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# The Antigenic Effect of Intravenous Injection of Diphtheria Toxin

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# THE ANTIGENIC EFFECT OF INTRAVENOUS INJECTION OF DIPHTHERIA TOXIN.

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We have found very little reference to intravenous injection of diphtheria toxin in recent literature and it appears to have been generally accepted that animals cannot be successfully immunised by intravenous injection of diphtheria toxin. Madsen (1923) wrote "according to the published experiments—which are however not very numerous and need revising—there is an essential difference in the antitoxic reaction occurring in a horse actively immunised against diphtheria by subcutaneous injection and that occurring as a result of intravenous injection. In the latter mode of procedure the antitoxin formation is almost *nil*." The following results show however the circumstances in which immunity response follows intravenous injection.

*Intravenous injection as a primary stimulus.*

Table I. shows the results of injecting three rabbits with a certain mixture of toxin and antitoxin of low antigenic efficiency. One rabbit received a subcutaneous injection of 25 c.c. while the others received 1 c.c. and 5 c.c. intravenously. The response to the intravenous injection of 5 c.c. was as good as that following the subcutaneous injection of 25 c.c. Two rabbits were then injected with 0·05 c.c. of a certain batch of modified diphtheria toxin, one rabbit intravenously and the other subcutaneously. No response judged by antitoxin production occurred in either rabbit. After two further injections of the same quantity of modified toxin given subcutaneously, both rabbits showed a rapid production of the same amount of antitoxin (1/25 unit). We concluded that the intravenous injection had acted as a primary stimulus and had produced as satisfactory ground immunity as the subcutaneous injection had done.

Another group of normal rabbits was injected with 0·1 c.c. and 1·0 c.c. respectively of modified toxin partially neutralised with antitoxin. The rabbit injected subcutaneously with 1·0 c.c. showed a response in 11 days, those receiving respectively 0·1 c.c. subcutaneously and 1·0 c.c. intravenously both responded to a lesser extent after a latent period of 3 weeks, while the fourth rabbit, which received 0·1 c.c. intravenously, showed no response.

TABLE I.

*Showing the antitoxic value of the blood of three rabbits after intravenous and subcutaneous injection of a primary stimulus consisting of a toxin antitoxin mixture.*

| Rabbit . . . | G 51. | G 52. | G 53. |
|---|---|---|---|
| Route . . . | Intravenous. | Intravenous. | Subcutaneous. |
| Volume . . | 1·0. | 5·0 | 25 c.c. |
| *Antitoxin content in units per c.c.* | | | |
| Before injection . . | Nil * | Nil | Nil |
| ½ week after . . | ,, | ,, | ,, |
| 1 ,, . . . | ,, | ,, | ,, |
| 1½ weeks after . . | ,, | ,, | 0·006 |
| 2 ,, . . . | ... | 0·001 | 0·006 |
| 2½ ,, . . . | 0·002 | 0·016 | 0·009 |
| 3 ,, . . . | 0·003 | 0·02 | 0·011 |
| 3½ ,, . . . | 0·0045 | 0·022 | 0·012 |
| 4 ,, . . . | 0·007 | 0·025 | 0·022 |
| 4½ ,, . . . | 0·011 | 0·025 | 0·022 |
| 5 ,, . . . | 0·012 | 0·035 | 0·025 |
| 5½ ,, . . . | 0·012 | 0·035 | 0·033 |
| 6 ,, . . . | 0·016 | 0·033 | 0·033 |
| 6½ ,, . . . | 0·022 | 0·03 | 0·033 |
| 7 ,, . . . | 0·018 | 0·022 | 0·033 |
| 7½ ,, . . . | 0·016 | 0·022 | 0·033 |
| 8 ,, . . . | 0·012 | 0·020 | 0·033 |

* Nil means either no antitoxin or less than 0·0005 unit, *i.e.*, the smallest amount that we ordinarily test for.

Normal rabbits injected with concentrated modified toxin have given the following results:—

| Dose, c.c. | Route. | Maximum antitoxin produced per c.c. of blood. | Latent period. |
|---|---|---|---|
| 1·0 | Subcutaneous | 0·1 unit. | 9 days |
| 0·1 | ,, | 0·04 ,, | 11 ,, |
| 1·0 | Intravenous | 0·004 ,, | 5 weeks |

In a further experiment five normal rabbits were injected subcutaneously, one with 0·1 c.c. of modified toxin alone and the other four with a mixture of 0·1 c.c. of modified toxin and antitoxin varying in the different rabbits from 1 unit to 7½ units. Six other normal rabbits were injected intravenously with similar mixtures. The amount of antitoxin produced in the eleven rabbits was scarcely detectable. The immunity response to the first stimulus was therefore judged by the degree of response to a secondary stimulus consisting of the subcutaneous injection of 0·1 c.c. of the same modified toxin.

The results given in table II. show that ground immunity is produced

equally well by intravenous and subcutaneous injection. It has already been shown (Glenny, Hopkins and Pope, 1924) that in an immunising mixture prepared with modified toxin the amount of antitoxin present does not, within wide limits, affect the antigenic power.

TABLE II.

*Comparing the secondary response of two groups of rabbits previously injected subcutaneously and intravenously respectively with mixtures of toxoid and antitoxin.*

| Amount of antitoxin in mixture injected. | Degree of secondary response in rabbits originally injected. | |
|---|---|---|
| | Subcutaneously. | Intravenously. |
| Nil | 1/10 unit | 1/5 unit |
| 1 unit | 1/10 ,, | 1/250 ,, |
| 2½ units | 1/5 ,, | 1/5 ,, |
| 5 ,, | 1/25 ,, | 1/10 ,, |
| 7½ ,, | 1/25 ,, | 1/100 ,, |
| 10 ,, | ... | 1/10 ,, |

Another group of rabbits recorded in table III. was injected with 5·0 c.c. of a batch of modified toxin of high antigenic value as determined by guinea-pig experiments. No antitoxin was detected in three different rabbits injected intravenously with unneutralised toxoid while five rabbits receiving intravenously the same volume of toxoid partially neutralised with antitoxin all produced an easily detectable amount of antitoxin.

TABLE III.

*Showing the primary stimulus response of ten rabbits to injections, given either intravenously or subcutaneously, of modified toxin with and without antitoxin.*

| Rabbit. | Composition of mixture injected. | | Route. | Antitoxin produced. | Latent period. |
|---|---|---|---|---|---|
| | Modified toxin. | Antitoxin. | | | |
| 1 | 5·0 c.c. | Nil | Intravenous | Nil | ... |
| 2 | 5·0 ,, | ,, | ,, | ,, | ... |
| 3 | 5·0 ,, | ,, | ,, | ,, | ... |
| 4 | 5·0 ,, | 5 units | ,, | 1/250 unit per c.c. | 20 days |
| 5 | 5·0 ,, | 15 ,, | ,, | 1/50 ,, ,, | 18 ,, |
| 6 | 5·0 ,, | 20 ,, | ,, | 1/25 ,, ,, | 20 ,, |
| 7 | 5·0 ,, | 20 ,, | ,, | 1/100 ,, ,, | 18 ,, |
| 8 | 5·0 ,, | 50 ,, | ,, | 1/500 ,, ,, | 24 ,, |
| 9 | 5·0 ,, | Nil | Subcutaneous | 1/12 ,, ,, | 9 ,, |
| 10 | 5·0 ,, | ,, | ,, | 1/2 ,, ,, | 9 ,, |

Two normal rabbits injected subcutaneously with 5 c.c. of the modified toxin alone responded so rapidly that antitoxin was detectable

in their blood 9 days after the injection. This great response of two rabbits (9 and 10) to a subcutaneous injection is in marked contrast to the failure of the three rabbits (1, 2 and 3) injected intravenously with the same dose, yet the intravenous injection of the same amount of modified toxin, when partially neutralised, acted as a successful stimulus to five other rabbits (4 to 8). A relatively poor response was given by rabbits 4 and 8 receiving intravenously the mixture containing the most and least antitoxin. A possible explanation is that the intravenous injection of unneutralised toxoid may fail to act as a powerful antigen because of too rapid elimination, and that the presence of antitoxin in the mixture injected may delay absorption or elimination.

*Intravenous injection as a secondary stimulus.*

The experiments so far have dealt with the action of intravenous injection as a primary stimulus to normal rabbits.

Table IV. shows that the intravenous injection of toxin into actively immune rabbits may act as a secondary stimulus. It will be seen that three rabbits, 11, 16 and 17, injected intravenously with enough toxin to neutralise from 10 to 20 per cent. of antitoxin present in

TABLE IV.

*Showing the antitoxic content of the blood of five immune rabbits after a secondary stimulus consisting of an intravenous injection of diphtheria toxin.*

| Rabbit . . . . | 10. | 11. | 17. | 16. | 13. |
|---|---|---|---|---|---|
| Volume of toxin injected in c.c. | 0·8 | 0·32 | 0·08 | 1·0 | 0·0064 |
| Number of Lo doses injected . | 5·0 | 2·0 | 0·5 | 6·25 | 0·04 |
| c.c. of rabbits' blood able to neutralise injection | 75·0 | 25·0 | 9·0 | 11·0 | 2·5 |
| Proportion of total blood able to neutralise injection | 50 % | 20 % | 13 % | 10 % | 2 % |
| Number of units per c.c. before injection | 0·06 | 0·07 | 0·055 | 0·55 | 0·017 |
| 2 hours after . . . . | 0·014 | ... | ... | ... | ... |
| 2 days after . . . . | 0·033 | ... | ... | 0·9 | ... |
| 3 ,, . . . . | 0·055 | ... | ... | ... | ... |
| 4 ,, . . . . | 0·14 | ... | ... | 15·0 | 0·02 |
| 6 ,, . . . . | 0·65 | ... | ... | 23·0 | 0·05 |
| 7 ,, . . . . | 0·9 | 3·5 | 2·75 | ... | ... |
| 8 ,, . . . . | 0·9 | ... | ... | 27·5 | 0·08 |
| 10 ,, . . . . | 0·9 | ... | ... | 15·0 | 0·04 |

the circulating blood of the animal responded to the secondary stimulus by the production of a considerable amount of antitoxin: 16 produced 27·5 units per c.c. A smaller response occurred in rabbit 13 injected with enough toxin to neutralise only 2 per cent. of the total antitoxin in the rabbit. Only a small response occurred in rabbit 10 receiving enough toxin to neutralise half the total antitoxin. It is

probable that the effect of the injection in this rabbit was too severe for a good response to be produced.

It appears obvious that the response to the intravenous injection of toxin in the actively immune rabbits is considerably lessened if the toxin injected is considerably over neutralised by the circulating antitoxin in the rabbit. It is therefore impossible to immunise successfully by the intravenous method animals that already contain several units of antitoxin per c.c. for the necessary volumes of toxin would be too great to allow of injection. It is of interest to note the relationship between a quantity of toxin and the amount of antitoxin that must be present before the power of the toxin to act as an antigen (*i.e.* to cause the production of antitoxin) is completely suppressed. The antitoxin may be present in the mixture of toxin and antitoxin made before injection or it may be already present in the circulation of an animal possessing some immunity.

Mixtures over neutralised 3- or 4-fold injected subcutaneously fail as antigens. Toxin injected intravenously can still act when there is enough antitoxin in the total circulation to neutralise the toxin 50-fold. Toxin injected subcutaneously into actively immune animals may induce a response even though there is enough antitoxin in the total circulation to neutralise many thousand times the amount of toxin injected. Thus a rise in antitoxic value of a horse already containing 1000 units of antitoxin per c.c. of serum may follow a subcutaneous injection of 100 c.c. of toxin, a quantity which could be fully neutralised by 1 c.c. of serum. In such a horse toxin injected subcutaneously can still act as an antigen when there is enough antitoxin in the total circulation to neutralise 30,000 times the amount of toxin injected.

The disproportion between total circulating antitoxin and size of an effective immunising dose of toxin injected subcutaneously may be compared with the similar disproportion, apparent in the Schick test, between circulating antitoxin and the amount of toxin injected intracutaneously that will produce an inflammatory reaction. A person possessing say 4 litres of blood containing 1/100 of a unit of antitoxin per c.c. still develops a red flush, *i.e.* a positive reaction, following an intradermic injection of that amount of toxin that would be completely neutralised by 1/10 c.c. of his blood. This disproportion is very much smaller when circulating antitoxin in a guinea-pig is compared with the amount of toxin necessary to kill the animal. A positive Schick reaction is produced in the local tissue cells; against this effect only a very small proportion of total circulating antitoxin is available; against the general lethal effects, a far greater proportion of antitoxin is available. The disproportion between total circulating antitoxin and the size of an effective immunising dose injected subcutaneously suggests that the antigenic stimulus of toxin is a local effect.

## Conclusions.

1. The intravenous injection of diphtheria toxin antitoxin mixture produces a primary stimulus response in normal rabbits.

2. Unneutralised modified toxin injected intravenously is less effective than the same toxin partially neutralised.

3. The intravenous injection of diphtheria toxin can act as a secondary stimulus.

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