8 (2240)

Non specific immunity to diphtheria and tetanus toxins not induced.

By WILLIAM N. BERG.

[*From the Berg Biological Laboratory, Brooklyn, N. Y.*]

Guinea pigs were injected subcutaneously with a number of antigens with the expectation that non specific as well as specific immunity would be induced. The non specific immunity was looked for by testing the guinea pigs' resistance to diphtheria and tetanus toxins and to several pathogenic organisms.

Suspensions of pure proteins (casein, edestin) and dead organisms *(B. coli, B. pyocyaneus)* were the antigens used. They were prepared as follows: a 1.000 gm. portion of pure dry protein was suspended in 20 cc. of 0.5 per cent acid sodium phosphate solution. The suspensions contained in sterile weighing bottle were incubated, tested for sterility, and when found free from contaminating organisms were diluted with 0.8 per cent sodium chloride. The calculated volume of protein suspension was then injected. Acid sodium phosphate solution was used for suspending the antigens in order to avoid disintegration or autolysis of protein with attendant formation of toxic products as suggested by Thomson.[1]

Cultures of *B. coli and B. pyocyaneus* were grown 18 to 24 hours on 1 per cent dextrose 3 per cent agar slants without peptone. Peptone was omitted because Hitchens[2] found that organisms grown on peptone agar are much more toxic than those grown on peptone free media. The growths were suspended in a solution containing 0.5 per cent phenol, 0.5 per cent chloroform and 0.5 per cent acid sodium phosphate. No heat was used to render bacterial suspensions sterile. These were diluted with 0.8 per cent sodium chloride solution just before subcutaneous injection. The weight of dry organisms injected was determined by drying a portion to constant weight, and making the necessary corrections.

---

1 Thomson, D., *Lancet*, 1919, June 28, p. 1102.

2 Hitchens, A. P., *Interstate Med. J.*, 1914, xxi, 537.

No attempt was made at bacterial counts. An approximate idea of the number of organisms injected was obtained from the data of Wilson and Dickson.[3] According to these investigators one milligram of dry *B. coli* contains 6.5 billions; one milligram of dry *B. pyocyaneus,* 3.5 billions of organisms.

The diphtheria and tetanus toxins and antitoxins used were the standard preparations furnished by the Hygienic Laboratory, Washington, D. C. One minimal lethal dose of Diphtheria Toxin No. 266 for 500-600 gm. guinea pigs was 0.015 cc. injected subcutaneously. One minimal lethal dose of Tetanus Toxin No. F 3 for 350 gm. guinea pigs was 0.0075 mg. subcutaneously.

Guinea pigs received as many as three injections of the same antigen. The doses were as large as was consistent with safety. That it was safe was judged by the weights of the experimental animals throughout the tests. A week or more after the last injection of antigen, the resistance of the guinea pigs to diphtheria and tetanus toxins was tested by injection of one to three minimal lethal doses. In none of the animals was the slightest non specific resistance detected—all died in the same time as the controls. Thus Guinea Pig No. 53 received three injections of dead *B. coli.* These organisms were chloroform killed, but not otherwise altered nor dried. The organisms in the doses injected weighed, 5, 10 and 10 mg. when dried. This did not protect the animal against a little more than one minimal lethal dose of tetanus toxin. (See Tables I and II.)

TABLE I.

NON RESISTANCE OF IMMUNE GUINEA PIGS TO TETANUS TOXIN.

Guinea Pig No.	Initial weight	Final weight	Number of minimal lethal doses of toxin injected	Time of death
	gm.	gm.	April 7	hours
8	—	585	control 2	88
3	—	645	" 3	64
5	—	540	" 2	88
4	—	640	" 3	88
			April 19 May 24	76
			GLYCININ mg.	
19	590	545	25 3	
22	570	555	25 3	67

[3] Wilson, W. J., and Dickson, Ch., *J. Hyg.*, 1912, xii, 49.

			April 21	April 28	May 4	May 24	
			CASEIN mg.				
30	530	540	5	7.5	10	3	67
36	560	575	5	7.5	10	3	92
			May 2	May 7	May 14	May 24	
			EDESTIN mg.				
41	570	575	10	15	15	3	67
47	520	550	10	15	15	3	69
			May 6	May 12	May 26	June 13	
			B. COLI dead mg.				
53	520	595	5	10	10	3	65
61	630	620	5	10	10	3	65
			May 17	May 25	June 6	June 13	
			B. PYOCYANEUS dead mg.				
65	600	605	5	5	10	3	65
70	600	605	5	5	10	3	73
86	—	545	control			2	65
87	—	635	"			3	65

TABLE II.

NON RESISTANCE OF IMMUNE GUINEA PIGS TO DIPHTHERIA TOXIN.

Guinea Pig. No.	Initial weight	Final weight	Number of minimal lethal doses of toxin injected				Time of death
	gm.	gm.				April 7	hours
2	—	565	control			1	64
7	—	580	"			1.3	64
6	—	565	"			1	64
1	—	515	"			1.3	44
			April 19			May 10	
			GLYCININ mg.				
10	505	510	25			1	46
13	690	645	25			1	66
15	500	495	25			1	50
			April 21	April 28	May 4	May 10	
			CASEIN mg.				
27	605	605	5	7.5	10	1	75
28	540	535	5	7.5	10	1	66
29	595	545	5	7.5	10	1	66
63	—	465	control			1	66
64	—	515	"			1	92
65x	—	550	"			2	66
			May 6	May 12	May 26	June 13	
			B. COLI dead mg.				
55	640	610	5	10	10	1	73
62	620	595	5	10	10	1	116
			May 17	May 25	June 6	June 13	
			B. PYOCYANEUS dead mg.				
69	670	675	5	5	10	1	70
74	595	605	5	5	10	1	90
88	—	675	control			0.7	116
89	—	620	"			1	90

The initial weight is weight immediately before first injection of antigen. Final weight is weight immediately before toxin injection. Weights were carefully watched to make certain that resistance to toxin was measured only in apparently normal healthy animals.

The above results are similar to those obtained by Cowie and Kempton[4] and by Kolle and Schlossberger.[5] They found that injecting guinea pigs with typhoid organisms or horse serum did not protect against small doses of diphtheria toxin, (3 mld.). That three guinea pigs receiving 100 times the fatal dose of diphtheria toxin were saved with normal horse serum as reported by Kastenmeyer,[6] is extremely doubtful.

Data on the non specific resistance of guinea pigs to several organisms are omitted. The difficulty of accurately determining the minimal lethal dose of a live culture obscured the results. Non specific agglutinins were looked for but not found. The methods of calculating and preparing the doses of standard antitoxins, together with several useful modifications of the standard or Hygienic Laboratory technic have already been described by Berg.[7]

9 (2241)

The antiketogenic influence of insulin in diabetes.

By JOHN A. KILLIAN.

[*From the Department of Biochemistry, New York Post-Graduate Medical School and Hospital, New York.*]

The object of this study was to determine the action of insulin upon the production and excretion of ketone bodies and upon the acid-base equilibrium of the blood of human cases of diabetes mellitus. This report presents the results of seventeen

4 Cowie, D. M., and Kempton, R. M., *J. Med. Research*, 1921, xlii, 227.
5 Kolle, W., and Schlossberger, H., *Med. Klin.*, 1919, 1, 83.
6 Kastenmeyer, B., *Deutsch. med. Woch.*, 1919, xlv, 1338.
7 Berg, W. N., *J. Infect. Dis.*, 1921. xxix, 86.