ACTIVE IMMUNITY TO DIPHTHERIA IN THE ABSENCE OF DETECTABLE ANTITOXIN.

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(With one Chart.)

An animal may be regarded as being immune against diphtheria if the presence of any antitoxin can be detected in its blood, or if it can tolerate the injection of toxin more easily than can a normal animal; the Schick test which is now so widely used as a means of differentiation of the immune from the non-immune is a modification of this test for immunity.

There are two simple types of immunity, active and passive, and the criterion, which distinguishes the former from the latter, is that an actively immune animal responds to the injection of an antigen by a more rapid production of circulating antitoxin than does a passively immune or normal animal after the injection of the same antigen. In other words an animal is actively immune if the injection of an antigen acts as a secondary stimulus (Glenny and Sudmersen, 1921). Though the idea that active immunity may exist in the absence of circulating antibody has been suggested at various times in the literature, we have been unable to find any clear experimental demonstration of the truth of the suggestion; the work here recorded was undertaken in order to investigate the matter and supply such a demonstration.

By means of the intradermic method of testing the antitoxic content of a serum (Glenny and Allen, 1921), it is possible to detect with certainty the presence of such a small quantity as 1/2000 of a unit of antitoxin per c.c.; we propose here to record results showing that an animal may be actively immune before even this small amount of circulating antitoxin can be detected in its blood.

Among a number of guinea-pigs and rabbits which had been used to test the antigenic value of toxin-antitoxin mixtures, several were found which had not responded to the injection of the primary stimulus by the production of even 1/2000 of a unit of antitoxin per c.c. of blood. This non-production of antitoxin may have been due to three causes, the injection of too small a dose of antigen, the weakness of the antigen, or to the lack of reactive capacity of the animal. In some instances sufficient time may not have elapsed since the primary stimulus was given.

These animals, however, on the injection of another toxin-antitoxin mixture showed by the rapid production of circulating antitoxin that the

primary stimulus had induced a condition of active immunity, though a detectable amount of antitoxin was not present in the circulation.

The results obtained with four rabbits are recorded in Table I and Chart 1.

Table I. *Showing the antitoxic content of four rabbits after the injection of toxin-antitoxin mixtures.*

Rabbit	*G* 7	*G* 12	*G* 22	*G* 33
Secondary stimulus	5·0 c.c. of *B* 234	5·0 c.c. of *B* 234	5·0 c.c. of *B* 234	5·0 c.c. of *B* 346
Antitoxic value in units per c.c. at time of injection	<0·0005	<0·0005	<0·0005	<0·0005
1 day later	—	—	—	—
2 days later	<0·0005	<0·0005	<0·0005	—
3 ,,	—	—	—	—
4 ,,	0·02	0·022	0·004	—
5 ,,	—	—	—	—
6 ,,	0·22	0·2	0·04	—
7 ,,	—	—	—	0·10
8 ,,	0·25	0·18	—	—
9 ,,	—	—	0·30	—
10 ,,	0·20	0·16	—	—
11 ,,	—	—	—	—
12 ,,	—	—	0·20	—
13 ,,	0·10	0·10	—	—

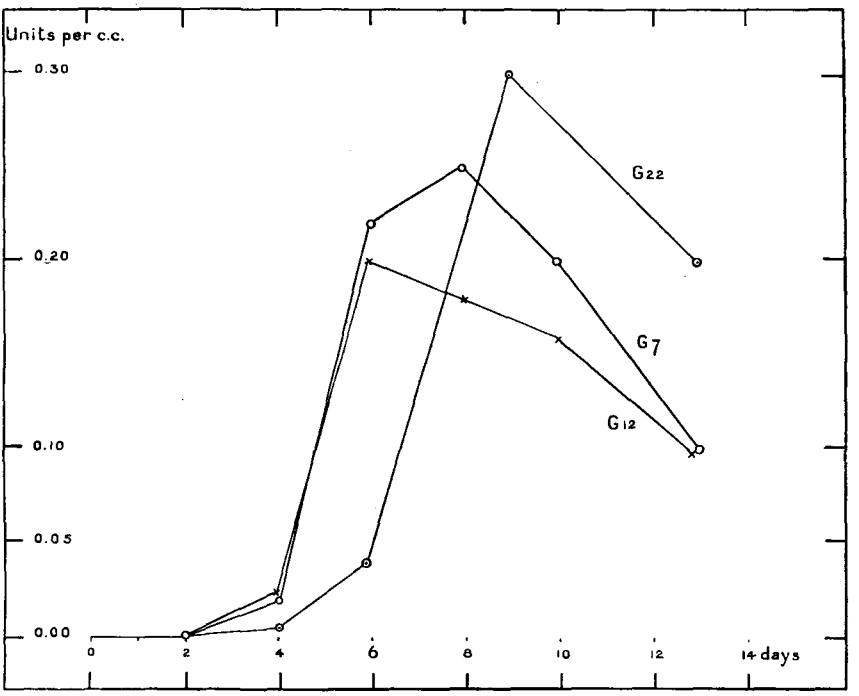

Chart 1.

The first three rabbits, *G* 7, *G* 12 and *G* 22 were all injected with the same volume of the same antigen, that is, 5 c.c. of a toxin-antitoxin mixture *B* 234, while rabbit *G* 33 was injected with 1 c.c. of *B* 346, another mixture of similar constitution. Both these antigens contained in each cubic centimetre three

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L_0 doses of toxin together with 3·5 units of antitoxin and passed the tests, recommended by the American authorities, for toxin-antitoxin mixtures destined for human immunisation, that is, 1 c.c. caused no effect when injected subcutaneously into normal guinea-pigs, but 5 c.c. caused late paralysis.

Of the four rabbits, *G* 7 had been under observation for over a year, and twelve months before the commencement of the present experiment had reached an antitoxic value of 1/5 of a unit of antitoxin per c.c. of blood. At the time of the injection of the toxin-antitoxin mixture recorded in Table I its antitoxic content had fallen to below 1/2000 of a unit of antitoxin per c.c., that is, all detectable circulating antitoxin had disappeared. Of the remaining three rabbits both *G* 12 and *G* 22 received the primary stimulus four months before, and *G* 33 four weeks before, the injection of the antigen recorded in Table I. These rabbits were tested at weekly intervals for circulating antitoxin and at no stage before the second injection could the presence of even 1/2000 of a unit of antitoxin be detected.

In all four rabbits the antitoxin production following the injection of the second toxin-antitoxin mixture was more rapid than ever occurs in a normal animal after the injection of such a mixture. This rapid response indicated that each animal was in a state of active immunity before the injection of the second antigen, although no circulating antitoxin could be detected, and that the second antigen had acted as a secondary stimulus. By reference to Chart I it is interesting to note the uniformity in the immunity response of the three rabbits receiving the same volume of the same antigen.

Table II records the results obtained from a series of three guinea-pigs which were injected with 1 c.c. of a toxin-antitoxin mixture *B* 234 which was repeated at intervals of four, five and six weeks after the first injection.

In the case of guinea-pig *ZZ*, four weeks after the first injection of the antigen, no circulating antitoxin could be detected, yet seven days after the second injection the presence of 1/15 of a unit of antitoxin was demonstrated. At that time no circulating antitoxin could be detected in the second guinea-pig *AA*, and again it was shown that the injection of the first antigen had induced a state of active immunity since the injection of the second antigen

Table II. *Showing the antitoxic content of three guinea-pigs injected with toxin-antitoxin mixtures.*

Guinea-pig	*ZZ*		*AA*		*XX*	
Primary stimulus	1 c.c. *B* 234		1 c.c. *B* 234		1 c.c. *B* 234	
Interval after primary stimulus	Injection	Value	Injection	Value	Injection	Value
4 weeks	1 c.c. *B* 234	<0·0005 units per c.c.	—	—	—	—
5 ,,	—	0·06	1 c.c. *B* 234	<0·0005 units per c.c.	—	—
6 ,,	—	—	—	0·06	1 c.c. *B* 234	<0·0005 units per c.c.
7 ,,	—	—	—	—	—	0·005

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caused an increase in antitoxic content from less than 1/2000 of a unit to 1/15 of a unit per c.c. in seven days. That this response was entirely due to the injection of the second antigen, acting as a secondary stimulus, and not due to any action of the primary stimulus, is proved by the fact that the third guinea-pig at this time still showed no circulating antitoxin.

Many additional examples of the phenomenon described above are recorded in "The Schick dose of Diphtheria Toxin as a Secondary Stimulus" (Glenny and Allen, 1922). That paper gives instances of the rapid production of antitoxin following the injection of a small dose of toxin into previously injected guinea-pigs and rabbits in many of which no circulating antitoxin could be detected before the injection of the secondary stimulus.

CONCLUSIONS.

(1) An animal may be in a state of active immunity before any circulating antitoxin can be detected and, further,

(2) The condition of active immunity continues after the disappearance of all circulating antitoxin produced by the animal in response to a previous stimulus.

REFERENCES.

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