constant in the plasma and increased in the cells. In one female there was a marked decrease in both the cells and plasma. Figure II gives a graphic illustration of the changes which occurred in a male and a female rabbit with a marked diphtheria intoxication.

The increases of the inorganic phosphorus of the plasma were enormous. In rabbit #288 the plasma inorganic phosphorus rose from 2.9 to 29.0 millegrams percent. This rabbit was hyperexcitable at

FIGURE II*



*In these figures the columns 1, 2, and 3 represent the total P as mg.% in the whole blood, cells, and plasma, respectively. In the columns the solid, cross-hatched, and blank portions indicate respectively the organic acid-soluble, the inorganic, and the acid-insoluble P. Non-protein nitrogen is expressed as mg.% in the whole blood. The figures for the Ester-P:RBC count ratio, the Hb:RBC count ratio, the Cl:RBC count ratio, and the RBC count in millions per c.mm. have been multiplied by 10 for the sake of clarity.

this time, and could be thrown into tetanic convulsions by a sudden noise or by a gentle tap on the hind quarters. Unfortunately no measurements of serum calcium were made. Presumably the calcium was reduced, as Peola (1930) found it to be in cases of severe clinical diphtheria accompanied by paralysis and toxemia.

The increases of the cell organic acid-soluble phosphorus were greater than those in the inorganic phosphorus of either the cells or the plasma. The greatest increase was observed in rabbit #288. The cell organic acid-soluble phosphorus rose from 82 mg.% to 124.6 mg.%, and the Ester-P:RBC count ratio increased from 5.94 to 9.00.

During a set of experiments on experimental diphtheria intoxication done earlier in this laboratory it was noted that the lipin phosphorus was reduced in the blood of rabbits receiving diphtheria toxin. Tashiro and Schmidt (1931) found marked decreases in lipin phosphorus and gastric ulcers in two rabbits which had received diphtheria toxin. The first rabbit died in ten days after receiving the toxin, and during the period of intoxication the lipin phosphorus decreased 26.6%. The second rabbit died in twenty-four days with a decrease of 51.2% in the lipin phosphorus.

Of the rabbits in the present series in which values for the distribution of phosphorus in the blood were obtained after diphtheria toxin injections only one developed gastric ulcers. No direct measurements of the lipin phosphorus by alcohol-ether extraction were made, but since there was no increase in leukocyte volume during the intoxication the changes in the acid-insoluble phosphorus fraction are

practically equivalent to the changes in the lipin phosphorus. This rabbit, #289 female, had multiple gastric and duodenal ulcers and, contrary to expectations, an increase in the acid-insoluble phosphorus of the whole blood, plasma, and cells.

		Whole Blood	Plasma	Cells
Acid-insoluble P	before toxin mg.%	8.5	6.5*	11.2
" " "	10th day of intoxication	11.0	6.7	20.2
Percentage change		+ 33%	+ 3.1%	+ 80%

* Total organic phosphorus

Rabbit #287, female, had thin patches in the stomach wall, but no ulcers. In this rabbit the acid-insoluble phosphorus was decreased.

		Whole Blood	Plasma	Cells
Acid-insoluble P	before toxin mg.%	10.6	6.6*	16.4
" " "	8th day of intoxication	8.0	5.6	12.0
Percentage change		- 25%	- 15.1%	- 26.8%

* Total organic phosphorus

Unfortunately five other rabbits which developed gastric ulcers during the course of diphtheria intoxication died suddenly or peritonitis from perforated gastric ulcers before any blood was obtained for analysis.

A possible explanation for the contradictory observations

made in the earlier and in the more recent experiments may be that in the earlier experiments done in this laboratory and in the two experiments reported by Tashiro and Schmidt toxin #390 was used. The action of this toxin, as stated earlier, was different from that of Schick Toxin 58M(51), the toxin used in the greater part of this study. #390 toxin produced paralysis in nearly all subacute intoxications, whereas toxin 58M(51) produced no paralysis.

Variations in the chloride content of the blood during diphtheria intoxication were not constant. In some cases there was a slight decrease in both the cells and plasma, in others a slight increase, and in still others a decrease in one and an increase in the other. The ratio of the organic-acid-soluble phosphorus of the cells to the chloride content of the cells was increased, however, in every case. If the organic acid-soluble phosphorus compounds of the blood behave as acids, and there is reason to believe that they do, this increase of organic acid-soluble phosphorus may be an important factor in the acidosis of diphtheria intoxication.

D. THE EFFECT OF DIPHTHERIA INTOXICATION AND REPEATED BLEEDING ON THE RELATIVE CELL VOLUME, ERYTHROCYTE COUNT, AND SOLIDS OF THE BLOOD

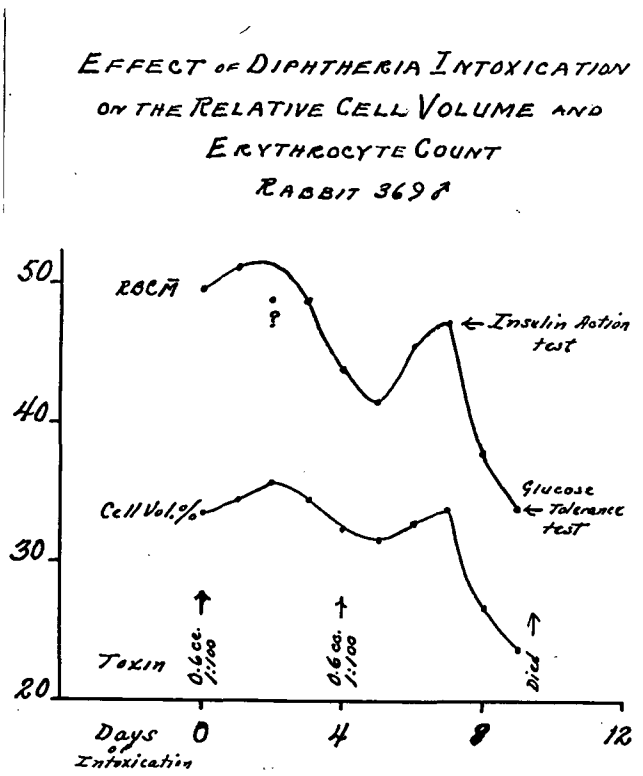
The effect of diphtheria intoxication upon the relative cell volume and erythrocyte count was determined in eight rabbits. Two rabbits were bled only one during the course of the intoxication.

Of these two rabbits the cell count and volume rose in one and fell in the other. In the six rabbits which were bled more than once during the development of the intoxication five showed an increase in cell volume and count in samples taken early in the intoxication, and a marked drop at the time of death. The latter results were obscured because large amounts of blood were taken from the rabbits during the course of the intoxication. It was not clear whether it was the intoxication or the loss of blood which caused this drop in cell volume and count in the late stages of the intoxication. For this reason three normal rabbits were taken for bleeding controls, and daily small samples of blood were taken for cell count and volume from one rabbit during the course of diphtheria intoxication.

Values for the cell volumes and cell counts of the three normal rabbits, and of four rabbits with diphtheria intoxication are given in Table II. The daily changes in cell count and volume in rabbit #369, during progressive diphtheria intoxication, are plotted in Figure III.

It can be seen from Table II that repeated bleeding did lower the cell count and volume in normal rabbits, but this lowering was not as marked as that which occurred in late stages of diphtheria intoxication. In the early stages of intoxication there was an increase in the cell count and cell volume, but as the intoxication progressed there was a decrease in both. Schwentker and Noel (1930) observed similar changes in rabbits following the injection of diphtheria toxin, and attributed the increase of cell count in acute severe intoxications

FIGURE III



RBC M Erythrocytes in millions per cu.mm. x 10
Cell Vol.% Relative cell volume

TABLE II

THE EFFECT OF REPEATED BLEEDING AND DIPHTHERIA INTOXICATION ON THE RELATIVE CELL VOLUME AND ERYTHROCYTE COUNT

PART I NORMAL RABBITS

RABBIT #	SAMPLE	RBC RBC M/c.mm.	REL. CELL Vol.%	c.c. BLOOD
269	initial sample	6.05	39.7	15
	48 hrs. later	5.30	35.9	15
289	initial sample	5.00	38.4	25
	96 hrs. later	4.90	37.7	25
	192 hrs. later	5.00	37.4	25
399	initial sample	5.41	42.4	30
	24 hrs. later	4.78	35.8	20
	72 hrs. later	4.73	35.5	20
	96 hrs. later*	3.98	31.1	20

*Bad snuffles, rather sick

Part II Diphtheria Rabbits

326	before toxin	5.80	43.6	30
	7th day of intoxicatio	5.97	42.9	20
	10th day of intox.	5.00	34.4	30
288	before toxin	4.65	33.8	20
	17th day of intox.	7.35	50.6	25
	20th day of intox.	6.00	42.5	30
283	before toxin	6.05	36.5	15
	8th day of intox.	5.83	37.8	30
	10th day of intox.	4.75	26.6	30
369	before toxin	4.95	33.3	10
	1st day after toxin	5.1	34.3	10
	2nd " " "	4.87 (?)	35.7	10
	3rd " " "	4.88	34.3	10
	4th " " " **	4.38	32.1	10
	5th " " "	4.15	31.3	10
	6th " " "	4.54	32.7	10
	7th " " "	4.69	33.6	25
	8th " " "	3.77	26.6	10
	9th " " "	3.48	24.4	10

**Second dose of Toxin

to a concentration of the blood by dehydration; the decrease in cell count in intoxications of longer duration was attributed to a toxic destruction of the erythrocytes. No evidence of toxic destruction of the erythrocytes, such as discoloration of the plasma, was observed in the present study. The reduction in the number of cells seemed rather to be the result of an inhibition of hematopoietic function by the intoxication, since the decrease in erythrocyte volume was far greater in diphtheria intoxication after repeated bleeding than it was in normal rabbits. As further control of this factor determinations of the total blood volume in diphtheria intoxicated rabbits are to be done in the near future.

Blood solids were determined in three rabbits during diphtheria intoxication. In every case there was an increase of solids in the plasma and cells. Whole blood solids were increased in two cases and decreased slightly in the third. Since the whole blood values are affected by changes in relative cell volume interpretations of the changes observed in the whole blood must be made in regard to changes in the cell volume.

The following are the values found for the blood solids in the three rabbits.

	Wh. Blood	Plasma	Cells	Cell Vol.%
#326 Normal	18.9%	8.2%	33.0%	43.6%
10th day of intoxication	18.3	9.7	37.5	34.4
#324 Normal	17.0	7.6	31.8	39.0
8th day of intoxication	21.3	11.1	33.4	45.9

	Wh. Blood	Plasma	Cells	Cell Vol.%
#288 Normal	16.9	7.9	34.0	33.8
20th day of intoxication	20.5	9.7	35.0	42.5

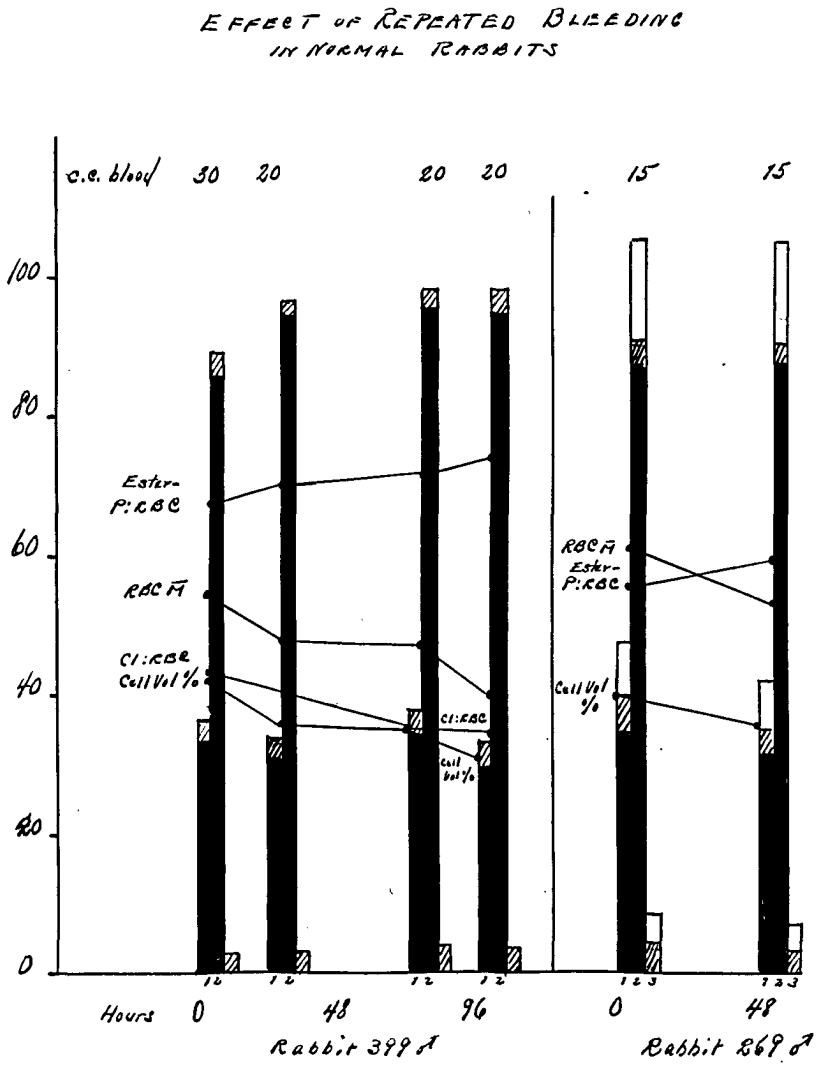
Diphtheria intoxication causes an increase in the solids of both plasma and cells, even though the relative cell volume is diminished. Blood solids, as they were measured in the present study, consist of the cell and plasma constituents minus the substances which are volatile at 110°C. The chief volatile constituent of blood is water. Therefore, diphtheria intoxication produces a relative increase in solids as compared to water in the plasma and cells.

E. THE EFFECT OF REPEATED BLEEDING ON THE DISTRIBUTION OF PHOSPHORUS AND ON THE CHLORIDE CONTENT OF THE BLOOD

Four rabbits were used as bleeding controls, two males and two females. Two samples of 15 c.c. each were taken from rabbit #269 forty-eight hours apart. Three samples of 25 c.c. each were taken from rabbit #294 two weeks apart. Rabbit #399 was bled four times:- 30 c.c. were taken the first day, and 20 c.c. at twenty-four hours, seventy-two hours, and ninty-six hours after the initial bleeding. Figure IV illustrates the changes which occurred in two of these rabbits.

The most significant changes which occurred following the loss of large amounts of blood was an increase in the organic acid-soluble phosphorus of the cells, and an increase in the Ester-P: RBC count ratio. Changes in the other phosphorus constituents of

FIGURE IV



were irregular and not of great magnitude. Changes in the chloride content of the cells and plasma were also irregular. The increase in the organic acid-soluble phosphorus of the cells was not nearly as marked after repeated bleeding as it was in diphtheria intoxication.

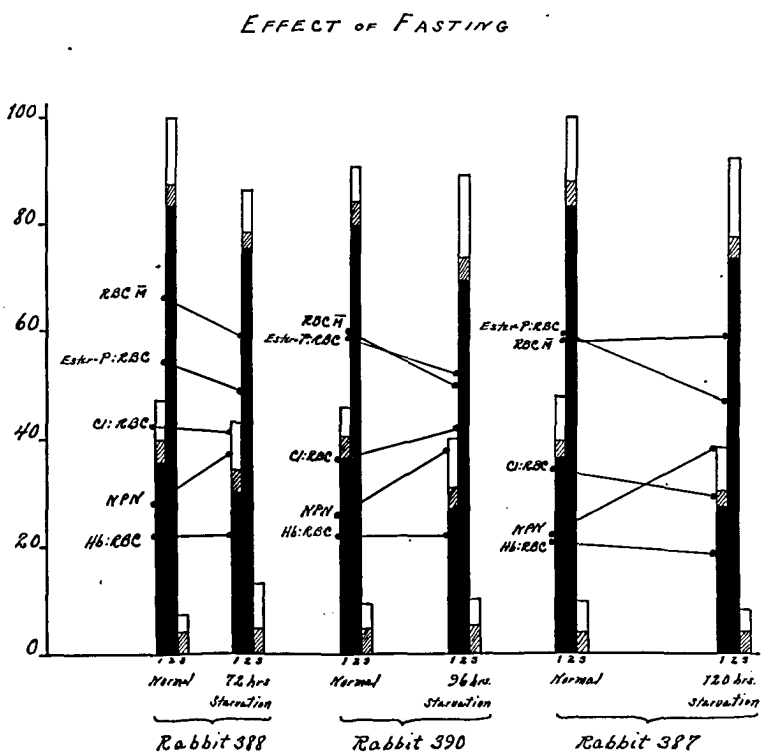
F. THE EFFECT OF FASTING ON THE DISTRIBUTION OF PHOSPHORUS AND ON THE CHLORIDE CONTENT OF THE BLOOD

It was necessary to determine the effect of fasting on the distribution of phosphorus and on the chloride content of the blood because during the course of diphtheria intoxication the rabbits usually refused all food a few days before death. Three normal rabbits were fasted for various lengths of time for this purpose. The results obtained from the analyses of their bloods are represented in Figure V.

The changes which occurred in the blood of fasting rabbits were slight in comparison to those which occurred in the blood of rabbits with diphtheria intoxication. There was a decrease rather than an increase in the organic acid-soluble phosphorus of the cells and in the Ester-P:RBC count ratio. The changes in the concentration of chloride in the cells and plasma were irregular, but there was an increase in the relative amount of chloride to organic acid-soluble phosphorus in the cells. This is in direct contrast to diphtheria intoxication. The non-protein nitrogen was slightly increased. Lennox, O'Connor, and Bellinger (1926) found a tendency for the non-protein nitrogen to increase in the early stages of starvation in human subjects.

From the results of these three experiments it may be said

FIGURE V



that the effect of fasting in rabbits is to minimize, rather than to augment, the effects of diphtheria intoxication on the phosphorus and chloride in the blood.

G. THE EFFECT OF BILATERAL URETER LIGATION ON THE DISTRIBUTION OF PHOSPHORUS AND ON THE CHLORIDE CONTENT OF THE BLOOD

To study the effect of the suppression of urine secretion upon the distribution of phosphorus in the blood bilateral ureter ligations were made in three male rabbits. The operations were performed under ether anesthesia with sterile technique. A single midline incision was made in two cases, in the third case two lateral incisions were made. The kidneys were decapsulated and a ligature was placed as near as possible to the point at which the ureter leaves the kidney. The incisions healed quickly and without infection. Blood samples were taken from the heart for the determination of phosphorus distribution a week or more before the operation, and at the moment that the rabbit ceased breathing. Small samples for the determination of inorganic phosphorus and non-protein nitrogen were taken daily from the ear. The rabbits survived the operation 132, 134, and 112 hours.

At autopsy the kidneys were swollen and necrotic, there was congestion and enlargement of the liver and spleen, congestion of the adrenals, and fluid in the abdomen. Necrosis of the kidneys was so advanced that the whole kidney surface was spongy. Probably much of the fluid which accumulated in the abdomen was forced through the

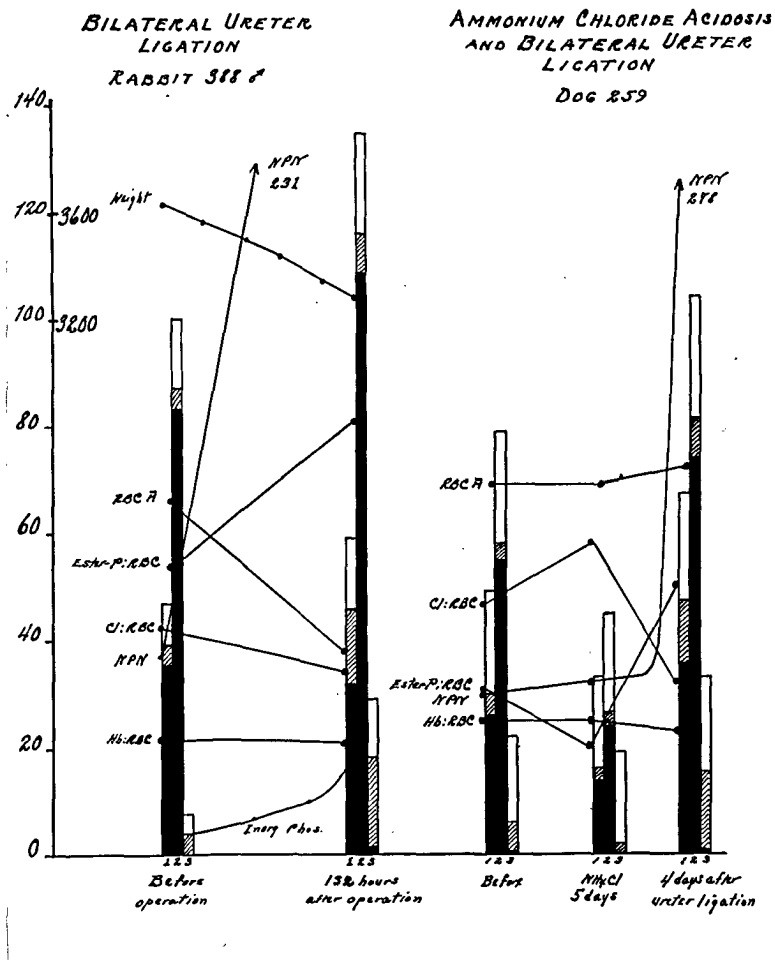
kidney tissue, since the ligatures about the ureters seemed to be intact.

The changes produced in the rabbits after bilateral ureter ligation were similar to those produced in the dog mentioned earlier in this paper. There was a marked rise in the inorganic, organic acid-soluble, and acid-insoluble phosphorus of both the cells and the plasma, the greatest changes being in the inorganic phosphorus of the plasma and in the organic acid-soluble phosphorus of the cells. The chlorides were greatly diminished, about equally in the plasma and cells. The erythrocyte counts and cell volumes were reduced. The non-protein nitrogen values were greatly increased. As in the dog, the relative amounts of organic acid-soluble phosphorus to chloride in the cells were increased. Figure VI represents graphically the changes observed in one of the rabbits (#388) and in the dog(#259) after bilateral ureter ligation. The effect of ammonium chloride administration to dog #259 is shown in the same figure for comparing the effects of bilateral ureter ligation with those of ammonium chloride administration on the organic acid-soluble phosphorus and the chloride content of the cells.

H. THE EFFECT OF DIPHTHERIA INTOXICATION ON THE CHANGES OF INORGANIC PHOSPHORUS AND SUGAR OF THE BLOOD FOLLOWING INTRAVENOUS ADMINISTRATION OF GLUCOSE

Seven rabbits were given glucose tolerance tests before and after the administration of diphtheria toxin and the inorganic phosphorus of the blood was determined in parallel with the blood sugar deter-

FIGURE VI



minations. Blood was obtained from the ear except late in the intoxication when the rabbit became prostrated and the blood pressure was so low that the blood did not flow freely from the ear vein. In these instances blood was obtained by cardiac puncture. The rabbits were not restrained when they were bled from the ear unless they became restless, in which case they were wrapped in towels throughout the test. This was done to minimize muscular movement.

The rabbits were allowed water but no food for twenty-four hours prior to the tolerance test. At least one test was made on each rabbit before the administration of toxin. When the rabbit showed signs of marked intoxication a glucose tolerance test was made, and tests were repeated every day or every other day until the rabbit died. Blood samples were taken according to one of two schedules. From rabbits #23 and #25 blood was obtained before the giving of glucose, and in 7 minutes, 30 minutes, 1, 2, and 3 hours after the glucose. The other rabbits were bled before, and in 3 - 5 minutes, 30 minutes, 1½, and 2½ hours after receiving glucose.

Rabbit #25 received a massive dose of toxin #390 (2 c.c. of a 1:20 dilution) intravenously two and one-half hours before receiving glucose. Blood sugar and inorganic phosphorus were followed at frequent intervals between the time that the toxin was given and the glucose was received. The toxin injection provoked a sudden rise in blood sugar in the first half hour, and then a slight fall. The inorganic phosphorus, which was rather high initially, fell steadily for the first hour and a half, and then remained constant until the glucose was

received. The toxin had little effect upon the rate of the disappearance of glucose and inorganic phosphorus from the blood, as shown in Figure VII. The inorganic phosphorus fell to a lower level than that which it reached in a previous test when the rabbit was normal, and the blood sugar remained slightly elevated even at the end of three hours. But the rate of the disappearance of the glucose from the blood was practically identical with that at which it had disappeared in the previous test.

Figure VIII shows the effect of toxin 58M(51) on the tolerance for glucose and the hypoglycemic action of insulin in rabbit #392. In this animal also the rate of the disappearance of sugar from the blood after its intravenous administration was not decreased in diphtheria intoxication. As the fasting level of the inorganic phosphorus rose with the progress of intoxication greater decreases of inorganic phosphorus followed the glucose injections. It seems, therefore, that the interaction of sugar and inorganic phosphorus during the process of the assimilation of intravenously administered glucose is not altered in diphtheria intoxication.

I. THE EFFECT OF DIPHTHERIA INTOXICATION UPON THE HYPOGLYCEMIC ACTION OF INSULIN AND UPON THE CHANGES OF THE INORGANIC PHOSPHORUS AND SUGAR OF THE BLOOD FOLLOWING THE ADMINISTRATION OF INSULIN

Studies of the action of insulin in diphtheria intoxication were made in two rabbits. Ten units of Lilly's Iletin were

FIGURE VII

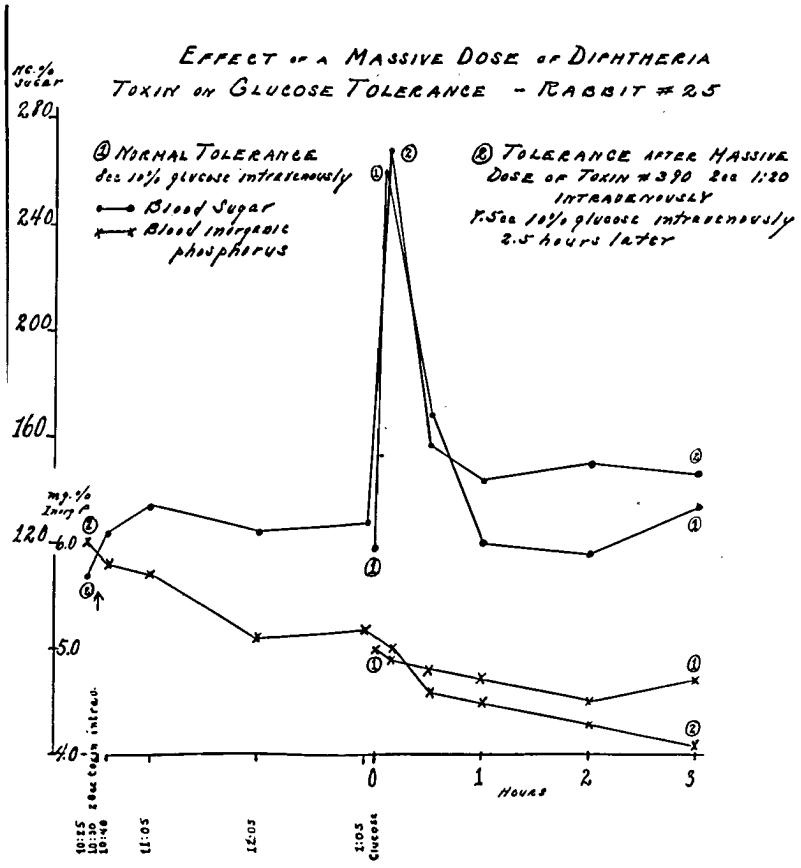
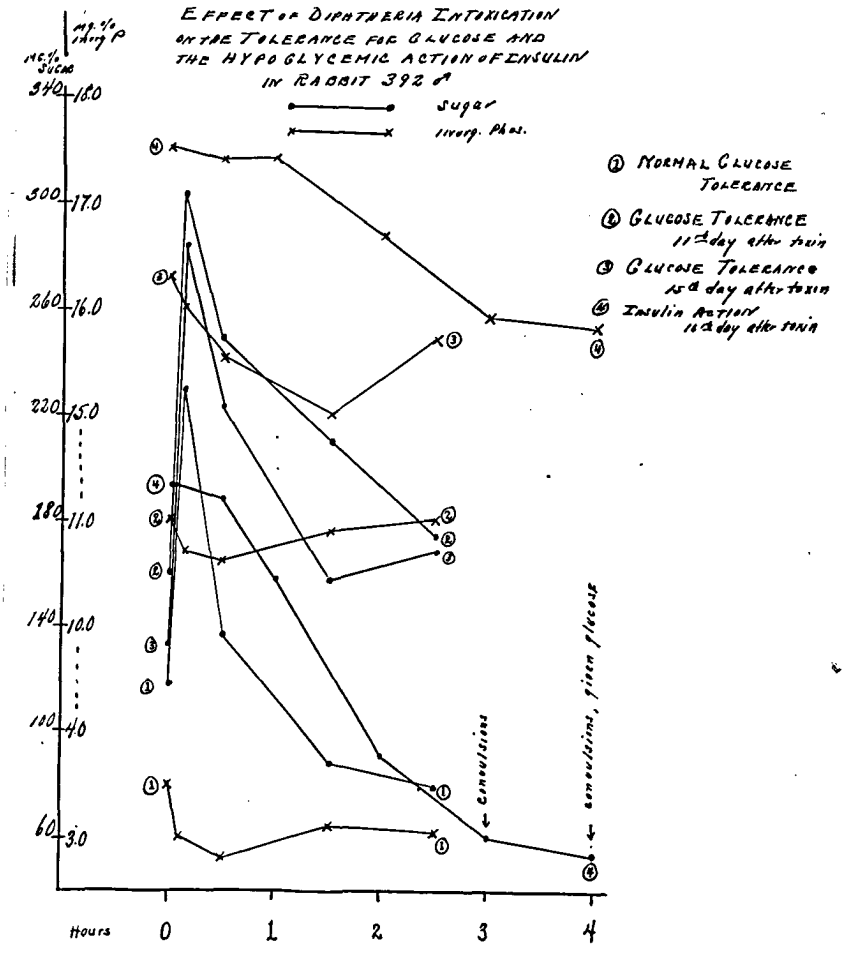


FIGURE VIII

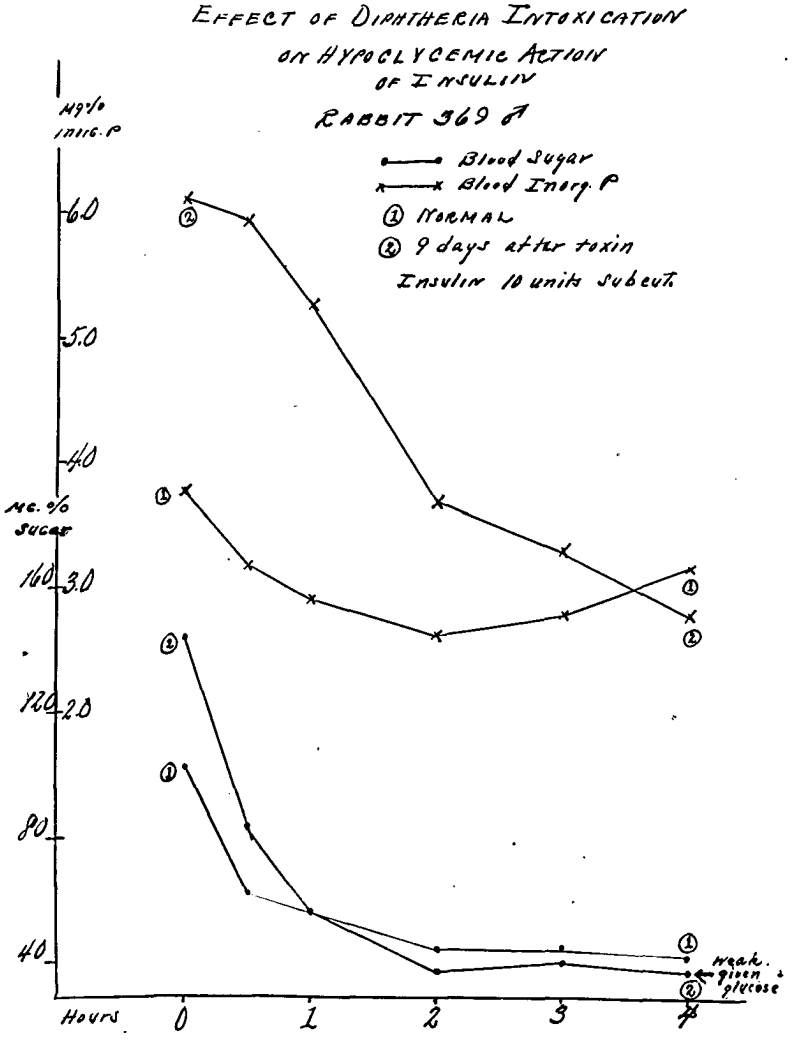


given subcutaneously. Blood was obtained from the ear before the giving of insulin, and in ½, 1, 2, 3, and 4 hours after the insulin. If convulsions occurred glucose was given intravenously.

Insulin, in the large doses in which it was administered in the present study, had no diminished hypoglycemic action in diphtheria intoxication. In fact it had a greater effect in the rabbits when they were in a state of severe intoxication than it did when they were normal. (See Figures VIII and IX.) In both rabbits the fasting blood sugar was elevated at the time of severe intoxication, yet hypoglycemic convulsions occurred following the administration of insulin, although no convulsions had occurred in the normal rabbits after a similar dose of insulin. There was a slight delay in the fall of the inorganic phosphorus of the blood following the administration of insulin in diphtheria intoxicated rabbits, but the fall was greater and of longer duration than that which occurred when insulin was given to the rabbits under normal conditions.

Netzley (1929) found an inhibition of the hypoglycemic action of insulin in rabbits with diphtheria intoxication when 0.2 units of insulin were employed. This he attributed to the interaction of the toxin with the insulin, and a failure of the insulin to prevent the formation of new sugar which is continually being mobilized in diphtheria intoxication because of the overstimulation of the adrenals. In the present study ten units of insulin were used. These doses are so large that any interaction of the toxin and insulin or any over-

FIGURE IX



stimulation of the adrenals probably have little effect upon the net activity of the insulin. The differences in the action of the insulin in normal and diphtheria intoxicated rabbits are perhaps, in the case of the present study, due to the fact that in the intoxicated rabbits liver glycogen has been depleted to a minimum, due to overstimulation of the adrenals. The convulsions which occur in diphtheria intoxicated rabbits may be due to the fact that the liver glycogen is depleted to such an extent that little or no sugar can be mobilized to combat hypoglycemia..

CONCLUSION

1. Diphtheria intoxication in rabbits caused a marked increase of the organic acid-soluble phosphorus of the cells, and of the inorganic phosphorus of the plasma. The organic acid-soluble phosphorus increase in the cells was not always accompanied by a decrease in the chloride content of the cells, but the ratio of the organic acid-soluble phosphorus to the chloride in the cells (expressed as the Ester-P:RBC count ratio and the Cl:RBC count ratio) was increased without exception. This indicates that there is some relation between the changes in the organic acid-soluble phosphorus and the chloride of the blood in diphtheria intoxication.

2. Fasting and repeated bleeding, though altering to some extent the organic acid-soluble phosphorus of the cells and other chemical constituents of the blood, did not affect materially the

changes which occurred in the blood in the course of diphtheria intoxication.

3. In diphtheria intoxication increases in non-protein nitrogen and inorganic phosphorus of the blood were accompanied by loss of weight, decreased urine volume, and decreased phosphorus content of the urine. The increased non-protein nitrogen and inorganic phosphorus content of the blood in diphtheria intoxication can be accounted for in part by retention and by tissue destruction.

4. During the early stages of diphtheria intoxication the erythrocyte count and relative cell volume increased. In later stages of the intoxication there was a decrease in both. The blood solids were increased.

5. The rate at which glucose disappeared from the blood was not altered in diphtheria intoxication in rabbits when the glucose had been given intravenously in the veins of the ear.

6. The inorganic phosphorus of the blood was markedly depressed following the intravenous injection of glucose in diphtheria intoxicated rabbits. The meaning of this exaggerated depression is not clear, but it is evident that there was no insulin insufficiency in these rabbits.

7. The hypoglycemic action of large doses of insulin was not diminished in diphtheria intoxication. The inorganic phosphorus of the blood was markedly depressed and the tendency to develop

hypoglycemic convulsions appeared to be increased in rabbits with diphtheria intoxication.

8. Bilateral ureter ligation produced changes in the blood in rabbits similar to those produced in diphtheria intoxication.

9. A failing urine secretion seems to be the most important factor contributing to the blood chemical changes which occurred in the diphtheria intoxication produced under the experimental conditions of the present investigation.

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ACKNOWLEDGMENT

I wish to express my appreciation of the guidance and helpful suggestions given by Dr. G. M. Guest throughout the present investigation, and to thank the Children's Hospital Pediatric Research Foundation for permitting me to carry out this work in its laboratory.

PROTOCOLS

PROGRESSIVE CHANGES IN DIPHTHERIA INTOXICATION

Rabbit #289

Date	Weight	Inorg.P	NPN	Urine Vol.	Urine P/24°	Remarks
3/10	2860					Placed in cage 9AM
3/11	2830			100	47.6	
3/12	2885			110	50.1	
3/13	2760			140	50.0	
3/14	2815	3.08	29	130	47.8	0.2cc. toxin 4PM
3/15	2860			120	50.3	
3/16	2810	3.79	25	240	87.4	0.4 cc. toxin 5PM
3/17	2775			lost	--	
3/18	2735			205	86.3	0.4cc. toxin 2PM
3/19	2730	3.65	41	170	44.4	
3/20	2710			120	15.5	
3/21	2640	6.67	108	lost (about 20 cc.)		Gluc. Tol. Test
3/22	2535	9.52	162	0	0	
3/23	2445	14.0	219	0	0	Gluc. T.T., P Dist. Died at Noon

Rabbit #369

3/28-3/31					43.5	Av. for four days
4/6	3035					Exp. started 8AM
4/7	3065	3.96	28	170	25.0	0.6cc. toxin 9AM
4/8	3170	4.88	25	0		
4/9	2960	4.23	30	230	25.0	
4/10	3130	4.52	29	110	88.0	
4/11	3110	3.69	29	75	16.8	0.6cc. toxin 9AM
4/12	3100	4.00	32	0		
4/13	3085	4.79	67	100	2.1	Losing appetite
4/14	2960	6.11	111	90	3.6	Insulin Test. No food
4/15	2750	7.56	180	45	1.8	No food
4/16	2650	10.6		90	4.4	No food. Died Noon

In these table inorganic P, non-protein nitrogen are expressed as milligrams percent, weight in grams, urine volume in cu.cm., and urine P as milligrams per 24 hours.

PROGRESSIVE CHANGES IN DIPHTHERIA INTOXICATION

Rabbit #327

Date	Weight	Inorg.P	NPN	Urine Vol.	Urine P/24°	Remarks
2/6	2857	2.74	31	0		
2/8	2645			250	127.5	0.2cc. toxin
2/9	2760			0		0.4 cc. toxin
2/10	2645			200	129.0	
2/11	2625			0		
2/12	2522			225	108.5	
2/13	2610			0		
2/14	2460			170	15.5	
2/15	2407			lost	----	
2/16	2440			20	5.0	
2/17	2485	5.37	73	0		
2/18	2248			lost	----	
2/19	2170	5.00	76	25	10.9	
2/20	2140	4.75	58	60	40.0	
2/22	2292	2.60	46	180	104.5	0.4cc. toxin
2/23	2265	2.13	35	0		
2/24	2225	1.47	30	180	40.5	
2/25	2435			0		
2/26	2460	1.76	35	160	1.7	
2/27	2450			0		
2/28	2360			175	2.7	
2/29	2480			0		
3/1	2385	3.10	38	165	18.8	
3/2	2465			0		0.5cc toxin
3/3	2365			175	43.0	
3/4	2480			lost	----	
3/5	2340			0		
3/6	2315			lost	----	not eating
3/7	2130			0		not eating
3/8	1935	11.3	238	160	22.4	Developing snuffles Not very sick, died fom heart puncture.

PHOSPHORUS DISTRIBUTION NORMAL AND AFTER DIPHTHERIA TOXIN

Rabbit #287 New Zealand Red, female. Diphtheria toxin 58M(51), diluted 1:100. Received 1/4 0.2cc., 1/7 0.2 cc., 1/8 0.4 cc., 1/9 0.2 cc., 1/10 0.2 cc., 1/11 0.2 cc., intravenously

Days after Toxin			Before	8 after 1st
Weight		grams	3188	3068
Blood cells,	Total	Vol.%	40.7	37.4
	RBC only,		40.0	36.5
	RBC millions/c.mm.		5.80	5.35
Hemoglobin,	Whole Blood	g.%	12.1	11.6
	Cells		30.2	31.8
Hb:RBC count ratio			2.09	2.17
Plasma Protein		g.%	7.22	7.42
Non-protein Nitrogen		mg.%	24	300
Phosphorus Distribution				
Whole Blood				
1. Inorganic		mg.%	3.31	17.4
2. Total acid-soluble			36.5	64.1
3. Organic acid-soluble			33.2	46.7
4. Total			47.1	72.1
5. Acid-insoluble			10.6	8.0
Ester-P:RBC count ratio			5.72	8.72
Plasma				
1. Inorganic			3.72	20.2
2. Total acid-soluble				22.8
3. Organic acid-soluble				2.6
4. Total			10.3	28.4
5. Acid-insoluble			6.6*	5.6
Cells				
1. Inorganic			2.70	12.8
2. Total acid-soluble			84.3	133.3
3. Organic Acid-soluble			81.6	120.5
4. Total			100.7	145.2
5. Acid-insoluble			16.4	12.0

*Total Organic

EFFECT OF REPEATED BLEEDING

Rabbit #399

			0°	24°	72°	96° *
Hours after initial bleeding						
Weight		grams.	3505	3465	3365	3325
Cc. Blood taken			30	20	20	20
Blood cells, Total		Vol.%	42.4	35.8	35.5	31.1
	RBC only		41.6	34.3	34.5	30.1
	RBC millions/c.mm.		5.41	4.78	4.73	3.98
Hemoglobin,	Whole Blood	g.%	14.3	11.9	11.0	9.6
	Cells		29.1	28.8	31.4	32.4
Hb:RBC count ratio			2.64	2.49	2.34	2.41
Chloride	Whole Blood	mEq./L.	82		81	84
	Serum		101.5		99.5	102
	Cells		55.5		48	45
Cl:RBC count ratio			4.34		3.56	3.52
Phosphorus Distribution						
Whole Blood						
1. Inorganic		mg.%	2.74	2.64	3.55	3.79
2. Total acid-soluble			36.2	33.8	37.6	33.3
3. Organic acid-soluble			33.5	31.2	34.0	29.5
Ester-P:RBC count ratio			6.69	7.06	7.19	7.41
Plasma						
1. Inorganic			2.56	2.87	3.95	3.76
Cells						
1. Inorganic			3.28	2.24	2.82	3.73
2. Total acid-soluble			88.7	96.6	98.6	98.6
3. Organic acid-soluble			85.4	94.4	95.8	94.9

* Has bad snuffles. Looks sick.

EFFECT OF A SINGLE BLEEDING

Rabbit #269

		0°	48°
Hours after initial bleeding			
Weight	grams	3120	3120
Blood taken	cc.	15	15
Blood cells, Total	Vol.%	39.7	35.9
RBC only		39.2	35.4
RBC millions/c.mm.		6.05	5.30
Hemoglobin Whole Blood	g.%	13.4	10.3
Cells		34.2	29.0
Hb:RBC count ratio		2.22	1.93
Phosphorus Distribution			
Whole Blood			
1. Inorganic	mg.%	4.26	3.03
2. Total acid-soluble		39.1	34.5
3. Organic acid-soluble		34.8	31.5
4. Total		47.1	42.1
5. Acid-insoluble		8.0	7.6
Ester-P:RBC count ratio		5.56	5.95
Plasma			
1. Inorganic		4.71	3.36
2. Total acid-soluble			
3. Organic acid-soluble			
4. Total		8.5	6.9
5. Acid-insoluble		3.8*	3.5*
Cells			
1. Inorganic		3.58	2.45
2. Total acid-soluble		91.3	90.1
3. Organic acid-soluble		87.7	87.6
4. Total		105.7	105.0
5. Acid-insoluble		14.4	14.8

*Total Organic

EFFECT OF FASTING

Rabbit #388

			0°	72°
Duration of Fast				
Weight		grams	3825	3600
Blood cells, Total		Vol.%	43.9	40.0
	RBC only		43.0	39.7
	RBC millions/c.mm.		6.6	5.9
Hemoglobin	Whole Blood	g.%	14.7	13.1
	Cells		34.2	32.9
Hb:RBC count ratio			2.23	2.21
Plasma Protein		g.%	6.65	
NPN		mg.%	28	37
Sugar			132	99
Chloride	Whole Blood	mEq./L.	86.5	86
	Serum		104	102.5
	Cells		64	61
Cl:RBC count ratio			4.24	4.15
Phosphorus Distribution				
Whole Blood				
1. Inorganic		mg.%	3.83	4.21
2. Total acid-soluble			39.4	34.2
3. Organic acid-soluble			35.6	30.0
4. Total			47.1	42.4
5. Acid-insoluble			7.7	8.2
Ester-P:RBC count ratio			5.40	5.09
Plasma				
1. Inorganic			3.88	4.91
4. Total			7.7	13.3
5. Acid-insoluble			3.8*	8.4*
Cells				
1. Inorganic			3.76	3.03
2. Total acid-soluble			86.9	78.0
3. Organic acid-soluble			83.1	75.0
4. Total			99.8	86.0
5. Acid-insoluble			12.8	8.0

*Total Organic

EFFECT OF FASTING

Rabbit #390

			Before	96°
Duration of fast				
Weight		grams	3715	3485
Blood cells, Total		Vol.%	44.9	38.6
	RBC only		44.3	36.2
	RBC	millions/c.mm.	6.0	5.15
Hemoglobin	Whole Blood	g.%	13.2	11.5
	Cells		29.8	31.8
Hb:RBC count ratio			2.20	2.23
Plasma Protein		g.%	6.49	6.03
NPN		mg.%	26	38
Sugar			116	118
Chloride	Whole Blood	mEq./L.	80.5	81.5
	Serum		107.5	97.5
	Cells		48	55.5
Cl:RBC count ratio			3.59	4.18
Phosphorus Distribution				
Whole Blood				
1. Inorganic		mg.%	4.67	4.12
2. Total acid-soluble			40.4	31.2
3. Organic acid-soluble			35.6	27.1
4. Total			45.7	40.0
5. Acid-insoluble			5.3	8.8
Ester-P:RBC count ratio			5.93	5.20
Plasma				
1. Inorganic			4.84	4.37
4. Total			8.9	9.4
5. Acid-insoluble			4.1*	5.0*
Cells				
1. Inorganic			4.66	3.73
2. Total acid-soluble			84.0	72.9
3. Organic acid-soluble			79.3	69.2
4. Total			90.6	88.6
5. Acid-insoluble			6.7	15.6

*Total Organic

EFFECT OF FASTING

Rabbit #387

			Before	120°
Duration of fast				
Weight		grams	4115	3804
Blood cells, Total		Vol.%	42.5	36.3
RBC only			42.1	35.9
RBC millions/c.mm.			5.95	5.8
Hemoglobin	Whole Blood	g.%	13.2	10.8
	Cells		31.3	30.0
Hb:RBC count ratio			2.21	1.86
Plasma Protein		g.%	6.08	9.16
NPN		mg.%	22	38
Sugar			76	91
Chloride	Whole Blood	mEq./L.	81	79
	Serum		105	97
	Cells		48.5	47
Cl:RBC count ratio			3.45	2.93
Phosphorus Distribution				
Whole Blood				
1. Inorganic		mg.%	3.69	3.24
2. Total acid-soluble			39.1	30.3
3. Organic acid-soluble			35.4	27.1
4. Total			47.8	38.5
5. Acid-insoluble			8.7	8.2
Ester-P:RBC count ratio			5.95	4.64
Plasma				
1. Inorganic			3.33	3.24
4. Total			9.7	7.8
5. Acid-insoluble			6.4*	4.6*
Cells				
1. Inorganic			4.19	3.25
2. Total acid-soluble			87.5	77.4
3. Organic acid-soluble			83.3	74.1
4. Total			100.0	92.3
5. Acid-insoluble			12.5	15.1

*Total Organic

EFFECT OF BILATERAL URETER LIGATION

Rabbit #388

			Before	132
Hours after Operation				
Weight		grams.	3615	3280
Blood cells,	Total	Vol.%	43.9	28.3
	RBC only		43.0	27.1
	RBC millions/c.mm.		6.6	3.8
Hemoglobin	Whole Blood	g.%	14.7	8.0
	Cells		34.2	29.6
Hb:RBC count ratio			2.23	2.11
Plasma Protein		g.%	6.65	7.9
Non-protein Nitrogen		mg.%	28	231
Sugar			132	187
Chloride	Whole Blood	mEq./L.	86.5	69
	Serum		104	78
	Cells		64	46
Cl:RBC count ratio			4.24	3.42
Phosphorus Distribution				
Whole Blood				
1. Inorganic		mg.%	3.83	14.2
2. Total acid-soluble			39.4	46.1
3. Organic acid-soluble			35.6	31.9
4. Total			47.1	59.1
5. Acid-insoluble			7.7	13.0
Ester-P:RBC count ratio			5.40	8.39
Plasma				
1. Inorganic			3.88	16.9
2. Total acid-soluble				18.5
3. Organic acid-soluble				1.6
4. Total			7.7	29.3
5. Acid-insoluble			3.8*	10.8
Cells				
1. Inorganic			3.76	7.3
2. Total acid-soluble			86.9	116.0
3. Organic acid-soluble			83.1	108.7
4. Total			99.8	134.6
5. Acid-insoluble			12.8	18.6

*Total Organic

EFFECT OF AMMONIUM CHLORIDE FEEDING AND OF

BILATERAL URETER LIGATION

Dog #259

Blood Sample			Normal	NH_4Cl 5th day	Ureter Lig. 4th day
Blood cells,	Total	Vol.%	43.7	55.0	47.6
	RBC only		42.4	53.4	45.1
	RBC millions/c.mm.		6.90	6.97	7.13
Hemoglobin	Whole Blood	g.%	16.7	17.9	16.2
	Cells		36.8	33.4	36.0
Hb:RBC count ratio			2.39	2.56	2.28
Plasma Protein		g.%	8.78	8.72	
Non-protein Nitrogen		mg.%	46	27	244
Sugar			75	97	140
Chloride	Whole Blood	mEq./L.	89	93	68
	Serum		106	117	86
	Cells		70	89.5	48
Cl:RBC count ratio			4.68	5.82	3.23
Phosphorus Distribution					
Whole Blood					
1. Inorganic		mg.%	4.76	2.34	11.2
2. Total acid-soluble			30.7	15.6	46.7
3. Organic acid-soluble			25.9	13.3	35.5
4. Total			48.8	33.4	66.7
5. Acid-insoluble			16.6	17.8	20.0
Ester-P:RBC count ratio			3.68	1.90	4.98
Plasma					
1. Inorganic			5.84	2.47	14.7
2. Total acid-soluble			6.09		15.3
3. Organic acid-soluble			0.25		0.6
4. Total			22.0	19.2	32.9
5. Acid-insoluble			15.9	16.8*	17.6
Cells					
1. Inorganic			3.55	2.24	7.43
2. Total acid-soluble			58.2	26.3	81.3
3. Organic acid-soluble			54.7	24.1	73.9
4. Total			78.8	45.0	103.9
5. Acid-insoluble			20.1	18.7	22.6

*Total Organic

EFFECT OF A MASSIVE LOSE OF TOXIN ON GLUCOSE TOLERANCE

Rabbit #25

Normal 6/7

Time interval after glucose	Bl. Sugar mg.%	Inorg. P mg.%
Before	119	5.06
Received 8 cc. 10% glucose intravenously		
3 min.	259	4.97
30 min.	170	4.85
1 hr.	119	4.74
2 hrs.	116	4.52
3 hrs.	134	4.63

After Toxin 6/28

Time	Bl. Sugar mg.%	Inorg. P mg.%
10:25AM	111	6.06
10:30	2.0 cc. #390 toxin dil. 1:20, intraven.	
10:40*	125	5.88
11:05	134	5.63
12:05	123	5.16
1:05	127	5.20
1:10	Received 7.5 cc. 10% glucose intravenously	
	267	5.00
	157	4.65
	143	4.55
	151	4.33
	150	4.15
10:00PM	Appeared weak	
10:45	40 cc. blood from heart	
11:00	Died.	

*Excited

EFFECT OF DIPHTHERIA INTOXICATION ON GLUCOSE TOLERANCE AND INSULIN ACTION

Rabbit #392

Test #		1	2	3	4
Day of Intoxication		Before	13th	16th	17th
Initial Blood Sugar	mg.%	118	160	132	191
" " Inorganic P		3.50	11.0	16.3	17.5
Amount Glucose intravenously or Insulin subcutaneously		3cc. 33% glucose	2cc. 50% glucose	2cc. 50% glucose	10 units insulin
Second Blood Sugar	mg.%	229	303	284	187
" " Inorganic P		3.05	10.7	16.0	17.4
Third Blood Sugar		135	248	222	158
" " Inorganic P		2.78	10.6	15.5	17.4
Fourth Blood Sugar		88	210	158	90
" " Inorganic P		3.10	10.9	15.0	16.7
Fifth Blood Sugar		78	174	167	59*
" " Inorganic P		3.01	11.0	15.7	15.9
Sixth Blood Sugar					53*
" " Inorganic P					15.8
Non-protein Nitrogen		23	146	171	216
Weight	grams	3450	3000	2730	2700

*Convulsions

In the glucose tolerance tests blood samples were taken before the injection of glucose and in 3 minutes, $\frac{1}{2}$, $1\frac{1}{2}$, and $2\frac{1}{2}$ hours after the glucose. In the insulin action test samples were taken before the administration of insulin, and in $\frac{1}{2}$, 1, 2, 3, and 4 hours after the insulin.

EFFECT OF DIPHTHERIA INTOXICATION ON THE HYPOGLYCEMIC ACTION OF INSULIN

Rabbit #369

Time interval after insulin	Normal		9 days after toxin	
	Sugar mg.%	Inorg. P mg.%	Sugar mg.%	Inorg. P mg.%
Before	102	3.75	143	6.11
Insulin subcutaneously	10 units		10 units	
30 minutes	62	3.16	83	5.93
1 hour		2.91	55	5.27
2 hours	43	2.51	37	3.66
3 hours	44	2.74	39	3.28
4 hours	41	3.14	37*	2.76
Non-protein nitrogen mg.%	29		111	
Weight grams	3085		2960	

*Weak, convulsions, given glucose intravenously.

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