

Simultaneous active and passive immunization of guinea-pigs against tetanus*

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Antitoxic horse serum when injected into man may induce hypersensitivity reactions to the horse serum proteins. Even though obvious reactions do not occur, the patient may become sensitized so that a reaction develops when antitoxin is given on a subsequent occasion. Furthermore, antibodies against horse serum may arise, which can cause antitoxin injected subsequently to be eliminated from the body too rapidly to be of prophylactic or therapeutic value. Injection of horse antitoxin may therefore leave the patient at a therapeutically induced disadvantage, the dangers of which can largely be overcome by ensuring that patients who receive horse serum are actively immunized against those diseases for which horse serum may be used. Such a procedure is also to be recommended since a person who has been actively immunized is more effectively protected than one who is passively protected at the time of infection.

The main diseases of man in which antitoxin is used are diphtheria and tetanus and, as a result of diphtheria immunization campaigns, tetanus prophylaxis is now the common reason for injecting horse serum. It is therefore considered that all who receive tetanus antitoxin in the form of horse serum should also be actively immunized against tetanus. This is most conveniently achieved by beginning the course of tetanus toxoid at the same time as the antitoxin is given.

Simultaneous active and passive immunization against tetanus has not been regularly practised because the antitoxin may interfere with the active response (Cooke & Jones, 1943; Barr & Sachs, 1955; Prudovsky & Turner, 1958; Philipson, 1959). On the other hand, evidence is available that interference is less evident when toxoid containing an adjuvant is used (Wolters & Dehmel, 1937; Gold & Bachers, 1943; Ericsson, 1948). The studies of Uhr & Baumann (1961), on diphtheria immunization in guinea-pigs, suggest that although interference with the immediate antitoxin response may occur when antitoxin is given with toxoid, nevertheless, a basal immunity develops which enables a booster response to be induced by a later dose of toxoid. Indeed simultaneous active and passive immunization using adsorbed toxoid is successfully practised for the protection of children exposed to diphtheria (Fulton, Wells, Taylor & Wilson, 1941).

It was therefore considered that simultaneous immunization against tetanus would probably be effective if toxoid containing adjuvant was used, and that, even though some interference might occur, the active response would probably

* Some of the results in this paper were first presented at an International Conference on Tetanus held in Bombay in November 1963.

be sufficient to provide acceptable levels of circulating antitoxin. Consequently a trial of simultaneous active and passive immunization against tetanus was made in man and the results, combined with those of another group, have already been reported (Smith, Evans, Jones, Gear, Cunliffe & Barr, 1963). In conjunction with these field studies, laboratory experiments were made using guinea-pigs in order to examine the response to simultaneous immunization carried out in different ways. The experimental results are reported here and the findings related to those obtained in man.

MATERIALS AND METHODS

Antitoxin and toxoid

Tetanus antitoxin (Wellcome) containing 1500 units per ml. was used. A subcutaneous injection of 2.0 ml. of a 1/10 dilution in normal saline was given into the shaved lateral aspect of the left thigh of guinea-pigs. The dose was therefore 300 units, i.e. 1/5th of the usual human dose.

Plain tetanus toxoid (Wellcome) containing 40 Lf/ml. was used. The dose given was 1 ml. of a 1/10 dilution in normal saline, i.e. 1/5th of the human dose, and was injected subcutaneously into a shaved area of the right anterior quadrant of the abdomen.

The aluminium hydroxide adsorbed tetanus toxoid (Wellcome) used also contained 40 Lf/ml. and 1.8 mg. of aluminium per ml. It was given in the same dilution and by the same route as the plain toxoid.

Tetanus antitoxin titrations were performed by the method of Glennly & Stevens (1938) and the results recorded in international units per ml. of serum. The smallest amount of antitoxin which was tested for was 0.01 units per ml.

Experimental animals

Female albino guinea-pigs were used and at the time of active immunization were approximately 6 weeks old, weighing between 300 and 400 g. They were housed five to a cage and fed on guinea-pig pellet food supplemented by cabbage daily.

Blood samples of 2.0 ml. were obtained from the guinea-pigs by cardiac puncture under ether anaesthesia, and the serum was separated after overnight storage at 4° C.

Immunization procedure

The guinea-pigs were randomly divided into six groups (A to F) each of fifteen animals. Serum samples were obtained from three animals of each group before immunization and none was found to contain detectable tetanus antitoxin. Toxoid was given in three doses spaced as in the schedule used in Britain for the immunization of children, the second dose being given 6 weeks after the first and the third dose 6 months after the second. Serum samples were taken 1 day before and 2 weeks after both the second and third doses of toxoid.

Group A was immunized with plain toxoid and group B with adsorbed toxoid. Group C was immunized with plain toxoid and group D with adsorbed toxoid, but in these two groups the first injection was accompanied by a dose of tetanus anti-toxin given into a different site as described above, i.e. simultaneous active and passive immunization was employed. In all groups, however, the third dose of toxoid, given 6 months after the second, was plain toxoid.

A fifth group, E, was immunized both actively and passively using plain toxoid, but in this group the injection of antitoxin was given 24 hr. after the toxoid. The sixth group, F, was given simultaneous active and passive immunization using adsorbed toxoid but, in addition, the guinea-pigs received an injection of tetanus antitoxin 3 weeks before the simultaneous immunization, i.e. when they were 3 weeks old.

The immunization régimes employed are summarized in Table 1.

Table 1. *Immunization régimes employed in six groups each of fifteen guinea-pigs*

Group	Preliminary procedure	1st injection	2nd injection (6 weeks after 1st)	3rd injection (6 months after 2nd)
A	None	Plain toxoid	Plain toxoid	Plain toxoid
B	None	Adsorbed toxoid	Adsorbed toxoid	Plain toxoid
C	None	Plain toxoid and simultaneous anti-toxin	Plain toxoid	Plain toxoid
D	None	Adsorbed toxoid and simultaneous anti-toxin	Adsorbed toxoid	Plain toxoid
E	None	Plain toxoid and anti-toxin 24 hr. later	Plain toxoid	Plain toxoid
F	Antitoxin 3 weeks before 1st toxoid injection	Adsorbed toxoid and simultaneous antitoxin	Adsorbed toxoid	Plain toxoid

RESULTS

Response to the first and second doses of tetanus toxoid

Table 2 shows the response of the guinea-pigs of groups A to D to the first and second doses of toxoid. These responses are plotted in Fig. 1 which also shows the responses obtained to the third dose of toxoid.

The response to immunization with adsorbed toxoid alone (group B) was considerably better than with plain toxoid (group A), the difference in response being approximately 60-fold after the first dose of antigen and 7-fold after the second. Furthermore, four of the guinea-pigs failed to show a response to the first dose of plain toxoid, whereas all the guinea-pigs immunized with adsorbed toxoid produced more than 0.2 units of antitoxin.

The antitoxin response to simultaneous active and passive immunization using the plain toxoid (group C) was very poor, thirteen of fourteen animals having less than 0.01 units of antitoxin 6 weeks after the first injection. On the other hand, when adsorbed toxoid was used (Group D) only three of twelve animals failed to

Table 2. *Tetanus antitoxin titres in guinea-pigs after receiving the different immunization régimes shown in Table 1*

Group	Serum sample taken after	No. of sera with antitoxin titres of (units per ml.)													No. of sera tested	Geometric mean titre			
		< 0.01	0.01-0.01	0.02-0.02	0.05-0.05	0.1-0.1	0.2-0.2	0.5-0.5	1.0-1.0	2.0-2.0	5.0-5.0	10-10	20-20	50-50			100-100	200	
A	1st injection	4	1	4	2	1	1	—	—	—	—	—	—	—	—	—	—	13	0.03
	2nd injection	—	—	—	—	—	—	—	2	—	1	4	3	—	—	—	—	10	11.0
B	1st injection	—	—	—	—	—	1	3	—	10	—	—	—	—	—	—	—	14	2.0
	2nd injection	—	—	—	—	—	—	—	—	—	—	1	1	2	5	—	—	9	79.0
C	1st injection	13	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14	< 0.01
	2nd injection	3	—	1	—	5	3	—	1	—	—	—	—	—	—	—	—	13	0.06
D	1st injection	3	1	1	—	1	1	4	1	—	—	—	—	—	—	—	—	12	0.12
	2nd injection	—	—	—	—	—	1	1	—	—	3	—	6	1	2	—	—	14	17.0

Serum samples taken 6 weeks after the 1st injection and 2 weeks after the 2nd injection of toxoid.

respond to the first dose. Two weeks after the second dose of toxoid three of the animals given plain toxoid still showed no response, whereas all the animals given adsorbed toxoid had more than 0.2 units of antitoxin. Furthermore, it may be seen that the response of guinea-pigs to simultaneous active and passive immunization using adsorbed toxoid (group D) was greater than that to immunization with plain toxoid given without any antitoxin (group A).

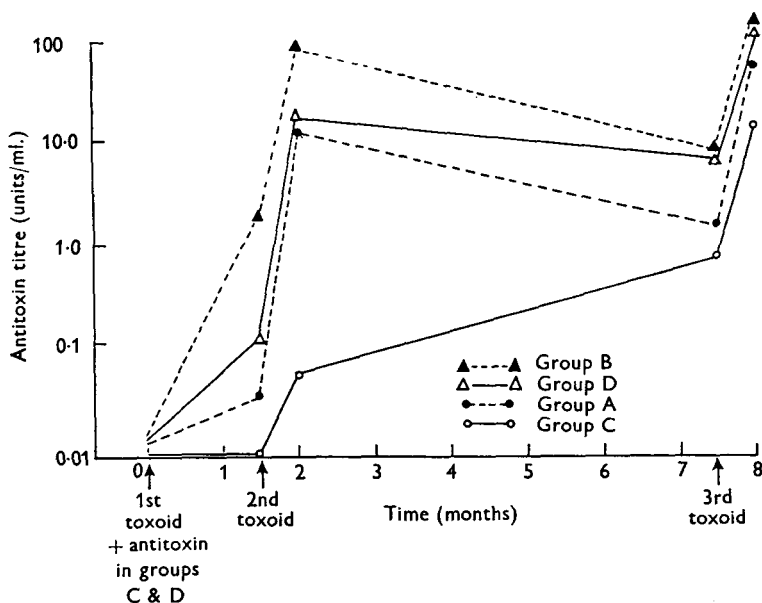


Fig. 1. Geometric mean tetanus antitoxin titres in guinea-pigs. Group A, plain toxoid; group B, adsorbed toxoid; group C, simultaneously, plain toxoid and antitoxin; group D, simultaneously, adsorbed toxoid and antitoxin.

Antitoxin titres 6 months after primary immunization and the response to the third dose of toxoid

The geometric mean antitoxin titres found when the guinea-pigs were bled 6 months after the second dose of antigen and the titres produced in response to a third dose of toxoid, are shown in Fig. 1.

It can be seen that the effect of interference by antitoxin was less evident at this later stage. The two groups in which adsorbed toxoid was used (groups B and D) had similar antitoxin titres 6 months after the second injection, and they showed a similar response to the booster of plain toxoid, even though in the early stages of immunization the group which also had antitoxin (group D) showed definite evidence of interference.

The animals of group C, which had simultaneous active and passive immunization using plain toxoid, responded well to the booster injection, but the effect of interference was still evident as the mean antitoxin titre was lower than in group A which had no passive antitoxin.

The guinea-pigs given simultaneous active and passive immunization with plain toxoid (group C and also group E shown in Fig. 2) had a higher antitoxin titre

at 6 months than at 2 weeks after the second injection of toxoid. Furthermore, the groups receiving simultaneous active and passive immunization with adsorbed toxoid (group D and also group F shown in Fig. 3) showed a slower decline in antitoxin titres after the second dose of antigen than guinea-pigs not given antitoxin (group B). It appears that the passive antitoxin not only reduced the antibody response to toxoid, but also caused the response to be delayed. A similar delaying effect has also been observed in diphtheria immunization both in guinea-pigs (Mason, Robinson & Christensen, 1955) and in man (Downie, Glenny, Parish, Smith & Wilson, 1941) and also in tetanus immunization in man (Tasman & Huygen, 1962).

The effect of delaying the injection of antitoxin for 24 hr.

Group E was included to test whether interference with plain toxoid could be avoided by delaying the antitoxin injection, so that the toxoid could have access to the antibody-forming cells for 24 hr. unhampered by antitoxin. Such a delay

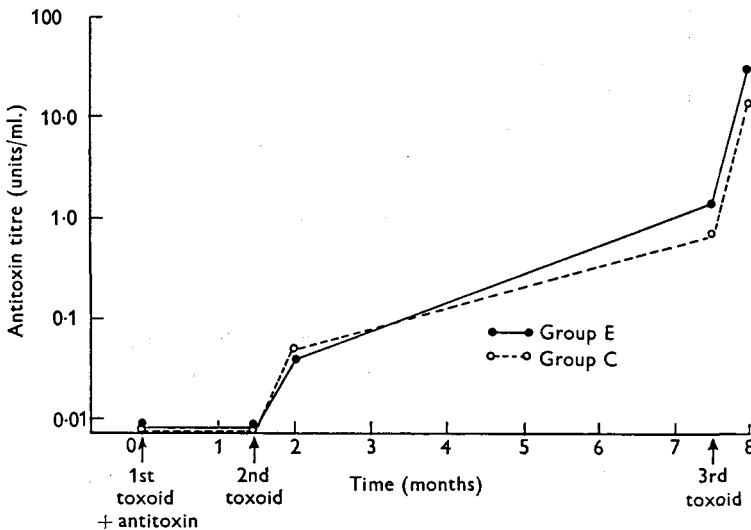


Fig. 2. Geometric mean tetanus antitoxin titres in guinea-pigs. Group C, simultaneously, plain toxoid and antitoxin; group E, plain toxoid and antitoxin 24 hr. later.

might be effective if interference is due to an afferent inhibition of the immunological response (Billingham, Brent & Medawar, 1956). The results obtained in group E are shown, together with those of group C, in Fig. 2.

It can be seen that the antitoxin response of group E was not appreciably different from that of group C, in which the antitoxin injection was not delayed.

The effect of an earlier injection of passive antitoxin

Group F was included to examine the possibility that simultaneous active and passive immunization might fail in animals already sensitized to horse serum, owing to the 'crowding out' effect of a secondary response to the horse serum (Glenny, Hopkins & Waddington, 1925).

The results obtained in group F are shown in Fig. 3 together with those of group D, which had no previous experience with horse serum. When adsorbed toxoid was used, instead of finding evidence of 'crowding-out', the responses, 6 weeks after the simultaneous immunization, were 10 times higher than in guinea-pigs with no previous experience of horse serum. This difference is highly significant ($P = < 0.005$).

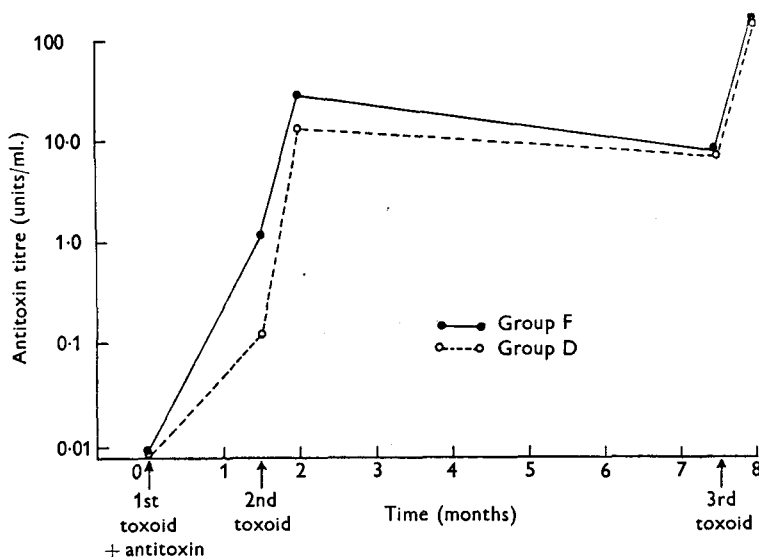


Fig. 3. Geometric mean tetanus antitoxin titres in guinea-pigs given, simultaneously, adsorbed toxoid and antitoxin. Group D, not sensitized to horse serum; group F, sensitized to horse serum by an injection 3 weeks earlier.

DISCUSSION

Whilst a direct comparison of results obtained in man and in experimental animals cannot be made, it is probably justifiable to use the results obtained in animals as a guide to what might be expected of an immunization régime in man. It is in an attempt to relate the laboratory to the field work that the results obtained in simultaneously immunized guinea-pigs are presented in Fig. 4, together with those obtained from simultaneous immunization with adsorbed toxoid in eighty-two patients (Smith *et al.* 1963). The titres shown are those found 2 weeks after the second dose of toxoid in both guinea-pigs and man.

Before making any comparison two points should be considered. First, the doses of toxoid and of antitoxin used in guinea-pigs, although given in the same relative proportions as in man, must be considered large in relation to the body weight of the animals. The doses were chosen to provide circulating passive antitoxin for a time similar to that in human patients given 1500 units (i.e. approximately 20 units per kg. body weight), as it was thought that the duration of passive immunity might be an important factor in determining the degree of interference. In preliminary experiments it was found necessary to give guinea-pigs 1000 units per kg. body weight to provide antitoxin levels just detectable two weeks later.

It appears that in guinea-pigs, and in rabbits, horse antitoxin is eliminated from the circulation more rapidly than is usually the case in man (Madsen, 1936; Suri & Rubbo, 1961).

The second point is the fact that in the clinical study, patients in whom antitoxin was detectable before the second dose of toxoid was given were excluded from

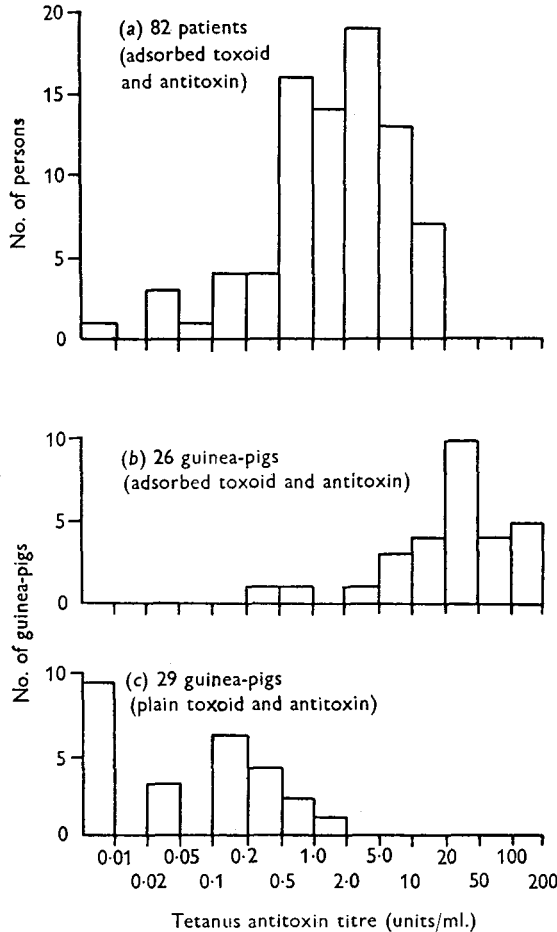


Fig. 4. Antitoxin titres 2 weeks after the second dose of toxoid in: (a) eighty-two patients immunized with adsorbed toxoid and antitoxin; (b) twenty-six guinea-pigs immunized with adsorbed toxoid and antitoxin, groups D and F; (c) twenty-nine guinea-pigs immunized with plain toxoid and antitoxin, groups C and E.

analysis. This was done to ensure that all previously immunized persons were excluded, but it is likely that some non-immunized persons were also excluded. Furthermore, the proportion of non-immunized patients excluded may have been high owing to the possibility, suggested by the results obtained in guinea-pigs (see Fig. 3), that patients previously injected with horse serum can respond particularly well to simultaneous immunization.

With these points in mind, the results obtained in guinea-pigs suggest that the response of man to simultaneous immunization using plain toxoid is likely to be

markedly inferior to the response using adsorbed toxoid. Thus if the same relative response is found in man as occurs in guinea-pigs, the use of the plain toxoid for simultaneous immunization in man is likely to yield titres of only 0.005–0.006 units per ml., 2 weeks after the second injection of antigen.

The deficiencies of plain toxoid for simultaneous immunization are most evident early in the course of immunization. After a third dose of toxoid, which should be considered an integral part of the tetanus immunization régime, the difference in responses obtained with plain and with adsorbed toxoid became less marked. In man therefore plain toxoid might produce satisfactory antitoxin titres after injection of the third dose. Nevertheless, it is considered that adsorbed toxoid is preferable for simultaneous immunization, since it should lead to the earlier development of immunity and to higher antitoxin titres after the third injection. Furthermore, patients who fail to return for either the third or second injection are more likely to be effectively immunized if they have received adsorbed toxoid.

A number of workers have examined the possibility that interference may be overcome by delaying the injection of antitoxin. Glenny, Buttle & Stevens (1931) reported that diphtheria antitoxin would cause interference when given to guinea-pigs 3 days after they had received diphtheria plain toxoid, and Uhr & Baumann (1961) found that interference occurred even when a 5-day interval was employed. On the other hand, Moloney & Fraser (1929) obtained no evidence of interference when antitoxin was given 2 hr. after toxoid, although interference occurred when the interval was 10 min. In the experiments reported here, tetanus antitoxin given to guinea-pigs 24 hr. after plain tetanus toxoid had no advantage over giving the antitoxin and toxoid at the same time.

The work of Glenny *et al.* (1925) and of Barr & Llewellyn-Jones (1953) suggests that, when two protein antigens are injected together, the antibody response to one antigen may be 'crowded-out' if the animal is in a state of secondary responsiveness to the other antigen. In the experiments reported here it was found that in guinea-pigs sensitized to horse serum, simultaneous active and passive immunization produced better responses than in guinea-pigs encountering horse serum for the first time. It therefore appears that, when one of the competing antigens (tetanus antitoxin) is an antibody to the second (tetanus toxoid), then a booster response to the first antigen can prevent it, to a large extent, from interfering with the second antigen. Whilst the mechanism responsible deserves investigation, the finding suggests that simultaneous active and passive immunization in man is unlikely to fail in patients who have previously received horse serum.

SUMMARY

1. Simultaneous active and passive immunization of guinea-pigs using tetanus toxoid adsorbed on to aluminium hydroxide produced a higher antitoxin response than simultaneous immunization using plain toxoid. The response was also better than that produced by plain toxoid alone.

2. A 24 hr. interval between the injection of plain toxoid and the subsequent injection of antitoxin gave no better results than when toxoid and antitoxin were given at the same time.

3. The response to simultaneous immunization, using adsorbed toxoid, of guinea-pigs sensitized to horse serum, was superior in the first 6 weeks to that of guinea-pigs which had not been sensitized.

4. The suggestion that simultaneous active and passive immunization of man should be performed with adsorbed toxoid rather than plain toxoid is supported by these results.

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