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Research Article

PHYSIOLOGICAL DISTURBANCES DURING EXPERIMENTAL DIPHTHERITIC INTOXICATION. I. BLOOD SUGAR, LACTIC ACID AND NON-PROTEIN AND AMINO-ACID NITROGEN[1]

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The therapy of diphtheria and control of the disease is largely based on outstanding achievements in the field of immunology, starting with von Behring's discovery of antitoxin, going on to Schick's method of measuring susceptibility to toxin and culminating in the development of means of producing active immunity by injection of a toxin-antitoxin mixture. Although the administration of antitoxin constitutes the chief mode of treating diphtheria, in a considerable number of severe cases this therapy alone is inadequate. It was believed, therefore, that additional study of the metabolic disturbances of diphtheritic intoxication was indicated and might supply data which would suggest additional lines of treatment. This and three subsequent papers (29, 30) will report studies in this field.

Disturbances in carbohydrate metabolism during experimental and clinical diphtheritic intoxication have been the subject of intensive investigation. In 1914, Rosenthal (1) demonstrated delayed sugar tolerance curves in rabbits following the injection of diphtheria toxin. This was confirmed by other investigators (2, 3, 4). With doses of toxin which lead to death in two to three days, Rosenthal and others (4, 5, 6) frequently found markedly reduced fasting blood sugar values, 12 to 24 hours before the death of the animals. Lawrence and Buckley (7), injecting smaller doses of diphtheria toxin (causing death in from 6 to 8 days), noted the occasional occurrence of a pre-lethal rise in the blood sugar. Schwentker and Noel (6) demonstrated a similar tendency towards hyperglycemia in the less severely intoxicated animals. They found a fairly close direct relationship between the period of survival and the elevation of the blood sugar. Impairment of the normal blood sugar depressing action of insulin was demonstrated in rabbits poisoned with diphtheria toxin by Lawrence and Buckley (7) and by Netzley (8). Sweeney (4), on the other hand, could find no diminution in the response to insulin, under similar conditions.

Investigations during the course of diphtheria in humans yielded results which are similar in many respects to those cited above. Hector (9), in 1926, found delayed sugar tolerance curves in 5 of 6 patients. These tests, when repeated during convalescence, were all normal. Similar findings were reported

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by Elkeles and Heimann (10), Prochàjka (11), Schwentker and Noel (6), Benn, Hughes and Alstead (12) and Brems (13). In occasional patients the fasting blood sugar values were found moderately elevated (9, 10, 13), in one patient reported by Elkeles and Heimann, as high as 400 mgm. per cent. The latter authors also reported extremely low blood sugar values in some of their patients. The injection of insulin prior to the administration of glucose for the sugar tolerance tests was found by Elkeles and Heimann to have no appreciable effect upon the resulting curve. Schwentker and Noel, on the other hand, found that the injection of insulin definitely altered the response in the direction of the normal sugar tolerance curve.

The occurrence of glycosuria during the clinical course of diphtheria has been noted. Hibbard and Morrissey (14) found reducing substances in the urine of 34 of 96 patients and in 8 of 9 fatal cases. The glycosuria in the nonfatal cases was transitory, disappearing during convalescence. Brems (13) also found spontaneous glycosuria in some of his patients. Elkeles and Heimann, on the other hand, failed to find urinary sugar in any of the cases, despite the presence of hyperglycemia.

The bearing of the above investigations upon the nature of the disturbance in carbohydrate metabolism during diphtheritic intoxication has been the subject of much speculation. The suggestions that have been offered fall principally into two groups, namely: (1) a disturbance in the adrenals or related glands of internal secretion; (2) a depression or dysfunction of the insulin mechanism. Rosenthal (1), Mikami (5) and Elkeles and Heimann (10) favored the former concept of adrenal damage, although Rosenthal felt that liver injury played an important but secondary rôle. Lawrence and Buckley (7), on the basis of histological evidence of injury to the thyroid and adrenals, implicated the latter glands as the responsible factors. Hector (9) considered that the basis for the disturbance lay in a derangement of the entire endocrine apparatus as well as moderate liver injury. The insulin mechanism was believed primarily responsible for the disturbances of carbohydrate metabolism by Sweeney (4), Sweeney and Lackey (3), Schwentker and Noel (6) and Benn, Hughes and Alstead (12). The latter two groups of investigators utilized insulin and glucose as an adjunct to the antitoxin treatment of the disease with apparently good results.

The literature on the nonprotein nitrogen constituents of the blood is less voluminous. In 1914, Karsner and Denis (15) found, following the injection of diphtheria toxin into cats, a slight increase of the nonprotein nitrogen during the first 2 days and then a marked rise up to 200 mgm. per cent 1 or 2 days before death (4 to 6 days). They attributed these results to the development of a nephritis, tubular in nature, during the early phase of the intoxication and vascular during the latter phases. Glesinger-Reischer and Glesinger (16) found the blood urea nitrogen greater than 30 mgm. per cent in 16 of 21 patients with diphtheria and over 50 mgm. per cent in seven. Following the administration of antitoxin, 20 to 50 per cent reductions in the urea values occurred in about half of the patients. Because of the essentially negative urinary findings they attributed the elevations in blood urea to the toxic destruction of body proteins. Prochàjka (11) found that the nonprotein nitrogen of the blood was a most valuable prognostic sign in diphtheria patients. From a study of 86 cases he cited the following statistics: (1) when the nonprotein nitrogen was less than 40 mgm. per cent, the mortality was 5 per cent; (2) when the nonprotein nitrogen was between 40 and 50 mgm. per cent, the mortality was 32 per cent; (3) when the nonprotein nitrogen was over 80 mgm. per cent, the mortality was 69 per cent.

In experimental animals and patients with diphtheria, Lereboullet, Gournay and Donato (17) found elevated blood urea but no disturbance in elimination of phenolsulphonephthalein nor marked histological changes in the kidneys. They emphasized the fact that the degree of azotemia was directly proportional to the severity of the diphtheritic intoxication and believed that the elevation in blood urea was brought about by a suprarenal disturbance. It is important to point out here that the phenolsulphonephthalein excretion test becomes difficult of interpretation in the presence of the severe liver damage that is usually found in diphtheritic intoxication. Hanner and Whipple (18) showed that liver injury produced by chloroform and phosphorus poisoning was accompanied by a definite increase in the renal excretion of the dye. A normal excretion during diphtheria, therefore, cannot be used as an indication of normal renal functions. Derot (19) demonstrated, in rabbits injected with diphtheria toxin, that the rise in blood nonprotein nitrogen was accompanied by a roughly parallel rise in blood creatinine.

No reports of amino-acid determinations in experimental diphtheritic poisoning or clinical diphtheria have been found in the literature.

PROCEDURE AND METHODS

Rabbits weighing from 1400 to 2000 grams were employed in this investigation as the experimental animals. The diet for approximately one week prior to the experimental period consisted of a liberal supply of greens and oats. All food was removed from the cages 24 hours before the injection of the diphtheria toxin, and none was given during the balance of the experimental period. Water was supplied in liberal quantity. The diphtheria toxin used was found to contain 62 minimal lethal doses per cubic centimeter. The toxin was diluted with sterile saline so that the calculated dose was contained in about 0.3 cc. of solution. This was injected intravenously.

One series of animals received approximately three quarters, and another series two to three minimal lethal doses. The smaller dose caused the death of the animals in from 4 to 7 days, the larger dose, in from 1 to 3 days. The animals were bled by cardiac puncture every day or every other day, depending on the severity of the intoxication. About 5 cubic centimeters of blood were drawn off and transferred to a bottle containing sufficient potassium oxalate to prevent clotting. Each bottle also contained a small quantity of powdered sodium fluoride to inhibit glycolysis. The Folin-Wu method of precipitation was used to obtain a protein-free filtrate.

For the blood sugar determinations the Shaffer, Hartmann and Somogyi (20) method was employed. Nonprotein nitrogen was determined colorimetrically by direct Nesslerization, following digestion of the protein-free filtrate with sulphuric acid and 30 per cent hydrogen peroxide. Lactate was determined by the method of Avery and Hastings (21).

Amino-acid nitrogen was determined by Folin's colorimetric method (22). It was found, using the quantities prescribed by Folin, namely, 5 cc. of the 1:10 filtrate and 1 cc. of the 0.5 per cent solution of sodium β-naphthoquinonesulfonate, that the largest concentrations of amino-acid nitrogen which could be determined with any degree of accuracy approximated from 6 to 10 mgm. per 100 cc. of blood. Schmidt (23) made this same observation in a paper published in 1929. It was found most practicable in cases where there was doubt as to the approximate concentration of the amino-acid nitrogen to set up a series of 3 tubes for each specimen of blood, containing 1, 3 and 5 cc. respectively of the filtrate, and to add 2 cc. of the reagent to each. The tube in which the color

that developed was most closely matched by the standard, was used for the colorimetric comparison. The error in the method is approximately 5 per cent in the lower values and 10 per cent when dealing with values in the vicinity of 40 to 60 mgm. per cent.

RESULTS

The results are summarized in Tables I and II, and representative experiments are graphically presented in Charts 1 and 2. It is evident that the data confirm the findings of previous investigators as to the changes that occur in the blood nonprotein nitrogen and sugar during the experimental intoxications. Briefly, the results can be described as follows:

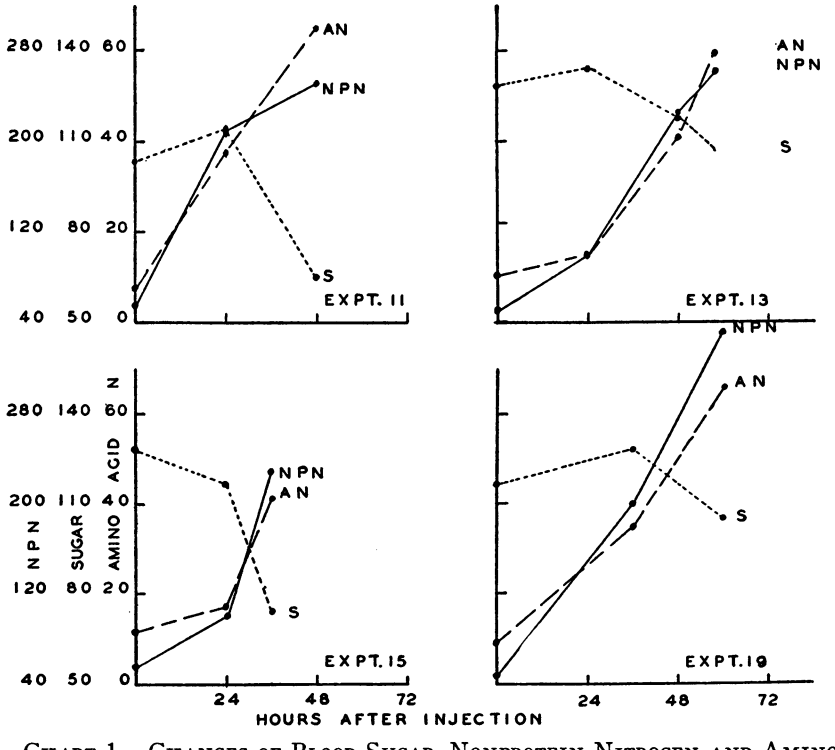

CHART 1. CHANGES OF BLOOD SUGAR, NONPROTEIN NITROGEN AND AMINO-ACID NITROGEN FOLLOWING THE INJECTION OF 3 MINIMAL LETHAL DOSES OF DIPHTHERIA TOXIN.

After a relatively large dose of toxin, i.e., one causing death in less than 3 days, there is a progressive increase in the nonprotein nitrogen which can be demonstrated as early as 24 hours after the injection; a definite tendency for the blood sugar to fall, in some cases, to relatively low levels; a rather abrupt rise in the amino-acid nitrogen (Chart 1). After a smaller dose of toxin, i.e., one causing death in from 4 to 7 days, the changes are slower in appearing, but just as definite (Chart 2). These are a gradual, but progressive rise in the nonprotein nitrogen; a tendency for the blood

sugar either to change little or to rise to definite hyperglycemic levels; and a slight to moderate rise in the amino-acid nitrogen early, with in some cases a tendency for the amino-acid nitrogen to decrease subsequently, or remain moderately elevated until the death of the animal.

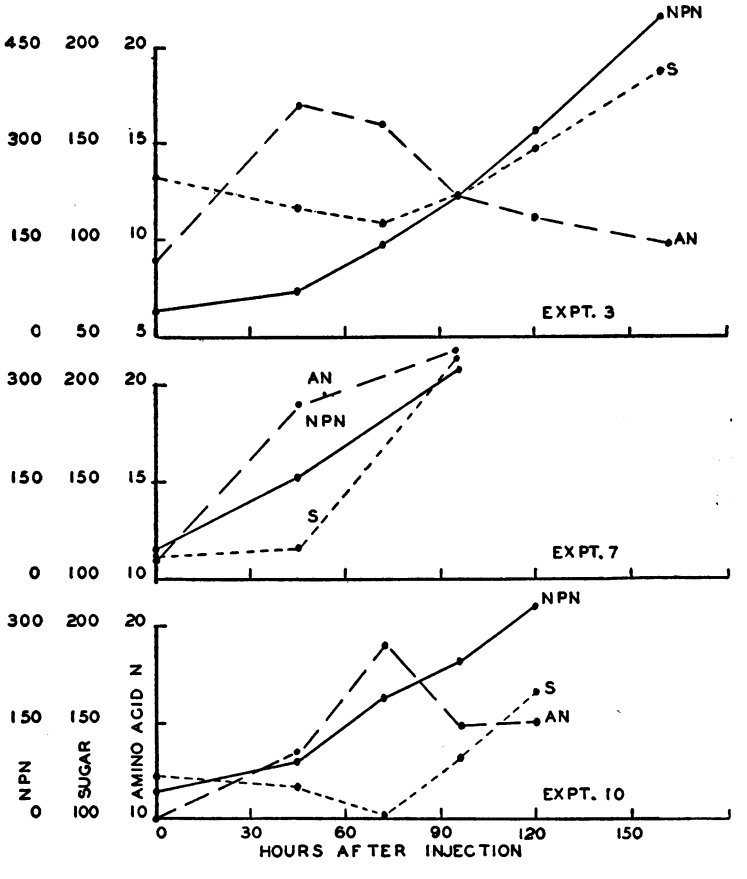

CHART 2. CHANGES OF BLOOD SUGAR, NONPROTEIN NITROGEN AND AMINO-ACID NITROGEN FOLLOWING THE INJECTION OF 0.8 MINIMAL LETHAL DOSE OF DIPHTHERIA TOXIN.

The nonprotein nitrogen rises at a regular rate with both doses of toxin. Although the nonprotein nitrogen increases more rapidly in the animals receiving the larger dose of toxin, it attains greater final concentration in the animals receiving the smaller dose of toxin. Apparently the final height of the nonprotein nitrogen is dependent chiefly on the duration of the intoxication.

The sugar and amino-acid nitrogen values present an interesting relationship. This can be more clearly observed in some of the individual experiments. With the larger dose of toxin, as the blood sugar fell, the

amino-acid nitrogen rose. For example, in Experiment 11 (see Chart 1), the blood sugar after 24 hours was 113 mgm. per cent and the amino-acid nitrogen 35 mgm. per cent. Coincident with the fall in sugar to 65, the amino-acid nitrogen rose to 55. Again, in Experiment 15, as the sugar fell from 117 to 73 the amino-acid nitrogen rose from 16.1 to 41.5. Similarly, in Experiment 13, the amino-acid nitrogen rose from 15 to 60 while the sugar fell from 135 to 108. In general it may be said that in the rabbits that die before the 3d day after having received relatively large doses of diphtheria toxin, the hypoglycemia is almost invariably accompanied by a marked amino-acidemia.

In the experiments in which the rabbits received smaller doses of toxin and survived from 4 to 7 days, the situation was somewhat different. The changes can best be pointed out in the experiments in which the animals survived the longest, namely, Experiments 3 and 10 (Chart 2). In these there is, at first, a very slight fall in the blood sugar values, coincident with a slight rise in the amino-acid nitrogen. Later, as the hyperglycemia develops, there is a tendency for the amino-acid to remain relatively stationary or to return to the pre-injection level. As has been pointed out, not all of the animals receiving the small dose of toxin develop hyperglycemia, nor do all show this relationship between the height of the blood sugar and the amino-acidemia as definitely as it has been described above. The significance of this will be discussed later.

PATHOLOGY

A number of histological sections were made of the various organs of the animals used in the chemical studies. Although these sections do not demonstrate any facts not already recognized, they permit correlation of the anatomical changes with the various stages of diphtheritic intoxication manifested in the chemical disturbances. Briefly, the histological changes in the animals receiving the larger dose of toxin were as follows: (1) Necrosis of the liver parenchyma involving in some cases the major part of the liver cells. (2) A tubular and glomerular nephritis which in extreme cases led to necrosis of large areas of the kidneys. (3) Degeneration of heart muscle fibers. (4) Hemorrhages in various organs including the adrenals and intestines. The last finding was not constant, but occurred in the animals most severely affected. The findings in the rabbits receiving the smaller doses were similar but less acute and extensive. The myocardial fibers were occasionally surrounded by round cells and some repair was apparently taking place. The liver in these, also, showed necrosis, which in some cases was limited to the area surrounding the central vein, with dilatation of the liver capillaries which gave a picture indistinguishable from that of chronic passive congestion. The kidney lesions were definite but did not seem to be necessarily irreparable. Lesions of the adrenal parenchyma, except in the presence of hemorrhage, could not be recognized with certainty after either dose. All the animals showed a

certain degree of dehydration as evidenced by diminished skin turgor, a relative increase in the concentration of hemoglobin (30) and loss of weight of 10 to 20 per cent in typical cases. All these findings have been described in human cases. We would particularly call attention to the liver and kidney lesions which have been overlooked most frequently.

DISCUSSION

The elevation of the nonprotein nitrogen can be most logically related to nephritis. This is confirmed also by the fact that the elevation of amino-acid nitrogen does not parallel that of the nonprotein nitrogen. Undoubtedly dehydration aggravated the renal insufficiency. Control experiments demonstrated that starvation alone may lead to an elevation of the nonprotein nitrogen, but this rarely exceeded 75 mgm. per cent.

Previous work indicates that liver injury is the chief cause of elevation of amino-acid nitrogen. Marshall and Rowntree (24) found elevated blood amino-acid nitrogen in the terminal stages of phosphorus and chloroform poisoning. Lewis and Izume (25) found a similar elevation in hydrazine poisoning in rabbits. Elevations of amino-acid nitrogen are also reported in acute yellow atrophy (Rabinowitch (26)), and in experimental yellow fever (Wakeman and Morrell (27)). Apparently the margin of safety in the deaminization function of the liver is so great that only extreme destruction of the liver leads to increase in the amino-acid nitrogen. Although an increase has been reported in rare cases of uremia, nephritis is not usually considered a cause of amino-acidemia. The degree to which the amino-acid nitrogen increased in the rabbits given diphtheria toxin is greater than that reported in severe hepatic disease in humans, except in a few instances (Rabinowitch). The data demonstrate marked disturbances of liver function corresponding to the extensive liver necrosis.

We have been unable to find reports of blood amino-acid nitrogen determinations in patients with diphtheria and have only had opportunity to make this determination ourselves in two cases of severe diphtheria. In neither was there an elevation of amino-acid nitrogen, although one patient developed definite myocardial degeneration and severe neuritis. Probably only a small number of the most severe cases will show an elevation of the amino-acid nitrogen.

With the recognition of the rôle of hepatic degeneration in severe diphtheria intoxication, the abnormality of carbohydrate metabolism receives an adequate explanation. With the greatest degrees of liver injury, the function of deaminization is diminished and presumably other related processes which serve to convert non-carbohydrate material to glucose fail. The loss of stored glycogen and the breakdown of the processes of glyconeogenesis lead to hypoglycemia. In Paper II of this series (29a) failure of the liver injured by diphtheria toxin to store glycogen is demonstrated. These findings are in agreement with the work of Corkill (28). This inability to store glycogen occurs in the animals receiving large and

small doses of toxin and can be demonstrated in animals at stages of the intoxication when no elevation of amino-acid can be demonstrated. With persistence of hepatic glyconeogenesis and impairment of hepatic glycogenesis, hyperglycemia develops. Since varying degrees of failure of these two hepatic functions may occur simultaneously, varying degrees of hypo- and hyperglycemia are found. As has been pointed out, hypoglycemia seems to be related to the rapidity of the increase of amino-acid nitrogen, and hyperglycemia to the period of survival when no increase in the amino-acid nitrogen occurs.

The relation of failure of hepatic glyconeogenesis to accumulation of blood lactic acid is presumably close. An attempt was made to study this by daily blood lactic acid determinations. The data are presented in Tables I and II. One hesitates to draw any conclusions from this material because

TABLE I

Changes in blood sugar, lactic acid and nonprotein and amino acid nitrogen following the intravenous injection of 3 minimal lethal doses of diphtheria toxin

Experiment number	Period following injection of toxin	Nonprotein nitrogen	Sugar	Amino-acid nitrogen	Lactic acid	Period of survival
	hours	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*m. Eq. per liter*	*hours*
		50	103	7.0	2.4	
	24	206	113	37.0	8.4	
11	48	250	65	65.0	12.7	50
		40	135	7.5	4.3	
12	24	170	77	37.0	14.8	28
		50	129	10.2	3.4	
	24	100	135	15.0	6.6	
	48	225	118	41.0	8.1	
13	54	265	108	60.0	9.0	59
		44	133	11.0	7.6	
14	24	90	72	27.5	9.7	34
		56	128	10.8	5.7	
	24	100	117	16.1	4.9	
15	36	220	73	41.5	5.8	40
		40	116	11.5	6.1	
16	24	92	63	32.0	7.2	27
		45	119	10.2	6.9	
17	24	100	82	27.8	10.3	32
		50	128	9.0	5.0	
18	24	133	101	29.5	7.2	36
		48	117	9.4	6.3	
	36	200	128	34.5	4.8	
19	60	350	106	66.0	12.0	63

TABLE II

Changes in blood sugar, lactic acid and nonprotein and amino-acid nitrogen following the intravenous injection of ¾ minimal lethal dose of diphtheria toxin

Experiment number	Period following injection of toxin	Nonprotein nitrogen	Sugar	Amino-acid nitrogen	Lactic acid	Period of survival
	hours	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*m. Eq. per liter*	*hours*
		40	123	10.3	9.7	
	48	148	122	15.0	6.2	
	96	320	150	14.6	5.8	
1	120	410	158	11.4	6.8	128
		43	136	9.5	10.7	
	48	80	112	13.2	6.2	
	96	125	137	14.0	8.2	
	120	210	218	14.9	7.8	
	144	260	196	12.5	5.0	
2	172	380		12.8	5.0	176
		40	133	9.4	9.3	
	48	71	116	17.3	8.2	
	72	144	109	16.6	5.6	
	96	215	122	12.3	4.8	
	120	320	149	11.6	5.6	
3	168	500	187	10.0	7.7	184
		44	105	8.8	7.1	
	48	91	123	15.8	6.3	
	72	240	129	14.0	7.5	
4	96	450	109	16.9	6.5	97
		42	127	9.7	8.6	
	48	100	118	14.4	7.3	
5	72	380	160	16.0	5.0	90
		33	119	8.8	9.2	
	48	105	118	15.4	8.7	
6	96	350	122	21.0	12.7	104
		45	111	11.0	4.8	
	48	154	114	19.5	10.5	
7	96	330	215	22.0	9.2	104
		44	131	9.8	9.5	
	48	85	120	13.9	8.2	
8	72	230	132	34.0		82
		48	140	9.5	6.0	
	48	73	116	13.7	6.4	
	72	185	134	27.0		
9	96	300	237	32.0		98
		40	122	9.9	5.6	
	48	83	116	13.4	12.5	
	72	190	100	19.0		
	96	250	132	14.8		
10	120	340	165	15.2		128

of the wide variation in blood lactic acid levels in the normal animals. The pre-injection levels were so frequently markedly elevated, due to struggling during the removal of blood samples from the hearts, that we regard lactic acid determinations in rabbits as unreliable indices of the state of blood lactic acid under resting conditions. In general, one may say that with the less severe intoxication there is less tendency for the lactic acid to reach the markedly elevated levels that are sometimes encountered in the severely intoxicated rabbits.

A similar unreliability of the blood sugar values might be suspected. However, essentially the same concentrations as those reported in this paper were found in blood taken from the ear when struggling was minimal. This fact, together with uniformity of the trend of the results, leads us to consider the blood sugar values reliable.

SUMMARY

1. The blood sugar, amino-acid nitrogen, nonprotein nitrogen, and lactic acid were studied in fasting rabbits which had intravenous injections of small and large doses of diphtheria toxin.

2. During the severe intoxication, hypoglycemia, marked amino-acidemia and azotemia were found.

3. A tendency was demonstrated for the hypoglycemia to occur while the amino-acid nitrogen was rapidly increasing.

4. During the mild intoxication hyperglycemia could be demonstrated in most of the animals that survived for 5 or more days. Under these circumstances the amino-acid nitrogen was only slightly elevated. Nonprotein nitrogen became markedly elevated.

5. It is pointed out that the azotemia is probably related to nephritis produced by the toxin and perhaps aggravated by dehydration. The amino-acidemia is apparently brought about by extensive liver necrosis. The varying degrees of hypo- and hyperglycemia probably are related to varying degrees of failure of hepatic glyconeogenesis and glycogenesis.

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