Vol. XXXIV, No. 2 (April, 1953) was issued on 24.iv.53.

THE BRITISH JOURNAL OF EXPERIMENTAL PATHOLOGY

VOL. XXXIV JUNE, 1953 NO. 3

INTERFERENCE WITH ANTITOXIC RESPONSES IN IMMUNISATION WITH COMBINED PROPHYLACTICS.

MOLLIE BARR AND MONA LLEWELLYN-JONES

From the Wellcome Research Laboratories (Biological Division), Beckenham, Kent.

Received for publication January 1, 1953.

In a previous paper (Barr and Llewellyn-Jones, 1953) we recorded experiments showing that interference with the response of animals to one component of a combined prophylactic occurred in certain circumstances In a single experiment, two groups of guinea-pigs received an injection of 0·05 ml. of T.A.B. vaccine (typhoid, paratyphoid A and B); 28 days later they received a dose of 0·05 ml. of a mixture of T.A.B. vaccine and tetanus toxoid (T.A.B.T.), which was repeated after a further 28 days. The second group received only two doses of 0·05 ml. of T.A.B.T. separated by an interval of 28 days. The tetanus antitoxin titres of blood samples taken 10 days after the second injection of T.A.B.T. were higher and less scattered in the second group than in the first.

In another experiment it was shown that guinea-pigs previously immunised with diphtheria toxoid had significantly lower tetanus antitoxin titres after immunisation with two doses of any of three combined diphtheria-tetanus prophylactics than normal guinea-pigs of the same weight. In an attempt to throw some light on the mechanism of the interference, caused apparently by the preliminary injections either of T.A.B. vaccine or diphtheria toxoid, we have carried out further experiments. This interference is a matter of practical importance. It is possible that in attempts to immunise against two or more diseases, two injections of a combined prophylactic might be given, and an additional dose of one component only, if the two doses were considered to produce insufficient immunity to this component. The spacing of such an extra dose, in relation to that of the injections of the combined prophylactic, might strongly influence the degree of immunity finally produced by both components of the preparation.

EXPERIMENTAL.

The Effect of a Preliminary Injection of T.A.B. Vaccine on the Response to Immunisation with Tetanus Prophylactics.

The following materials were used :

(*a*) T.A.B. vaccine containing 10^9 *S. typhi*, 5×10^8 each *S. paratyphi* A and B per ml., 0·5 per cent phenol.

(*b*) Crude formol tetanus toxoid, 9 Lf/ml., 0·5 per cent phenol.

(*c*) T.A.B.T., which was a mixture of the vaccine (*a*) and the toxoid (*b*).

The vaccine was added as a concentrated suspension, so that in the final mixture the toxoid was diluted about 5 per cent.

All injections were of 0·05 ml. diluted in saline to 1 ml. and administered subcutaneously. Eight groups of guinea-pigs of approximately equal weight were injected with one or more of these prophylactics given in different order. The scheme of injection is set out in Table 1.

TABLE I.—*The Tetanus Antitoxin Titres of Guinea-pigs after Immunisation with Two Doses of Tetanus Prophylactics.*

Group.	1st inj.	2nd inj.	Over / Under	Number of guinea-pigs with titres (units/ml.)— .. 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.	Geometric mean titre.
1	Toxoid	Toxoid		–	–	–	–	–	–	1	2	5	9	6	3	6·8
2	,,	T.A.B.T.		–	–	–	–	–	–	–	–	4	2	4	2	8·7
3	T.A.B.T.	Toxoid		–	–	–	–	–	–	–	1	2	5	4	1	7·7
4	,,	T.A.B.T.		–	–	–	–	–	–	–	1	9	8	4	6	7·9
5	Toxoid	Toxoid		–	–	–	–	–	–	–	3	3	6	3	–	5·0
6	,,	T.A.B.T.		–	–	–	–	–	–	–	2	3	7	2	1	5·9
7	T.A.B.T.	Toxoid		1	0	0	0	2	0	1	3	4	2	–	–	<1·1
8	,,	T.A.B.T.		1	0	0	0	1	1	1	0	7	3	–	–	<1·5

Groups 5–8 had received an injection of T.A.B. vaccine 28 days before immunisation with tetanus prophylactics.

Groups 1 to 4 received two injections of 0·05 ml. of tetanus toxoid and/or T.A.B.T. ; groups 5 to 8 received the same materials preceded by an injection of 0·05 ml. of T.A.B. vaccine. The interval between injections was in all cases 28 days. The guinea-pigs were bled 10 days after the last of the series of injections and the tetanus antitoxin content of the serum of each animal titrated by the mouse method (Glenny and Stevens, 1938). Titrations were made at approximately two-fold differences and the results are shown in Table 1. Geometric means were calculated, taking each titre as the geometric mean of the limits within which it lay.

There is no significant difference between the responses of groups 1 to 6, but it is evident that groups 7 and 8 gave an undoubtedly poorer response. This is shown by the lower geometric mean titre, and what is more significant, the greater scatter of values. It is clear that the poor response is associated, not with the effect of the previous injection of T.A.B. vaccine alone, but with the use of a primary stimulus for tetanus containing T.A.B. vaccine, for groups 5 and 6 (which received the preliminary T.A.B. injection) gave substantially the same response as groups 1 to 4, which received no preliminary injection.

The Effect of Pre-existing Diphtheria Immunity on the Response to Immunisation with Tetanus Prophylactics.

The materials used were :

1. Purified tetanus toxoid completely adsorbed on a mineral carrier, and containing 6 Lf/ml.
2. Combined diphtheria-tetanus toxoid similarly adsorbed on the same amount of carrier, and containing 60 Lf purified diphtheria toxoid and 6 Lf purified tetanus toxoid/ml.

The dose injected was 0·005 ml., and the interval between the two injections was 28 days. The guinea-pigs were bled 10 days after the second injection of tetanus or combined prophylactic. Two large groups of guinea-pigs were used. One consisted of animals immunised with two injections of diphtheria prophylactic some 6 months previously, the other of normal animals of approximately the same weight. These groups were further subdivided and injected as shown in Table II.

The results resemble those in Table I. When the primary stimulus to tetanus contained diphtheria toxoid, a much poorer response to tetanus toxoid was shown

TABLE II.—*The Tetanus Antitoxin Titres of Normal and Diphtheria-Immune Guinea-pigs after Immunisation with Two Doses of Prophylactic containing Tetanus Toxoid.*

Guinea-pigs.	1st inj.	2nd inj.	Number of guinea-pigs with tetanus antitoxin titres (units/ml.)— Over .. / Under	0·01.	0·02.	0·05.	0·1.	0·2.	0·5.	1·0.	2·0.	5·0.	10.	20.
				0·01.	0·02.	0·05.	0·1.	0·2.	0·5.	1·0.	2·0.	5·0.	10.	20.	50.
Normal	D + T	D + T		–	–	–	–	–	–	1	3	6	2	3	–
Diphtheria-immune	D + T	D + T		2	1	0	1	2	0	1	6	1	1	–	–
Normal	D + T	T		–	–	–	–	–	–	–	1	2	3	0	1
Diphtheria-immune	D + T	T		2	1	0	1	1	2	3	2	1	–	–	–
Normal	T	D + T		–	–	1	1	0	0	1	2	3	3	0	1
Diphtheria-immune	T	D + T		–	–	2	0	0	0	1	1	5	4	1	–

D + T = combined diphtheria-tetanus prophylactic.
T = tetanus prophylactic.

by guinea-pigs previously immunised with diphtheria toxoid than by normal guinea-pigs. It is, however, of interest that both groups injected with tetanus followed by combined prophylactic contained two poor responders, while the remaining animals had titres distributed over the same levels as the normal control groups above them in Table II. It is possible that this result could be accounted for by the weight of these guinea-pigs.

The average weight of these two groups of animals was 785 g., compared with 575 g. for those that received two injections of combined prophylactic and 675 g. for those injected with combined prophylactic followed by tetanus alone. The dosage injected, in terms of body weight, was therefore somewhat lower for the groups injected with tetanus prophylactic followed by the combined preparation. It is, however, possible that the presence of diphtheria toxoid in the first dose of prophylactic injected into the other four groups of guinea-pigs exerted a synergic effect upon the tetanus response, though when this prophylactic was injected in the same dosage into guinea-pigs of more normal weght (250–400 g.) no synergic effect was observed. The results of this test are shown in Table III.

TABLE III.—*The Tetanus Antitoxin Titres of Guinea-pigs* (250–400 *g.*) *after Immunisation with Two Doses of Tetanus Prophylactic and Diphtheria-Tetanus Prophylactic both containing* 6 *Lf/ml. Tetanus Toxoid.*

Prophylactic	Number of guinea-pigs with tetanus antitoxin titres (units/ml.)—				Geometric mean titre.
	Over 1.	2.	5.	10.	
	Under 2.	5.	10.	20.	
Tetanus . .	2 .	4 .	4 .	4 .	3·73
Diphtheria-tetanus	2 .	5 .	5 .	3 .	3·41

When the dosage in terms of body-weight was reduced, *i.e.*, the same dose injected into animals of about double the weight, a synergic effect might have been obtained. We have already shown that although the addition of a T.A.B. vaccine improved the response of guinea-pigs to immunisation with tetanus toxoid at one level of dosage, no synergic effect occurred when the dosage of the same combined preparation was increased (Barr and Llewellyn-Jones, 1953). It appears probable that the somewhat poor response of these animals in the last two groups of Table II was due to the effect of weight, for the general distribution of titres of the remaining animals is not typical of that shown by groups in which interference has occurred. In such groups the distribution tends to be more irregular and includes some very poor responders, while the peak value is lower than that of the controls.

Some groups of animals in which interference with a response had occurred were bled and tested again 28 days after the second injection in order to determine whether the response had been delayed. It was found that those guinea-pigs which had produced antitoxin by the 10th day after the second injection had given secondary responses, because a fall in titre occurred thereafter. It would therefore appear that the magnitude, not the speed, of the response had been reduced by interference.

It is clear that the interference with the tetanus response shown in Tables I and II must have been brought about by a secondary response to the T.A.B. vaccine or diphtheria toxoid present in the first injection of combined prophylactic. Interference with the development of potential immunity to tetanus toxoid might have occurred through crowding out of the immunity-producing mechanism by a speedy secondary response to the other component. Alternatively or in addition this response might still have been actively occurring at the time of the second injection of tetanus toxoid or combined prophylactic : this might have reduced the secondary response to the tetanus antigen. Thus interference may have occurred with the first or the second or both injections of the tetanus toxoid component, as a result of the superior speed or the prolonged effect of the secondary response to the other antigen present in the first dose of combined prophylactic. In view of the fact that in our earlier experiment (Barr and Llewellyn-Jones, 1953) tetanus antitoxin production was better in diphtheria-immune than in normal animals after the *first* injection of combined diphtheria-tetanus toxoid, it is perhaps more reasonable to believe that most if not all of the interference was with the second injection. It cannot however be assumed that antitoxin production after primary stimulation constitutes a measure of the degree of potential immunity established.

The Effect of an Injection of Vaccine between Two Injections of Combined Prophylactic.

This experiment was concerned with the effect on tetanus antitoxin production of an injection of T.A.B. vaccine given between two injections of T.A.B.T. The batches of these antigens were the same as those used in the first experiment, in which the additional injection of vaccine was given before the course of combined immunisation.

Four groups of guinea-pigs of comparable weight were used. Two of these groups received two injections, each of 0·02 ml. T.A.B.T., separated by an interval of 6 weeks, and the other two had doses of 0·05 ml. given at the same interval. One group at each level of dosage received in addition an injection of 0·02 or 0·05 ml. T.A.B. vaccine 4 weeks after the first injection. All the animals were bled 10 days after the second injection of T.A.B.T. and their tetanus antitoxin titres are shown in Table IV.

In both series the intermediate injection of vaccine had a beneficial effect on the response, although at the higher level of dosage the difference between the geometric means of the titres for the two groups is not significant (the *t*-test applied to the logarithms of the titres gives $0{\cdot}05 < P < 0{\cdot}10$). The scatter of titres shown by the control group 1, injected with doses of 0·02 ml., was considerably greater than that of group 2, which received an additional dose of vaccine

TABLE IV.—*Tetanus Antitoxin Titres of Guinea-pigs after Immunisation with Two Doses of T.A.B.T. given at an Interval of 6 Weeks.*

Group.	Dose.	Number of guinea-pigs with titres (units/ml.)— Over / Under 0·02. 0·05. 0·1. 0·2. 0·5. 1·0. 2·0. 5·0. 10. 20. / 0·05. 0·1. 0·2. 0·5. 1·0. 2·0. 5·0. 10. 20. 50.	Geometric mean titre.	*t*.	*P*.
1	0·02	2 1 1 0 0 4 2 1 0 1	0·818	2·249	0·02–0·05
2	0·02	– – – – 1 2 6 3 2 –	3·73		
3	0·05	– – – – – – 3 5 4 –	7·29	1·893	0·05–0·10
4	0·05	– – – – – – – 6 4 2	11·44		

Groups 2 and 4 received in addition an injection of T.A.B. vaccine 4 weeks after the first injection of T.A.B.T.

only; there is a difference between the geometric means for the two groups. It is possible that this was due to some neutralisation of the T.A.B. antigen in the second dose of T.A.B.T. by circulating antibodies produced in response to the injection of vaccine alone, given 14 days earlier. It is probable that this second dose of T.A.B.T. was given at a time when the titre of such antibodies was at its height. If this were the case, groups 2 and 4 in Table IV would receive in effect a dose of combined prophylactic in which the tetanus toxoid was in considerable excess, while groups 1 and 3, having had no injection of either component for 6 weeks, received a dose of a well-balanced mixture. If this explanation is correct, it is possible that the T.A.B. component of the second dose of combined prophylactic may have had little antigenic effect, and that the synergic effect on one component may have occurred at the expense of the response to the other. If the time interval between the injection of vaccine and the second injection of combined prophylactic had been longer a different result might have followed, for the titre of circulating antibodies to the T.A.B. vaccine might have fallen.

DISCUSSION.

It is evident from the results presented in Tables I and II that interference with the development of immunity to tetanus toxoid may occur in animals immunised with two injections of a combined prophylactic containing another antigen to which they have some pre-existing immunity. Similar interference occurred when the first injection was of the combined prophylactic and the second injection of tetanus toxoid, but not when the order of these injections was reversed.

Thus it would appear that if a primary injection of one antigen has already been given, satisfactory reinforcement of the immunity to it and primary immunisation with another antigen can be brought about by giving one injection of the new antigen alone followed later by a dose of combined prophylactic. If an adequate dose of the new antigen is used interference with the response would not be expected to occur.

It is probably not generally realised that the antigenic effect of heterologous antitoxic protein can interfere with the development of immunity to toxoid in combined active and passive immunisation : such interference is distinct from, though it may be additional to, any that may occur through partial neutralisation of the toxoid by its specific antitoxin.

One of us (M.B.) was concerned in an investigation of methods for the protection of army personnel against tetanus. It was found that the response of previously unimmunised men to two injections of tetanus toxoid was reduced if they received a dose of 500 units of tetanus antitoxin at the same time as the first injection of toxoid, but in a different site. This result has already been reported briefly (Sachs, 1952). A comparison of the distribution of the titres of these men with those of a control group who had no injection of antitoxin suggested that some interference was due to a reduction in the effective primary stimulus through partial neutralisation of the toxoid by antitoxin. Some of the active-passive group, however, showed accelerated elimination of the injected antitoxin, and their response to the tetanus toxoid was poorer than that of men who showed a normal rate of elimination. It was therefore suggested that precipitin formation had brought about interference with the response of these men to immunisation with tetanus toxoid, and indeed some of those who eliminated the antitoxin with abnormal rapidity were thought to have had a previous injection of horse serum, and might therefore have responded rapidly to the injection given in combined active and passive immunisation. The full results of this work will be the subject of a joint report to be published by the War Office. It was this finding that led us to do the experiments on interference in combined prophylaxis, for it suggested that one component of a combined antigen might confer little benefit on persons who had little or no basal immunity to it, but well-developed basal immunity to another component of the antigen. There appears to be a similarity between the results of this active-passive work and of the experiments on combined prophylactics that we have recorded here. Interference with primary immunisation with toxoid occurred by preferential response to another antigen administered simultaneously with the first injection.

Mention has also been made (Sachs, 1952; Barr and Sachs, 1952) of interference (due to precipitin formation) with the response of an actively immunised man to

a boosting dose of tetanus toxoid given at the same time as an injection of 500 units of tetanus antitoxin. This man had had an earlier injection of horse serum, and thus had immunity to both tetanus toxoid and horse protein.

An animal with good potential immunity to an antigen A and poor potential immunity to another antigen B might respond sufficiently rapidly to A after an injection of A + B, for interference with antibody formation to B to occur. If, however, the immunity to both components was of a high order, and there was a considerable amount of circulating antibody to A and no antibody but good potential immunity to B, a reversed result might conceivably occur because of neutralisation of antigen A by circulating antibody after injection. It is therefore possible that the presence or absence of circulating antibody to one component might play a considerable part in determining whether an increased or decreased response to another component would occur after an injection of both antigens. The man referred to by Barr and Sachs had a high titre of actively produced tetanus antitoxin at the time of the injections of tetanus toxoid and antitoxin given in combined active and passive immunisation. He probably, however, had no circulating precipitin, as he had had no injection of horse antitoxic protein for some years. Neutralisation of some of the toxoid by circulating antitoxin, thus reducing the effective dose of tetanus toxoid, may have facilitated a preferential secondary response to the dose of horse protein administered at the same time.

It is reasonable to suppose that both dosage and spacing of injections may affect the responses in combined prophylaxis. In an unfinished experiment we have found that a preliminary injection of *H. pertussis* vaccine may bring about interference with the diphtheria response to immunisation with two doses of a combined alum-precipitated diphtheria-pertussis prophylactic at a certain level of dosage. When the dosage of both antigens is increased considerably, no interference occurs. The interference at low dosage may be due to crowding-out by the secondary response to the vaccine contained in the first dose of combined prophylactic. The absence of interference at high dosage might be due to a reduction in the effect of the vaccine in this dose through neutralisation by very large amounts of circulating antibody produced in primary stimulation, or to the dosage of diphtheria A.P.T. being sufficient to overcome the crowding-out by the pertussis responses.

If the interference with response to tetanus toxoid, shown in our experiments, was due to reduction of the secondary response to the second dose by prolonged immunological activity connected with the secondary response to another antigen in the first dose of combined prophylactic, this interference might be reduced or abolished by delaying the second dose of toxoid. More work is needed on the spacing of injections and on the application of these considerations to human prophylaxis.

It is perhaps a matter of interest that a secondary response to T.A.B. vaccine, to horse antitoxic protein and to diphtheria toxoid, can all interfere with immunisation with tetanus toxoid. Little is known of the character of the responses of guinea-pigs to subcutaneous injections of bacterial vaccines, or indeed of the nature and number of antibodies produced thereby. It is known, however, that certain antibacterial bodies developed in horses after intravenous injections of cultures such as pneumococci are not associated with the same serum protein as the antitoxins. It would appear that the production of one particular kind

of antibody may interfere with the production of another, even though these two antibodies may represent modifications of different serum proteins.

SUMMARY.

Interference with the development of immunity to tetanus toxoid occurred in animals immunised with two injections of a combined prophylactic containing another antigen to which they had some immunity Similar interference occurred when the first injection was of the combined prophylactic and the second injection of tetanus toxoid alone, but not when the order of these injections was reversed.

This interference was due to the secondary response to the other component of the combined prophylactic, and occurred whether this was T.A.B. vaccine or diphtheria toxoid.

An injection of vaccine given 14 days before the second of two injections of a combined prophylactic containing the same vaccine resulted in an increased response to the toxoid component of the prophylactic.

We are indebted to Brigadier A. Sachs, C.B.E., M.D., for permission to refer to the results of combined active and passive immunisation of man. We should like to thank Mr. P. A. Young for statistical work on Table IV, and Mr. A. T. Glenny, F.R.S., for his help in discussing the results.

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