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# PHYSIOLOGICAL DISTURBANCES DURING EXPERIMENTAL DIPHThERIC INTOXICATION. IV. BLOOD ELECTRO- LYTE AND HEMOGLOBIN CONCENTRATIONS<sup>1</sup>

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In previous papers of this series (1) the authors have shown that derangements of the functions of the liver and kidneys play important rôles in the physiological disturbances following the intravenous injection of diphtheria toxin in rabbits. The following paper reports the results of the investigation of the changes in concentration of serum electrolytes and hemoglobin.

## PROCEDURE

Rabbits weighing approximately two kilograms were used as the experimental animals. They were maintained on a fasting regime, which began 24 hours before the injection of the toxin and continued thereafter. Water was always present in the cages. Three groups were studied. The first group of 5 animals served as fasting controls. Each of the second group (6 animals) received intravenously 3 minimal lethal doses of diphtheria toxin. The third group (8 animals) each received 0.8 intravenously. (Previous work had demonstrated that these quantities of toxin would cause death in from 2 to 3 days and 4 to 7 days, respectively.) The blood electrolyte studies were made 1 or 2 days before the expected death of the animals. Because of the relatively large amount of blood needed (20 to 25 cc.), cardiac punctures were employed. The blood was collected under oil, allowed to clot, centrifuged, and the serum removed as soon as possible. Before withdrawal of the blood, the animal was anesthetized by the intraperitoneal injection of 0.5 cc. of 10 per cent sodium amytal solution per kilogram of body weight. This procedure was used in order to eliminate the effects of struggling. The hemoglobin determinations were made before the injection of the toxin, and every day thereafter. The blood was obtained from a freely flowing wound made by a small incision through a marginal ear vein.

The methods employed for the chemical determinations were the following: bicarbonate, manometric method of Van Slyke and Neill using 0.2 cc. (2); chloride, Patterson micromodification of the open Carius method (3); phosphate, Benedict and Theis (4); total base, Stadie and Ross modification of the Fiske method (5) without removal of phosphate; sodium, Barber and Kolthoff method (6); potassium, Shohl and Bennett method (7); nitrogen, Kjeldahl method, using 1 cc. (the factor 6.25 was used to obtain per cent protein); non-

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protein nitrogen, Folin-Wu method; amino-acid nitrogen, Folin method (8) (see Paper I for description of slight modification employed); hemoglobin, Cohen and Smith method (9). All determinations were run in duplicate except amino-acid nitrogen, nonprotein nitrogen, phosphate and hemoglobin.

The various concentrations are expressed as milliequivalents of univalent base per liter of serum. The following conversion factors were used: total protein per cent  $\times 2.48$ ; carbon dioxide, volumes per cent  $\times 0.423$  (bicarbonate at pH 7.38); phosphate, mgm. per cent  $\times 0.58$ . Where only sodium and potassium were determined, total base was calculated by assuming a calcium value of 5.5 m. Eq. and adding this value to the potassium and sodium. (Determinations of serum calcium carried out on a number of both normal and diphtheritic rabbits showed but slight variations from this value, and were subsequently discontinued because of the limited quantity of available serum.)

### RESULTS

The results of the electrolyte determinations are graphically summarized in Chart I. This has been prepared in the usual manner, the height of each column representing the concentrations of cations and anions respectively in milliequivalents per liter of serum, the total anion concentration being considered equal to the determined total base. The first double column in each group (marked AV) represents the average values for the entire group. Rabbit Number 2 was not included in estimating the average since the sodium and chloride are considerably lower than in the other rabbits of the control group as well as other similar analyses made in normal rabbits in this laboratory.

Significant differences between the normal and diphtheritic animals are found in the bicarbonate, chloride, sodium and phosphate concentrations. The changes are of a similar nature in both diphtheritic groups. In the group injected with the large dose of toxin (3 minimal lethal doses) there is an average decrease of 8.7 m. Eq. of bicarbonate, 12.6 m. Eq. of chloride and 12.2 m. Eq. of sodium, while the phosphate is increased by 4.2 m. Eq. (7.2 mgm. per cent P). In the group injected with the smaller dose (0.8 minimal lethal dose) the decreases average 7.3 m. Eq., 10.8 m. Eq. and 11.6 m. Eq. for the bicarbonate, chloride and sodium respectively, while the phosphate increase averages 5.4 m. Eq. (9 mgm. per cent P).

There are no significant changes in the total protein or potassium concentrations. The nonprotein nitrogen concentrations are markedly elevated in both diphtheritic groups. The amino-acid nitrogen, which is only moderately increased in the animals receiving the smaller dose of toxin, attains very high values in the groups injected with the larger dose. In making Chart I, the rabbits were arranged in order of increasing concentration of blood nonprotein nitrogen and amino-acid nitrogen. The chart shows that the phosphate concentration tends to increase with the nonprotein nitrogen and amino-acid nitrogen. Previous work demonstrated that the degree of intoxication could be fairly accurately predicted from the amino-acid and

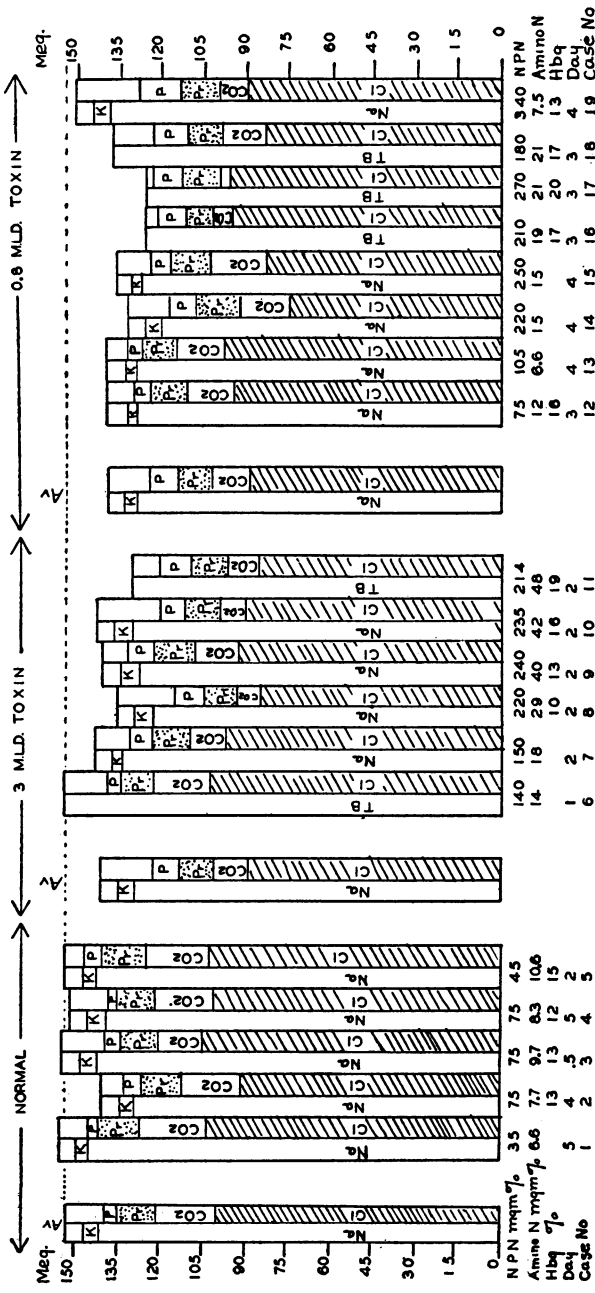


CHART I

THE CONCENTRATION OF ELECTROLYTES IN NORMAL RABBITS AND RABBITS INJECTED WITH DIPHTHERIA TOXIN

Height of columns represents concentration in m. Eq. per liter of serum. Columns marked Av. represent the average of the respective group. Pr. = protein in combining equivalents. T. B. = total base. All animals were starved during the course of the experiment. The day on which the blood sample was taken is given below; in the normal group this represents the period of starvation; in the diphtheritic groups this represents the period following the injection of toxin.

nonprotein nitrogen values. For this reason, it is felt that the elevation in inorganic phosphate parallels the degree of diphtheritic intoxication.

Table I gives the results of analyses of gastric contents. The total contents were recovered, weighed, and filtered through gauze. The acidity was titrated with phenolphthalein as indicator, and the total base and chloride were determined by the methods used in the analyses of serum. The re-

TABLE I  
*Chloride and total base of gastric contents in normal and diphtheritic rabbits*

Rabbit number	Toxin	Blood nonprotein nitrogen	Weight	Gastric contents			
				Concentration		Total amounts	
				Cl	Total base	Cl	Total base
	<i>minimal lethal dose</i>	<i>mgm. per cent</i>	<i>grams</i>	<i>m. Eq. per liter</i>	<i>m. Eq. per liter</i>	<i>m. Eq.</i>	<i>m. Eq.</i>
1	0		82	106	19	8.6	1.6
2	0		116	190	26	22.0	3.0
3	0		52	116	26	6.0	1.4
4	0		100	174	25	17.0	2.5
AVERAGE				146 ± 36	24 ± 2	13.4 ± 6.1	2.1 ± 0.6
5	0.8	340	78	180	49	14.0	3.8
6	0.8	180	103	133	32	14.0	3.3
7	0.8	75	56	143	36	8.0	2.0
8	0.8	210	42	152	31	6.3	1.3
9	0.8	270	65	147	42	9.6	2.7
AVERAGE				151 ± 12	38 ± 6	10.4 ± 3.5	2.6 ± 0.8
10	3.0	220	154	106	25	16.0	3.8
11	3.0	240	151	153	51	23.0	7.6
12	3.0	235	143	147	29	21.0	4.2
13	3.0	259	66	129	35	8.5	2.3
14	3.0	214	106	128	34	14.0	3.6
AVERAGE				133 ± 14	35 ± 7	15.9 ± 4.3	4.3 ± 1.3

sults are expressed both in terms of concentration and total amount. Since the dried weight, including the food present in the stomach, only constituted about ten per cent of the total weight, the results were calculated in terms of total weight of gastric contents. This did not introduce an error significant for our purposes. The table demonstrates in the three groups no significant difference in either concentration or total amount of electrolytes in the gastric juice.

In Chart II are graphically presented the daily hemoglobin values in two normal and four diphtheritic rabbits, two of which were injected with the larger dose and two, with the smaller dose of toxin. A total of 4 normal and 10 diphtheritic animals were studied in this manner. The results agree sufficiently so that only representative experiments need be cited. One may note a progressive hemoconcentration, as evidenced by the in-

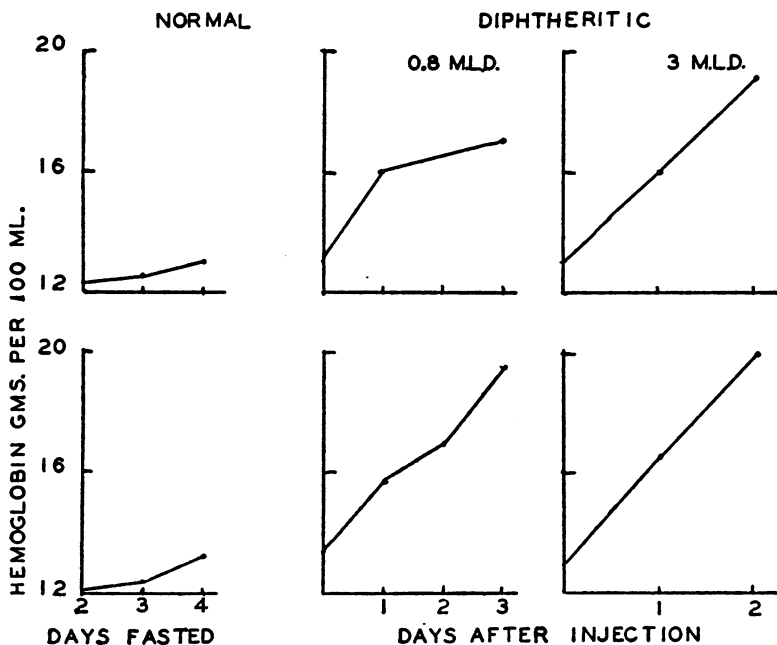


CHART II

THE CONCENTRATION OF HEMOGLOBIN IN NORMAL AND DIPHTHERITIC RABBITS

crease in hemoglobin values during the course of the intoxication. Unfortunately, accurate measurements of the fluid intake were not made. However, no apparent difference in this respect between the control and experimental animals was noted except that the fluid intake of the diphtheritic rabbits was low during the last 24 hours. Since the increase in concentration of hemoglobin is 30 to 45 per cent of the initial concentration, the lack of change in serum protein indicates a decrease in the total amount of circulating serum protein.

DISCUSSION

While the changes in concentration of serum electrolyte and hemoglobin found in rabbits following intravenous injection of diphtheria toxin cannot

be assumed to represent exactly the condition of the blood in diphtheria, analogous disturbances have been reported in patients. Martinson et al. (10) found reduction in whole blood chloride, whole blood total base and serum bicarbonate. While the electrolyte determinations on whole blood cannot be interpreted with certainty without knowledge of the cell volume, the results are suggestive.

Martinson et al. (10) and Wladimirowa (11) found organic acid in the blood of diphtheritic patients increased as compared to that of normal individuals. These results were obtained by titration with the application of a correction for creatin. While this method is subject to errors it probably represents approximately the change in organic acids. Csapó (12) found an increase in urinary organic acids.

Harding (13) and Hottinger (14) called attention to the high hemoglobin concentration and high erythrocyte count in malignant diphtheria.

Low concentration of inorganic phosphate was found in malignant diphtheria by Lesné, Zizine and Briskas (15). If this finding should be confirmed in severe cases with elevation of nonprotein nitrogen, it constitutes a definite difference from the reactions in rabbits injected with toxin. Feigl (16) found 16, 20 and 24 mgm. per cent of inorganic phosphorus in 3 cases of acute yellow atrophy of the liver. Since pathological and chemical evidences of liver injury are so marked in diphtheritic intoxication, elevation of phosphate may depend on hepatic injury. However, since high serum phosphate frequently occurs in renal disease accompanied by azotemia, the hyperphosphatemia in diphtheritic intoxication may be referable to the renal lesions of the disease.

The changes in rabbits are closely analogous to those found in shock, where diminution of blood volume is accompanied by decrease in the concentration of serum electrolytes. Low concentration of serum electrolytes is also characteristic of febrile states and a number of toxic conditions (17). The changes are apparently not principally dependent on an inadequate supply of salts but represent an unexplained disturbance in the usual concentration of the electrolytes. Since nephritis is frequently accompanied by low serum electrolytes, the renal lesions in the diphtheritic rabbits might produce similar disturbances. The studies of gastric contents demonstrate that the diminution of chloride and sodium is not brought about by an increase of these substances in the stomach, as might be anticipated from the work of Gamble and McIver (18) on pyloric obstruction in rabbits. Although some of the diphtheritic rabbits passed a few loose stools containing mucus, it was not felt that the diarrhea was sufficient to explain the changes in serum electrolyte. Retention of water was apparently not the cause of the reduction in concentration of electrolytes, since marked loss of weight occurred in all toxic animals. While the water intake was not measured, the animals seemed to drink freely except during the day before death.

Since the decreases in sodium and chloride were approximately equal, base deficit does not explain the low bicarbonate. Although increase of inorganic phosphate replaces part of the bicarbonate, the diminution of bicarbonate must be considered, by exclusion, as due chiefly to accumulation of organic acids and possibly sulfate. A few specimens of serum were distilled into Scott-Wilson (19) reagent but no ketone bodies were detected. Undoubtedly lactic acid accounts for part of the diminution of bicarbonate but the previous studies (1), while indicating a probable increase in lactic acid, were not convincing, because of the difficulty of obtaining from normal rabbits blood specimens which represented the resting state.

#### SUMMARY

The serum electrolyte and hemoglobin concentration of fasting rabbits after intravenous injection of diphtheria toxin is reported. The diphtheritic rabbits showed the following changes: (1) progressive increase in hemoglobin concentration; (2) approximately equal decrease of sodium and chloride; (3) decrease in bicarbonate; (4) marked increase in serum inorganic phosphate which paralleled the severity of the intoxication.

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