THE SCHICK DOSE OF DIPHTHERIA TOXIN AS A SECONDARY STIMULUS.

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For the effective control of diphtheria there are two modern weapons with distinctive uses, toxin-antitoxin mixtures for the active immunisation of the non-immune and the Schick test for the differentiation of the immune from the non-immune. The object of the present paper is to show that the very small amount of toxin used for the Schick test, apart from its action as a diagnostic agent, may also play an active part in immunisation.

That a succession of small doses of toxin would immunise was known from the work of Bauer (1918). Cowie (1916) also concluded that small doses of toxin had an immunising action, because of the reduction in the size of the skin reaction in human patients, after the intradermal injection of a series of doses of very dilute toxin. Unfortunately, the necessity for a heated toxin control was not known at the time; otherwise much clearer deductions could be drawn from the series of careful measurements and observations made by this author.

In a previous paper (Glenny and Sudmersen, 1921) "secondary stimulus" was the term applied to the injection of toxin into an actively immune animal. It was there shown that "in immune animals, whether naturally immune or artificially immune, a single dose of toxin or of a toxin-antitoxin mixture is followed by a latent period of about four days and the maximum immunity is reached in about ten days." In the experiments recorded in that paper the secondary stimulus consisted either of a toxin-antitoxin mixture containing one or more test doses of toxin or of a dose of unneutralised toxin containing many minimal lethal doses.

The dose of toxin used for the Schick test contains only 1/50 of a minimal lethal dose for a guinea-pig. Can such a small dose of toxin act as a secondary stimulus?

The Schick test consists in injecting intradermally a certain small amount of toxin which produces a red flush in the skin of an animal containing no antitoxin, or an amount of antitoxin less than a certain level, stated by various observers to be, in man, from $\frac{1}{100}$th to $\frac{1}{130}$th of a unit per c.c. of blood; if the subject's blood contains more than this amount, the toxin used in the Schick test is neutralised and no red flush results. The Schick test is thus an indicator of antitoxin concentration in the blood. If a different amount of toxin is used for the test the presence or absence of a reaction indicates a

different level of immunity. It is possible, therefore, to determine the degree of immunity of an animal by injecting it with several different concentrations of toxin at the same time; the degree of immunity of the animal lies between that indicated by the lowest concentration of toxin causing a reaction and the highest concentration failing to give a reaction.

If the small amount of toxin used as an indicator becomes also an active agent in increasing the immunity of the animal, then subsequent tests will show a higher level of immunity than would have occurred in the absence of the first tests.

This was found to be the case in the course of some work on immunity when an attempt was made to plot out, by this method, the immunity curves of guinea-pigs that had received various mixtures of toxin and antitoxin. The curves were found to be much steeper than any previously plotted by periodical antitoxin determinations upon blood.

This suggested that the diagnostic dose of toxin had acted as a secondary stimulus, and, by rapidly increasing the immunity, had materially altered the shape of the curve. The level of immunity of a guinea-pig seven weeks after the injection of a toxin-antitoxin mixture was tested by the intradermal injection of 1/5000 and 1/500 of a test dose of toxin. The former dose gave no reaction and the latter a positive reaction indicating a level of immunity lying between two fairly wide limits. In the attempt to narrow down these limits it was found that, five days after the first test was made, not only did 1/500 of a test dose fail to cause a reaction, but no reaction was given by 1/100 of a test dose of toxin.

This rapidity of increase of immunity appeared far greater than would result from the primary stimulus alone, and may therefore have resulted from a secondary response to the first test injection of 1/5000 and 1/500 of a test dose of toxin. The Schick dose of the particular toxin used for the test was 1/1000 of a test dose (L+) so that in this experiment, given as an example of a number of similar cases, a dose of toxin equivalent to just over two Schick doses acted as a secondary stimulus.

Other preliminary indications were also given by rabbits. Two immune rabbits, injected intradermally with a Schick dose of toxin to determine the suitability of rabbits for intradermal work, showed an increase in antitoxic value after the injection. One rabbit previously immunised by antigenic mixtures had reached a maximum value of 1/5 of a unit of antitoxin per c.c.; it commenced to fall in value, and contained 1/40 of a unit of antitoxin per c.c. at the time of injection with the Schick dose. Two weeks after this injection the value was found to be 1/12 of a unit per c.c.

The antitoxic content of the other rabbit had fallen to less than 1/500 of a unit per c.c. at the time of the Schick injection. Three days after this injection an antitoxic content of 1/250 of a unit was found, while at the end of ten days 1/5 of a unit was reached.

This phenomenon may account for references in literature to discordant

results after the injection of the Schick dose on the same individual, the discrepancy frequently being that a positive reaction has been followed by a negative reaction. It appeared to us, therefore, advisable to investigate further the possibility of the Schick dose as a secondary stimulus. Such immune animals as were available were injected with a Schick dose of diphtheria toxin and the antitoxic value of their blood determined immediately before and at intervals after the injection. These animals were guinea-pigs and rabbits which had been used for testing the antigenic value or the toxicity of toxin-antitoxin mixtures destined for human immunisation. The animals were all in various stages of immunity; these stages may conveniently be divided according to the scheme shown in Table I.

Very few of the animals had been tested at regular intervals, consequently the stage of immunity, *i.e.* whether on a rising or a falling curve, was not always known.

Table I.

Stage of immunity	Antitoxic value at time of injection	Schick reaction
A. Early stage—no antitoxin previously demonstrated B. Late stage—antitoxin previously demonstrated but now disappeared from circulation	No antitoxin detectable, that is, less than 1/2000 unit per c.c.	Positive
C. Early stage—rising curve D. Later stage—falling curve	Small amount of antitoxin present	Positive
E. Early stage—rising curve F. Later stage—falling curve	Considerable amount of antitoxin present	Negative

Table II records the results obtained after the injection of a dose of Schick toxin into two rabbits *G* 29 and *G* 31 both of which were in immunity stage A (see Table I), at the time of the secondary stimulus.

These rabbits had each received a dose of an antigenic mixture and had been tested at weekly intervals for the presence of any circulating antitoxin. Eleven weeks after its primary stimulus rabbit *G* 29 had not shown any detectable antitoxin and was injected with a Schick dose of toxin. The injection of this secondary stimulus induced the production of 1/100 of a unit of antitoxin per c.c. of blood in eight days.

Rabbit *G* 31 received its secondary stimulus eight weeks after its primary stimulus and at no time between the two injections had antitoxin been detected. This rabbit showed a greater production of antitoxin, a value of 1/50 of a unit per c.c. being detectable after six days' interval and 1/30 of a unit per c.c. after eight days' interval.

The only definite example of an animal whose immunity stage was B, that is, which had been previously immunised and whose circulating antitoxin had fallen to below a detectable level was rabbit *G* 7. In April 1920 this rabbit received as a primary stimulus two doses of a toxin-antitoxin mixture, and four weeks after these injections it showed an antitoxic content of 1/5 of a unit per c.c. of blood. After this rise a rapid fall in antitoxic content

occurred and in August and again in October a weak antigen was injected without any response. In February 1921 not even 1/2000 of a unit of circulating antitoxin could be detected, and the injection of a toxin-antitoxin mixture of known antigenic utility failed to produce any response by the

Table II. *Showing the effect on antitoxin production of the injection of a Schick dose of toxin into rabbits which had never produced any detectable antitoxin after the injection of an antigen.*

	G 29	*G* 31
Immunity stage	A	A
Secondary stimulus	Schick dose of toxin. Reaction + +	Schick dose of toxin. Reaction + +
Interval between primary stimulus and secondary stimulus ...	11 weeks	8 weeks
Antitoxic content at the time of secondary stimulus	<0·0005 units per c.c.	<0·0005 units per c.c.
1 day later	—	—
2 days later	<0·0005 ,,	<0·0005 ,,
3 ,, ...	—	—
4 ,, ...	<0·0005 ,,	<0·0005 ,,
5 ,, ...	—	—
6 ,, ...	<0·0005 ,,	0·02 ,,
7 ,, ...	—	—
8 ,, ...	0·01 ,,	0·035 ,,
9 ,, ...	—	—
10 ,, ...	0·01 ,,	0·03 ,,
11 ,, ...	—	—
12 ,, ...	—	—
13 ,, ...	—	—
14 ,, ...	—	0·018 ,,

A Schick injection given to rabbit *G* 31, 19 days after the first Schick injection, gave a negative reaction.

Table III. *Table comparing the results obtained by using a toxin-antitoxin mixture and a Schick dose as a secondary stimulus.*

Rabbit *G* 7. (Stage of immunity – B.)

Secondary stimulus	5·0 c.c. of toxin-antitoxin mixture *B* 234	Schick dose of toxin. Reaction + +
Date of injection	May	August
Antitoxic value at time of injection	<0·0005 units per c.c.	<0·0005 units per cc.
1 day later	—	—
2 days later	<0·0005 ,,	—
3 ,, ...	—	—
4 ,, ...	0·02 ,,	—
5 ,, ...	—	—
6 ,, ...	0·22 ,,	—
7 ,, ...	—	0·45 ,,
8 ,, ...	0·25 ,,	—
9 ,, ...	—	—
10 ,, ...	0·20 ,,	—

A Schick dose injected seven days after the injection of the first Schick injection gave a negative reaction.

production of any circulating antitoxin. Three months later in May the injection of the same dose of the same antigen acted as a secondary stimulus inducing a rapid response by the production of 1/5 of a unit of antitoxin in six days. Again after this rise a rapid fall occurred; by July the antitoxic content was 1/200 of a unit per c.c. and in August no circulating antitoxin

was detectable. At this time the rabbit was injected intracutaneously with a dose of Shick toxin and the injection of this small dose of toxin induced a rise in antitoxic content, greater than that produced after the injection of the toxin-antitoxin mixture in May. Details of the comparison between the results obtained by these two injections are given in Table III.

The results obtained from a further group of rabbits are shown in Table IV. It will be seen that two of the rabbits, *G* 20 and *G* 22, were in the same stage of immunity (A); one received a Schick injection and the other 5 c.c. of a toxin-antitoxin mixture. In the former case approximately 1/5 of a unit and in the latter case 1/4 of a unit per c.c. was produced.

Table IV. *Comparing the effect on antitoxin production of injection of* (*a*) *a Schick dose of toxin,* (*b*) *one-tenth of a Schick dose of toxin,* (*c*) *a toxin-antitoxin mixture as a secondary stimulus.*

	G 20	*G* 21	*G* 23	*G* 22
Immunity stage	A	F	C or E	A
Secondary stimulus	Schick dose of toxin. Reaction + +	Schick dose of toxin. Reaction −	1/10 of a Schick dose of toxin. Reaction −	Toxin-anti-toxin mixture. 5·0 *B* 234
Interval between primary stimulus and secondary stimulus	14 weeks	14 weeks	14 weeks	14 weeks
Antitoxic content in units per c.c. at the time of secondary stimulus	<0·0005	0·3	0·02	<0·0005
1 day later	—	—	—	—
2 days later	<0·0005	—	0·02	<0·0005
3 ,, ...	—	—	—	—
4 ,, ...	0·02	—	—	0·004
5 ,, ...	—	—	—	—
6 ,, ...	0·14	0·3	0·09	0·04
7 ,, ...	—	—	—	—
8 ,, ...	—	—	—	—
9 ,, ...	0·18	0·3	0·09	0·25
10 ,, ...	—	—	—	—
11 ,, ...	—	—	—	—
12 ,, ...	—	—	—	—
13 ,, ...	0·12	—	0·08	0·2
14 ,, ...	—	—	—	—

G 21 showed the highest antitoxic value at the 8th, 9th and 10th week after the primary stimulus and was gradually declining in value at the time of the Schick injection. It is suggested that the absence of immunity response to the secondary stimulus was due to the high antitoxic content, 1/3 unit per c.c., compared wtih the small amount of toxin injected.

The other rabbit, *G* 23, showed a higher antitoxic content at the time of the secondary stimulus than on any previous occasion. It must therefore be regarded as on a rising curve of immunity. In this case a full Schick dose was not given and so no decision can be made as to whether the animal falls into immunity group C or E. One-tenth of a Schick dose, that is, 1/500 of a minimal lethal dose of toxin, produced a rise in antitoxic content of from 1/50 of a unit to about 1/10 of a unit per c.c.

Table V shows the results obtained with two other rabbits, *G* 32 and *G* 34,

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both of which had been injected with a toxin-antitoxin mixture *B* 346; four weeks later *G* 32 received a Schick injection which produced a positive reaction, a week later a Schick injection was followed by a negative reaction.

Rabbit *G* 34 received its Schick injection after eight weeks interval and gave a positive reaction followed by a negative when tested a week later.

The tests for the antitoxic content of the blood confirmed the readings of the Schick reaction and showed that the first Schick injection had increased the antitoxic content from less than 1/2000 of a unit per c.c. to 1/12 of a unit in rabbit *G* 32 injected four weeks after the primary stimulus, and from less than 1/2000 of a unit to 1 unit per c.c. in rabbit *G* 34 injected eight weeks after the primary stimulus.

In two companion rabbits a second injection of the toxin-antitoxin mixture was given as a secondary stimulus, and both rabbits showed a lower antitoxic response than the two rabbits injected with a Schick dose of toxin.

Table V. *Comparing the value of a Schick dose of toxic as a secondary stimulus at intervals of four and eight weeks after the primary stimulus.*

Rabbit	*G* 32		*G* 34	
Primary stimulus	1·0 c.c. *B* 346		1·0 c.c. *B* 346	
	Value units per c.c.	Secondary stimulus	Value units per c.c.	Secondary stimulus
1 week later	—	—	—	—
2 weeks later	—	—	—	—
3 ,,	<0·0005	—	<0·0005	—
4 ,,	<0·0005	Schick dose of toxin. Result + +	<0·0005	—
5 ,,	0·08	Schick dose of toxin. Result −	<0·0005	—
6 ,,	0·125	—	<0·0005	—
7 ,,	—	—	<0·0005	—
8 ,,	0·05	—	<0·0005	Schick dose of toxin. Result + +
9 ,,	—	—	1·0	Schick dose of toxin. Result −
10 ,,	—	—	1·75	—
11 ,,	—	—	—	—
12 ,,	0·0125	—	0·6	—

Table VI records the results of weekly Schick injections into guinea-pigs starting at different intervals after the injection of a toxin-antitoxin mixture.

It will be seen that all four guinea-pigs gave a negative reaction after the third Schick injection except *PP*, whose first Schick injection was given only two weeks after the primary stimulus. That the immunity indicated by the negative reaction was the result of the antigenic action of the first Schick injections is shown by the fact that at the fifth week after the primary stimulus guinea-pigs *PP* and *OO*, after three and two Schick injections, gave negative reactions, while guinea-pigs *SS* and 3 *B*, one of whom had received previously only one Schick injection and the other no injection, were still giving positive reactions.

Again, guinea-pig *SS*, having received Schick injections on the fourth and fifth weeks, gave a negative reaction on the sixth week when guinea-pig 3 *B* gave its second positive reaction. It is possible that the second Schick injection

in each guinea-pig did not contribute markedly to the increase in immunity, that the response to the first Schick injection given three, four or five weeks after the primary stimulus had not reached its maximum seven days later, but that at the end of fourteen days the immunity produced by the Schick injection would have been sufficient to enable the guinea-pig to give a negative reaction to a subsequent Schick test.

Table VI. *Table showing the effect on antitoxin production of the injection of Schick doses of toxin into guinea-pigs previously injected with toxin-antitoxin mixtures.*

Guinea-pig	PP		OO		SS		3 B	
Primary stimulus	1·0 c.c. *B* 234 T + AT mixture		1·0 c.c. *B* 234 T + AT mixture		1·0 c.c. *B* 234 T + AT mixture		1·0 c.c. *B* 234 T + AT mixture	
	Reaction caused by Schick injection	Antitoxic value units per c.c.	Reaction caused by Schick injection	Antitoxic value units per c.c.	Reaction caused by Schick injection	Antitoxic value units per c.c.	Reaction caused by Schick injection	Antitoxic value units per c.c.
1 week later	—	—	—	—	—	—	—	—
2 weeks later	+	—	—	—	—	—	—	—
3 ,,	+	—	+	—	—	—	—	—
4 ,,	+ –	<0·0005	+	—	+	—	—	—
5 ,,	–	0·1	–	0·025	+	<0·0005	+	<0·0005
6 ,,	—	—	—	—	–	0·0125	+	0·001
7 ,,	—	—	—	—	—	0·1	–	0·01
8 ,,	—	—	—	—	—	—	—	0·04

Table VII shows the rise in antitoxic content of a number of immune guinea-pigs one and two weeks after a Schick injection. The majority of the animals in the table fall into immunity groups E or F and show a definite rise in antitoxic content after the injection of a Schick dose of toxin which gave a negative reaction.

Table VII.

Guinea-pig	*FF* 19. v	*LL* 17. vi	*HH* 17. vi	*DD* 17. vi	*KK* 17. vi	*D* 9. vi	*CC* 24. v	*K* 19. v
Primary stimulus ...	1·0 *B* 346	1·0 *B* 346	1·0 *B* 346	5·0 *B* 346	5·0 *B* 346	0·5 *B* 335	5·0 *B* 325	1·0 *B* 326
Interval between primary and secondary stimulus	8 wks.	8 wks.	8 wks.	8 wks.	10 wks.	10 wks.	11 wks.	12 wks.
Immunity stage	A	C or D	E or F	E or F	E	E or F	E or F	E or F
Antitoxic content in units per c.c. at time of Schick injection	<0·001	0·002	0·01	0·01	0·02	0·2	0·05	0·06
Schick response	+	+ –	–	–	–	–	–	–
Antitoxic content in units per c.c. 1 week later ...	—	0·002	0·02	0·06	0·08	1 to 2	0·2	0·125
2 weeks later ...	0·01	0·02	0·5	0·25	—	—	1·0	0·8

Summary.

1. The injection of the small amount of diphtheria toxin used in the Schick test may act as a secondary stimulus.

A Schick test may therefore cause a great and rapid increase in the immunity of the animals tested.

Examples are quoted of six rabbits and twelve guinea-pigs in Tables II to VII.

2. A fraction of a Schick dose may act as a secondary stimulus. Rabbit *G* 23 quoted in Table IV, injected with 1/10 of a Schick dose, showed an immunity response, the antitoxic content of its blood rising from 1/50 to nearly 1/10 unit per c.c. in six days.

3. The action of a Schick dose as a secondary stimulus may cause an animal to give a negative reaction when tested seven days or more after the first positive reaction.

This is illustrated by rabbits *G* 7 in Table III, *G* 31 in Table II, *G* 32 and *G* 34 in Table V and four guinea-pigs in Table VI.

4. The antigenic value of a Schick dose of toxin as a secondary stimulus may be as high as that of a reasonable dose of a toxin-antitoxin mixture suitable for human immunisation. Examples are given comparing the results of the injection of a Schick dose of toxin and of a toxin-antitoxin mixture in the same rabbit in Table III, in different rabbits, *G* 20 and *G* 22 in Table IV and reference is made to the companion rabbits to those quoted in Table V.

5. The antigenic value of a Schick dose as a secondary stimulus can be demonstrated:

A. In animals which have not produced a detectable quantity of antitoxin (that is less than 1/2000 of a unit per c.c.) as the result of a primary stimulus.

See both rabbits in Table II, rabbit *G* 20 in Table IV, both rabbits in Table V, and guinea-pig *FF* 19. v in Table VII. The four guinea-pigs in Table VI probably come under the same heading.

B. In animals whose actively produced antitoxin has fallen below a detectable level.

See rabbit *G* 7 in Table III.

(These results add further confirmation to the phenomenon reported in the paper "Active immunity to diphtheria in the absence of detectable antitoxin" (Glenny and Allen, 1922).

6. A Schick dose of toxin which gives a positive reaction may, by acting as a secondary stimulus, produce a rapid increase in the antitoxic value of animals already containing some actively produced antitoxin.

See guinea-pig *LL* 17. vi in Table VII.

7. A Schick dose of toxin which causes no reaction may, by acting as a secondary stimulus, produce a rapid increase in the antitoxic value of animals already containing some actively produced antitoxin.

See guinea-pigs in Table VII.

8. A Schick dose of toxin may fail as a secondary stimulus if the antitoxic content at the time of injection is comparatively high.

See rabbit *G* 21 in Table IV.

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