IMMUNOLOGIC SIGNIFICANCE OF VITAMINS

V. RESISTANCE OF THE AVITAMINIC ALBINO RAT TO DIPHTHERIA TOXIN; PRODUCTION OF ANTITOXIN AND BLOOD PRESSURE EFFECTS

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No adequate explanation has been offered to account for the greater susceptibility of avitaminic animals to bacterial infection although the rupture in resistance is definite and determinable. Among the explanations that have been offered are: (1) the presence of "degenerative changes and feeble leukoblastic reaction seen in the bone marrow of chronic scurvy guinea-pigs;[1] (2) inability to produce antibodies (agglutinins);[2] (3) reduced bactericidal activity of the blood serum;[3] (4) lowered body temperature and comcomitant effects, i. e., depressed phagocytosis,[4] better growth conditions for the micro-organism.[5] From the work done, it is apparent that several factors are of undoubted importance in accounting for the failure of animals deficient in vitamin to resist infection, as well as normal healthy individuals, and that resistance is not wholly dependent on any one factor.

The present paper is a study of the effect of diphtheria toxin on avitaminic albino rats; their resistance to toxin injections, their ability to react by the production of antitoxin, and the effect of the toxin on the blood pressure of avitaminic B rats and normal controls.

Although a vitamin deficiency in an animal may lead to infection, it is quite conceivable that the effect may be the result of superimposing the injurious effects of a small relatively unimportant infection on the injurious vitamin deficiency effects, resulting in a depressed metabolism that prevents the animal from exerting a normal effort to overcome the infection or toxemia. This we shall see may be particularly true for toxin effects.

Toxin experimentally injected into B-minus and normal control rats may exert quantitatively the same effect, but in the case of the

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1 Findlay: Jour. Path. & Bacteriol., 1923, 26, p. 1.
2 Guerrini: Ann. d'Ig., 1921, 31, p. 596.
3 Smith and Wason: Jour. Immunol., 1923, 8, p. 195.
4 Werkman: Jour. Infect. Dis., 1923, 32, p. 263.
5 Werkman: Ibid., 1924, 34, p. 447.

animals deficient in vitamin the effect may be superimposed on that already produced as the result of vitamin deficiency. Such a summation of depressions of physiologic activities would then result in a seriously reduced metabolism. Either injury acting alone would not prove fatal, assuming the degree or intensity of the injury not to increase. Of course, both "injury loads" may be increasing and either alone given time produce death. It is readily possible to produce in animals a vitamin deficiency which when added to a definite toxin effect or "load" results in death although the same "toxin load" is well borne by normal healthy animals. The "toxin load" is a function of toxin intensity (dosage) and time; similarly, with "vitamin deficiency load."

In case one or both "loads" is progressing to a fatal termination and both are present, the critical minimum in the depressed metabolism is more quickly reached, and death results earlier. This condition will be exemplified in the work on blood pressure of normal and avitaminic rats injected with diphtheria toxin.

EXPERIMENTS

Month old albino rats were used in these experiments. The basal ration consisted of casein (alcohol extracted) 18%, dextrin 74%, McCollum's salt mixture 3%. Yeast (3%) or filtered butterfat (3%) supplied vitamin B or A as required by the experiment.

The diphtheria toxin used in this work possessed an MLD of 0.0027 c c. and an L+ of 0.198 c c. At the time of use, it had been aged for 2 years. The toxin was furnished by Dr. W. H. Park of the New York Department of Health.

Resistance of the Avitaminic Albino Rat to Injection of Diphtheria Toxin.—Coca, Russel, and Baughman,[6] working with mature rats weighing almost 300 gm., found that 4,000 MLD regularly proved fatal, although 1,000 MLD were usually successfully resisted. Since young rats were employed in the present studies, the minimal lethal dose was less than that found by the previous investigators when considered from the standpoint of units of toxin required to kill, although comparable when considered as units of toxin per gram of rat.

Table 1 summarizes the resistance of normal and avitaminic albino rats to diphtheria toxin. These results were substantiated by several similar experiments using diphtheria and botulinus toxins. Difficulty in maintaining a fixed toxicity of the botulinus toxin led to less consistent results than were obtained with the diphtheria toxin. The results in table 1 were compiled from two similar experiments, which accounts for the two distinct weights of the animals. In general, the greater the degree of vitamin deficiency, the greater the susceptibility to infection or toxemia. This may be seen in the case of animals 29 and 30. Both rats received the same B-deficient ration, but, due to the maturity of the heavier animal, it did not suffer the effects of the vitamin deficiency to the extent that the younger, more actively growing animal did, and therefore successfully resisted an injection of toxin not withstood by the younger rat in urgent need of the vitamin.

[6] Jour. Immunol., 1921, 6, p. 387.

Ordinarily rats will not tolerate injections of toxin directly proportional to their body weights. With increase in weight, the toxin units per gram of rat required to kill decreases somewhat. However, in the case of the heavier rat mentioned above, its survival is due, in all probability, to the fact that it was in relatively good condition. This is indicated by its rectal temperature.

TABLE 1

RESISTANCE OF THE AVITAMINIC ALBINO RAT TO DIPHTHERIA TOXIN

Rat No.	Treatment	Weight in Gm.	Rectal Temperature, F.	Dosage per 10 Gm. of Rat, C c.	Dose, C c.	Toxin Units per 10 Gm. of Rat	Results
1		110	101.6	0.050	0.55	18.5	Lived
2		85	102.1	0.050	0.43	18.7	Lived
3		80	101.7	0.075	0.60	27.8	Lived
4		80	101.5	0.100	0.80	29.6	Lived
5		50	99.9	0.125	0.63	46.6	Lived
6		135	102.1	0.150	2.04	55.7	Died after 135 hours
7	Control	85	100.4	0.175	1.49	64.9	Lived
8		75	100.8	0.175	1.31	64.7	Lived, paralysis
9		80	101.9	0.175	1.40	64.9	Lived, paralysis
10		80	101.4	0.200	1.60	74.1	Died after 120 hours
11		70	102.3	0.230	1.61	84.7	Died after 72 hours
12		130	101.4	0.300	3.90	111.1	Died after 45 hours
13		110	100.8	0.400	4.40	148.1	Died after 30 hours
14		120	102.8	0.500	6.00	185.2	Died after 30 hours
15		80	101.1	0.050	0.40	18.5	Lived
16		65	101.4	0.100	0.65	29.6	Died after 105 hours
17		55	102.1	0.125	0.69	46.3	Died after 54 hours
18		90	100.7	0.150	1.35	55.5	Died after 56 hours
19		80	99.3	0.175	1.40	64.8	Died after 48 hours
20	A minus	65	99.7	0.175	1.14	64.8	Died after 50 hours
21		60	97.0	0.200	1.20	74.1	Died after 24 hours
22		80	100.0	0.230	1.84	85.2	Died after 38 hours
23		140	100.2	0.300	4.20	111.1	Died after 33 hours
24		105	99.0	0.400	4.20	148.1	Died after 30 hours
25		120	100.0	0.500	6.00	185.2	Died after 32 hours
26		110	99.4	0.050	0.55	18.5	Lived
27		90	94.0	0.050	0.45	18.5	Lived
28		120	99.6	0.073	0.88	27.3	Died after 96 hours
29		65	93.7	0.100	0.65	29.6	Died after 74 hours
30		110	100.8	0.100	1.10	29.6	Lived, paralysis
31	B minus	45	99.6	0.125	0.56	46.1	Died after 48 hours
32		55	97.0	0.150	0.83	55.8	Died after 45 hours
33		130	100.3	0.150	1.95	55.5	Died after 64 hours
34		90	100.7	0.175	1.58	65.0	Died after 36 hours
35		60	94.3	0.175	1.05	64.8	Died after 30 hours
36		40	99.5	0.200	0.80	74.1	Died after 30 hours

MLD (250 gm. guinea-pig) = 0.0027 c c.

The A-deficient rats do not show the even progressive drop in resistance manifested by the B-deficient rats. Their break in resistance is more sudden, corresponding to the suddenness with which symptoms of A-deficiency often show up.

Calculated on the basis of units of toxin per 10 gm. of rat necessary to produce the death of the animal, there were required approximately 30 units for the A-minus; a similar number (28) of units for the B-minus, whereas the normal rats required from 56 to 75 units.

These results might lead one to believe that the result is due to an actual rupture in the immunity mechanism of the rat. It is readily possible that

the cells of avitaminic rats absorb more toxin than the cells of normal animals. The cells of the normal rat are quite impermeable or do not attach to diphtheria toxin, and the poison continues to float in the blood stream. If cells of avitaminic rats were permeable to the toxin or the toxin attached itself more readily to the cells of avitaminic animals, the toxin would be removed from circulation. This point was determined, however, and the toxin was not found to be removed from circulation, testing the content of toxin in the serums on guinea-pigs. The permeability of the cells for the toxin was not determinably increased, since the toxin was found floating unattached for hours in the serum after injection. The avitaminic rats are not, then, more susceptible to diphtheria toxin because of any increased absorption. The

TABLE 2

EFFECT OF THE LACK OF VITAMINS ON THE PRODUCTION OF DIPHTHERIA ANTITOXIN BY THE ALBINO RAT

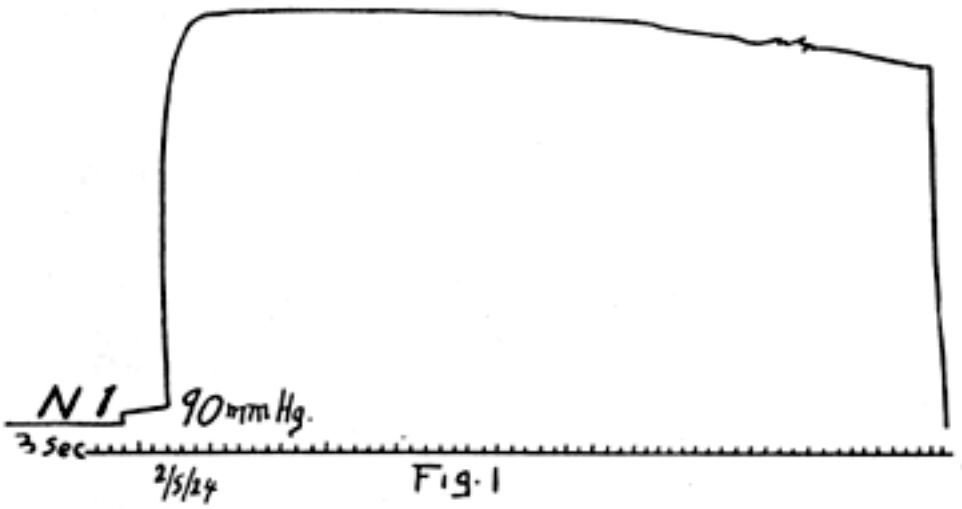
Guinea-Pig No.	Weight, Gm.	Antitoxin (in 2 c c.) C c.	Treatment of Antitoxin Rats	Toxin (L + in 1 c c.)	Results	Antitoxin per C c. Units
9	240	0.8	Control (Mixed Serums from 4 Rats)	0.198	Died after 44 hours	0.80
11	230	0.9		0.198	Died after 40 hours	
13	255	1.0		0.198	Died after 46 hours	
14	255	1.05		0.198	Died after 55 hours	
15	245	1.1		0.198	Died after 64 hours	
16	250	1.15		0.198	Died after 3 days	
17	255	1.2		0.198	Died after 3 days	
18	250	1.25		0.198	Died after 101 hours	
19	245	1.3		0.198	Died after 107 hours	
20	255	1.4		0.198	Lived	
33	260	0.9	A-minus (Mixed Serums from 4 Rats)	0.198	Died after 54 hours	0.77
35	270	1.0		0.198	Died after 54 hours	
36	265	1.1		0.198	Died after 55 hours	
37	255	1.15		0.198	Died after 60 hours	
38	245	1.2		0.198	Died after 73 hours	
39	240	1.25		0.198	Died after 77 hours	
40	250	1.3		0.198	Died after 93 hours	
41	250	1.35		0.198	Lived	
45	235	1.0	B-minus (Mixed Serums from 4 Rats)	0.198	Died after 55 hours	0.83
47	250	1.15		0.198	Died after 86 hours	
48	245	1.20		0.198	Died after 103 hours	
49	260	1.25		0.198	Died after 6 days	
50	255	1.3		0.198	Lived	
51	245	1.35		0.198	Lived	

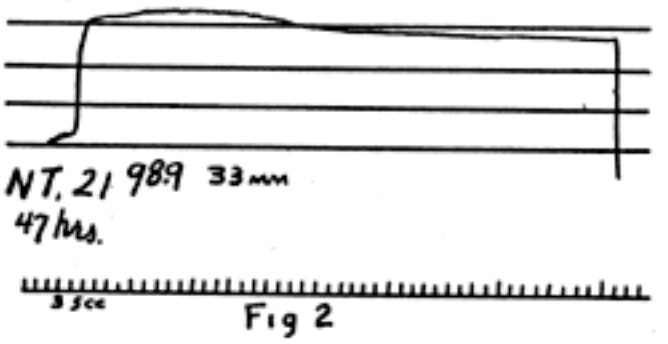
quantitative determination of the toxin content established no differences in the amounts absorbed by avitaminic and normal control rats. This will be referred to again when the effect of vitamin deficiency and diphtheria toxin injections on blood pressure of the albino rat is considered.

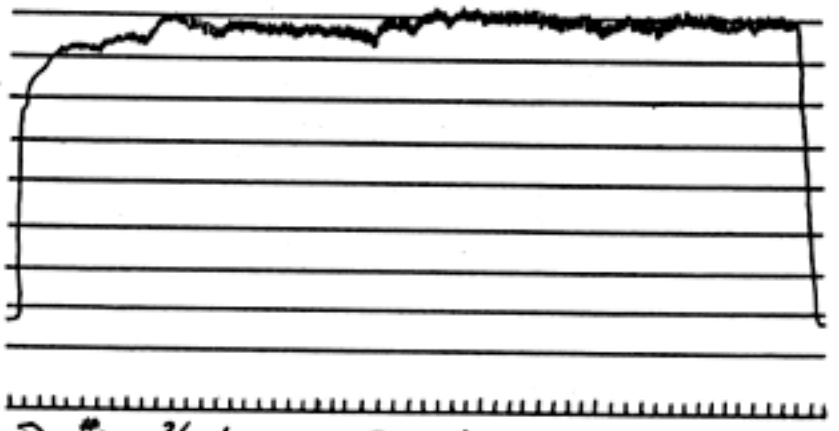
Effect of Vitamin Deficiency on the Production of Diphtheria Antitoxin by the Albino Rat.—The production of diphtheria antitoxin in the albino rat occurs in relatively small amounts. Coca, Russel, and Baughman[6] have shown the production of approximately 0.4 unit per c c. to result after 4 injections of toxin. The effect of vitamin deficiencies shown in table 2, was determined in 3 series of 4 rats each, including A and B minus and normal animals. Each rat received 5 three day intraperitoneal injections of 0.2, 0.4, 0.6, 0.8 and 1.0 c c. of toxin, and it was bled by heart puncture on the 9th day after the last injection. The antitoxin content of the serums was determined on guinea-

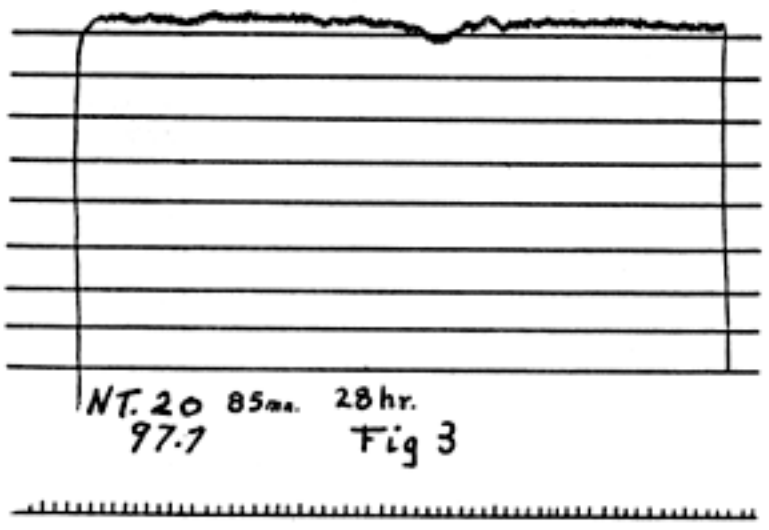
pigs after heating for 30 min. at 56 C. Toxin-antitoxin mixtures were allowed to stand for 1 hour at room temperature before injections were made. Table 2 shows no significant variations in the antitoxin productions by avitaminic and normal rats. Confirmatory results were obtained by injecting arbitrarily a definite quantity of antitoxin from avitaminic and normal rats into a series of rats of equal weight, determining the quantity of toxin required to kill in 6 days. The amounts necessary did not vary more than 0.2 c c. for the avitaminic and normal rats when as much as 7 c c. were required to kill in 6 days.

Effect of Diphtheria Toxin and the Lack of Vitamin B on the Blood Pressure of Albino Rats.—Although the normal albino rat is resistant to









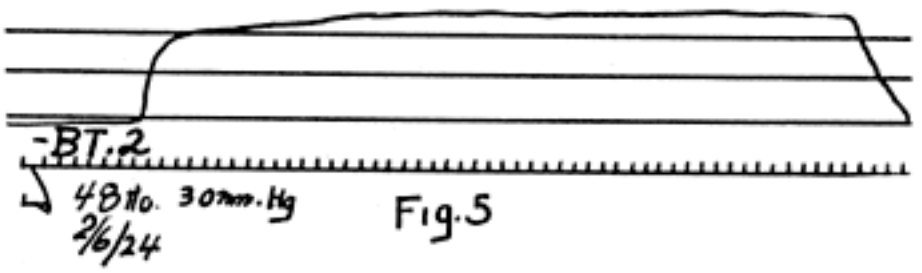


PLATE 1

Fig. 1.—Blood pressure of a normal rat, 90 mm. Hg.

Fig. 2.—Blood pressure of a normal rat 47 hours after lethal injection of diphtheria toxin, 33 mm. Hg.

Fig. 3.—Blood pressure of a normal rat 28 hours after lethal injection of diphtheria toxin, 85 mm. Hg.

Fig. 4.—Blood pressure of a rat lacking vitamin B six weeks on ration, 70 mm. Hg.

Fig. 5.—Blood pressure of a rat lacking vitamin B, six weeks on ration, 48 hours after lethal injection of diphtheria toxin, 30 mm. Hg.

enormous injections of diphtheria toxin, that the effect on the blood pressure is similar to that in man when injected in quantities sufficient to cause injury in the animal is shown by the kymograph records in figures 1 and 2 and by table 3. Fig. 1 shows normal pressure obtained from a 75 gm. rat by the

direct mercurial manometer method.[7] Fig. 2 indicates the pressure in a 75 gm. rat 47 hrs. after the injection of a minimal lethal dose of diphtheria toxin for the rat. The rectal temperature of this rat was 98.9 F. Death would probably have resulted on about the 6th day. The drop in blood pressure following diphtheria toxin injection usually occurs on the second or third day after injection. The drop sometimes occurs on the 1st day, but may be delayed until the 4th day. It is often marked and occurs suddenly. Delay in the drop of blood pressure in diphtheria in man often occurs.[8] Antitoxin administered in time inhibits the fall and prevents death. Meyers and Wallace[9] have shown that the arterioles and capillaries in the splanchnic domain fail to react

TABLE 3

EFFECT OF DIPHTHERIA TOXIN ON THE BLOOD PRESSURE OF AVITAMINIC ALBINO RATS

Rat No.	Weight, Gm.	Treatment	Rectal Temperature (F.)			Blood Pressure in Mm. Hg		
			Before Injection	24-35 Hours after Injection	35-50 Hours after Injection	Before Injection	24-30 Hours after Injection	30-50 Hours after Injection
1	75	Normal Control	102.0	.....	....	90	..	..
2	80		101.6	.....	....	100	..	..
3	80		102.2	.....	....	95	..	..
4	70		101.9	.....	....	93	..	..
5	90		102.3	.....	....	87	..	..
6	55	B-minus	97.3	.....	....	60	..	..
7	60		99.7	.....	....	70	..	..
8	65		93.8	.....	....	37	..	..
9	70		94.6	.....	....	48	..	..
10	75	Normal + toxin	100.7	103.1	98.8	..	..	77
11	75		101.0	102.4	98.9	..	..	33
12	75		100.2	.....	96.2	..	..	85
13	70		101.8	102.9	97.7	..	..	47
14	75		102.6	103.3	....	..	95	..
15	70		101.5	98.9	....	..	85	..
16	60	B-minus + toxin	97.0	102.7	90.0	..	..	20
17	50		97.3	101.0	95.9	..	..	60
18	70		98.7	100.3	97.3	..	..	55
19	55		100.1	.....	98.3	..	..	15
20	60		98.5	99.7	....	..	60	..
21	55		99.8	100.4	....	..	70	..

Toxin = calculated MLD for the animal in c c.

normally to epinephrin and are engorged. Peripheral splanchnic paralysis may therefore account for the hypotension.. The fall in pressure is generally proportional to the severity of the toxemia; it does not, however, bear any definite relationship to the intensity of the fever.

In Fig. 2, the animal showed a pressure of approximately 33 mm. Hg 47 hrs. after toxin was injected, and a rectal temperature of 98.9 F., whereas the pressure of one of its mates, shown in Fig. 3, 28 hrs. after injection showed a pressure of 85 mm. Hg and a rectal temperature of only 97.7 F.

The injection of toxin into the albino rat is followed by fever during the first day; the fever disappears and is followed by subnormal temperatures.

[7] Baldwin, Cook and Nelson: Am. Jour. Physiol., 1924, 68, p. 379.
[8] Beck and Slapa: Wien. klin. Wchnschr., 1895, 18, p. 323.
[9] Proc. Soc. Exper. Biol. and Med., 1914, 12, p. 43.

Subnormal temperature and low blood pressure may exist for several days before death intervenes.

Baldwin, Cook, and Nelson[7] have reported subnormal pressures in B-minus rats. Fig. 4 indicates the blood pressure in a 50 gm. rat on a B-deficient ration for 6 weeks, showing an average pressure of about 70 mm. Hg. The drop due to B-deficiency is progressive and ranges from normal down to 15 mm. Hg before death.

The blood pressure of a B-deficient rat 48 hours after the injection of an MLD of toxin for the rat, is shown in fig. 5. The temperature of this rat was 96.4 F. A summary of results on blood pressures is given for a few typical animals in table 3. The drop in resistance of avitaminic rats to diphtheria toxin is the superimposing of deleterious effects rather than the break in the immunity mechanism; the effect of toxin injections, alike in normal and avitaminic animals, is, in the case of avitaminic animals, superimposed on that of vitamin deficiency.

The serious effects of vitamin deficiency on the myocardium have been pointed out by Baldwin, Cook, and Nelson;[7] the injection of diphtheria toxin adds the myocardial lesions of diphtheria. Warthin,[10] in a recent study of the myocardial lesions of diphtheria, has concluded that "the essential lesion of the heart in diphtheria is a toxic parenchymatous hyaline degeneration or necrosis, associated frequently with fatty degenerative infiltration and less frequently with cloudy swelling or a simple necrosis," and that the histologic picture will depend among other factors on "the associated nutritional conditions."

SUMMARY

Rats suffering from the lack of vitamins A or B succumb to the effects of smaller injections of diphtheria toxin than do normal animals on a complete diet. The susceptibility of the avitaminic animal is not due to any rupture of the immunity mechanism that normally serves to prevent an injurious action by the diphtheria toxin. The ability of the animals to produce antitoxin is not disturbed, and a small production of between 0.5 and 1.0 unit per c c. occurs after about 5 sublethal injections of toxin. The absorption of diphtheria toxin by the cells of B-minus rats is not greater than occurs in normal animals, and the toxin is found floating unattached for hours in the serum after injection.

The injection of diphtheria toxin into the the rat produces a marked and sudden drop in blood pressure, usually occurring on the second or third day after the injection is made. If a fatal dose of toxin has been injected, the drop is progressive to the point of death. The rat tolerates markedly low blood pressures and body temperatures several days before death intervenes.

[10] Jour. Infect. Dis., 1924, 33, p. 32.

In agreement with the results of Baldwin, Cook and Nelson, the blood pressure of the rat suffers a progressive drop during vitamin B-deficiency proportional to the severity of the deficiency. The injection of diphtheria toxin into B-minus rats serves to hasten death, or results in a fatal termination when the additive effects of vitamin deficiency and toxin are sufficient to produce a depression of physiologic activities below a critical minimum.