COMBINED PROPHYLACTICS

VARIATIONS IN RESPONSE, IN CHILDREN, TO DIPHTHERIA TOXOID PRODUCED BY ADDITION OF TETANUS TOXOID AND *H. PERTUSSIS* VACCINE

BY

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There are three recognized ways in which the immunological response to a primary dose or course of an antigen may possibly be reduced (Parish, 1955). These are: (1) the presence of passive immunity, as in the case of maternally bestowed specific antibody in very young infants; (2) the giving of a primary stimulus of one antigen simultaneously with a secondary dose of another similar antigen; and (3) an imbalance of antigens in a mixture—for example, in animals an excess of tetanus toxoid may seriously reduce the response to the diphtheria toxoid component of a mixture (Barr and Llewellyn-Jones, 1953) while an excess of pertussis vaccine depresses the diphtheria antitoxin response as compared with a maximum obtained from a mixture containing less vaccine when a fixed dose of fluid diphtheria toxoid is employed (Barnes and Holt, 1955). On the other hand, the response to a primary dose or course of fluid toxoid may be significantly increased by admixture with a vaccine—for example, pertussis vaccine and diphtheria toxoid, or typhoid vaccine and tetanus toxoid. Moreover, Ungar (1956) found that the addition of diphtheria and tetanus toxoids to pertussis vaccine had no adverse effect on the immunity response of mice to injections of the mixture as compared with results obtained when the plain vaccine was used.

Little is known of the effect on the response to the diphtheria toxoid of variations in the ratio of vaccine to toxoid in children, and even less in respect of triple mixtures.

The primary purpose of the present work was to observe, in children, the effect on the response to a fixed amount of purified fluid diphtheria toxoid (30 Lf), of the addition of different quantities of (1) *H. pertussis* vaccine, and (2) purified tetanus toxoid. The criterion adopted was the conversion rate obtained by the Schick test (S.C.R.) measured one month after one dose. All children used in this work were under 2 years of age, originally Schick-positive, and each group was carefully balanced for age. All injections were given by the subcutaneous route, and all Schick tests were performed with Wellcome toxin and control. It should perhaps be mentioned that the work reported in this paper progressed over a period of time exceeding two years, which accounts for the use of different primary reagents at various stages. Each investigation in which comparisons are made, however, involved the use of identical antigens and, of equal importance, were self-contained.

First Investigation

Part 1

This embraced the use of varying doses of *H. pertussis* vaccine in combination with a single sample of purified diphtheria toxoid (Batch No. E. 3977; 1,900 Lf/mg. P.N.). The results (Table I) show most clearly that the adjuvant

action of the vaccine increases progressively with increase of dose of vaccine, and with no hint of an optimum ratio of vaccine to toxoid, up to the maximum of *H. pertussis* vaccine dosage used—that is, 20,000 million.

TABLE I.—Effect of Varying Pertussis Dosage with 30 Lf of Diphtheria Toxoid on S.C.R., One Month After One Dose. All Children Under 2 Years of Age, Originally Schick-positive

Diphtheria Toxoid Dosage	<i>H. pertussis</i> Organisms	No. of Cases	Post-Schick		S.C.R.
			Positive	Negative	
30 Lf (control)	None	124	62	62	50.0%
30 Lf ..	5,000 mil.	101	30	71	70.3%
30 Lf ..	10,000 ..	109	24	85	78.0%
30 Lf ..	20,000 ..	101	5	96	95.0%

Part 2

This was to ascertain to what extent, if any, the addition of different doses of purified tetanus toxoid altered the response to the fixed dose of diphtheria toxoid. A purified tetanus toxoid (Batch No. C.T. 87/142; 1,100 Lf/mg. N.) was used for these mixtures. The groups of children tested were in every way comparable to those in Table I. The results (Table II) show that in these children the purified

TABLE II.—Effect of Varying the Dose of Tetanus Toxoid Added to 30 Lf of Diphtheria Toxoid, on S.C.R., One Month After One Dose

Diphtheria Toxoid Dosage	Tetanus Toxoid Dosage	No. of Cases	Post-Schick		S.C.R.
			Positive	Negative	
(1) 30 Lf (control)	None	124	62	62	50.0%
(2) 30 Lf	3 Lf	105	53	52	49.5%
(3) 30 Lf	6 Lf	111	51	60	54.1%
(4) 30 Lf	12 Lf	111	40	71	64.0%
(5) 30 Lf	25 Lf	111	53	58	52.3%

χ^2 mixture 4/control = 4.66, d.f. = 1, P = 0.05.

tetanus toxoid had a significant adjuvant effect in the ratio of 12 Lf tetanus toxoid to 30 Lf diphtheria toxoid, but when the dose of tetanus toxoid was increased to 25 Lf the response to the diphtheria toxoid component was only approximately equal to the control—that is, 30 Lf plain diphtheria toxoid.

From the results shown in Table II it appears that the addition of purified tetanus toxoid improved the diphtheria antigenicity of the mixture up to a point. Without a series of cases in which the dose of tetanus toxoid lies between 12 and 25 Lf it is not possible to infer exactly at what dose the response diminishes. However, on account of considerations mentioned below, this point is of no more than theoretical interest. The important point is that the dose of tetanus toxoid should not be too high relative to that of diphtheria toxoid.

Part 3

Having shown (Tables I and II) that both pertussis vaccine and tetanus toxoid independently increased the antigenicity of diphtheria toxoid, it was decided to investigate the further series of comparable infants inoculated with a mixture corresponding to the current-issue composition of triple prophylactic. The same primary reagents were used and the tests were performed as before, one month after a single injection. For the sake of comparison, certain results have been taken out of the other two tables and set forth again, these being the mixtures which approximate most closely to those employed in the triple prophylactic.

The results (Table III) show that both pertussis vaccine and tetanus toxoid, when added to diphtheria toxoid separately and used as dual prophylactics, tended to improve the behaviour of the diphtheria toxoid, but when both were added together and used as a triple antigen the S.C.R. fell from 95% in the case of the diphtheria-pertussis mixture to 83.6% for the triple antigen.

It seemed possible that the improvement in the diphtheria toxoid antigenicity produced by the addition of pertussis vaccine was due to adsorption of some of the toxoid on to

TABLE III.—Comparative Efficiency of 30 Lf of Diphtheria Toxoid Alone and Combined with Pertussis Vaccine and Tetanus Toxoid. S.C.R. One Month After One Dose

Material	Diph. Toxoid	<i>H. pertussis</i> Organisms	Tetanus Toxoid	No. of Cases	Post-Schick		S.C.R.
					Pos.	Neg.	
1. F.T. control	30 Lf	None	None	124	62	62	50.0%
2. Diph. and tet.	30 Lf	„	6 Lf	111	51	60	54.1%
3. Diph. and pert.	30 Lf	20,000 mil.	None	101	5	96	95.0%
4. Diph., pert., and tetanus	30 Lf	20,000 mil.	5 Lf	110	18	92	83.6%

χ^2 mixture 3/mixture 4=7.05, d.f.=1, P=0.01.

the vaccine despite the failure to demonstrate any adsorption, employing ordinary laboratory procedures to measure toxoid.

In the routine preparation of triple antigen, the diphtheria and tetanus toxoids are mixed before addition to the vaccine. If the tetanus toxoid was more readily adsorbed on to the vaccine than the diphtheria toxoid, the latter might be left at an antigenic disadvantage. To obtain further information on this point it was therefore decided to prepare triple prophylactic in three different ways.

Second Investigation

For these mixtures a fresh lot of primary reagents was used (diphtheria toxoid Batch No. B.P. 51/53, 2,050 Lf/mg. P.N., and tetanus toxoid B.P.T. 3, 1,130 Lf/mg. T.N., and a fresh stock of *H. pertussis* vaccine). The mixtures were made in the following three different ways, but always giving a final product containing per ml. (one dose): 30 Lf of purified diphtheria toxoid, 5 Lf of purified tetanus toxoid, and 20,000 million *H. pertussis* organisms.

Sample A.—Diphtheria toxoid was added to half the volume of *H. pertussis* vaccine. Tetanus toxoid was added to the other half-volume of vaccine. After three days the two lots were mixed and bottled.

Sample B.—Tetanus toxoid was added to the *H. pertussis* vaccine. After three days the diphtheria toxoid was added, and the mixture bottled.

Sample C.—Diphtheria toxoid was added to *H. pertussis* vaccine. After three days the tetanus toxoid was added and the mixture bottled.

These three mixtures were tested as before, the results being shown in Table IV.

TABLE IV.—Results of Post-Schick Tests One Month After a Single Injection of Triple Prophylactic Prepared in Three Different Ways

Mixture	No. of Cases	Post-Schick		S.C.R.
		Pos.	Neg.	
A. (F.T. + $\frac{1}{2}$ vacc.) + (T.T. + $\frac{1}{2}$ vacc.)	150	25	125	83.3%
B. (T.T. + vacc.) + F.T.	166	33	133	80.0%
C. (F.T. + vacc.) + T.T.	145	26	119	82.0%

It will be seen that the S.C.R. from the mixture made nearest to the "normal" way (mixture A) is almost exactly that found with the previous set of primary reagents. There is no significant difference between mixture A and the other two: these findings therefore show that the method of preparing the triple prophylactic does not affect the immunological response to it, but they endorse the conclusion that the addition of 5 Lf of tetanus toxoid to a mixture of 30 Lf of diphtheria toxoid and 20,000 million *H. pertussis* produces a significant reduction in the S.C.R.

Third Investigation

From the preceding results it can be inferred that the addition of 5 Lf of tetanus toxoid in some way militates against the most effective diphtheria response. One explanation of

this could be that the triple antigen constitutes a stimulus which is beyond the capacity of the local antibody-forming tissues to handle adequately.

An attempt to solve this problem was made by utilizing different sites for different antigens. The diphtheria toxoid mixed with 20,000 million *H. pertussis* organisms had already given an excellent result. Thus it seemed reasonable to inject this into the left arm, and, at the same time, to administer the appropriate dose of tetanus toxoid separately into the right arm. On a further group precisely the same materials were mixed as a triple antigen and given in one arm. Finally, a third, or control, group was necessary, consisting of children inoculated with the same mixture of diphtheria toxoid and *H. pertussis* vaccine but without any administration of tetanus toxoid.

If the method in which separate arms are used for the diphtheria-pertussis mixture on the one hand and tetanus toxoid on the other gives an S.C.R. which approximates to that obtained from the diphtheria-pertussis mixture alone, it seems logical to assume that the lower S.C.R. following the use of triple antigen is due to overloading of the local immunity mechanism. If, however, the result from administration into separate arms is still poor, it could then be inferred that one is asking too much of the whole body defensive mechanism at one time. The primary reagents used in this third investigation were (1) purified diphtheria toxoid Batch No. B.P. 51/53 as before, (2) purified tetanus toxoid Batch No. C.T./5, 1,150 Lf/mg. N., and (3) a fresh batch of pertussis vaccine common to all the mixtures. The results of this investigation are shown in Table V.

TABLE V.—Effect of Administering Diphtheria Pertussis Prophylactic Into One Arm and Tetanus Toxoid Into the Other, Controlled by a Group in Which the Triple Prophylactic is Given Into One Arm. S.C.R. One Month After One Dose

Group	Left Arm	Right Arm	No. of Cases	Post-Schick		S.C.R.
				Pos.	Neg.	
A	30 Lf diphtheria toxoid plus 20,000 mil. <i>H. pertussis</i>	Nil	120	15	105	87.5%
B	30 Lf diphtheria toxoid plus 20,000 mil. <i>H. pertussis</i>	5 Lf tetanus toxoid	128	17	111	86.7%
C	30 Lf diphtheria toxoid plus 20,000 mil. <i>H. pertussis</i> plus 5 Lf tetanus toxoid	Nil	138	31	107	77.5%

χ^2 Group C/Group A=4.34, d.f.=1, P=0.05.

χ^2 Group C/Group A+B=5.97, d.f.=1, P=0.02.

Comment

Two points arise from these results: The first is that, although different primary reagents were employed in the first and second investigations, the results from the two triple prophylactics were virtually identical. On the other hand, in the third investigation the result from the triple mixture (Table V, Group C) was significantly lower at 77.5%, and similarly with the double mixtures from 95% in the first investigation to 87.5% in the third. The most probable reason for these differences is thought to be due to variations, from batch to batch, in the adjuvant properties of the pertussis vaccine. The second point is the finding that there is no significant difference between the diphtheria responses in children receiving (1) diphtheria-pertussis mixture in one arm and tetanus toxoid in the other, and (2) those receiving the diphtheria-pertussis mixture only. But the diphtheria responses from those children receiving the triple prophylactic are significantly lower—77.5% against 87.5%.

Although the third investigation was designed to show whether or not the giving of a triple prophylactic constituted an overloading of the local immunity mechanism of the child, the findings do not enable us to arrive at a definite conclusion, despite the fact that the addition of 5 Lf of tetanus toxoid to the diphtheria-pertussis mixture does cause a significant fall in the S.C.R. Since one triple prophylactic mixture gave rise to an S.C.R. of 83.6% and another, of simi-

lar composition, to only 77.5% we must concede that 83.6% is at least attainable. When, therefore, we do not obtain 83.6% we must blame the preparation and not the immunity mechanism of the child. And yet with *both* triple prophylactic mixtures the omission of the tetanus toxoid component resulted in a significantly greater S.C.R. It would seem possible that the triple prophylactic does not overload the immunity mechanism but that the adjuvant action of the vaccine is shared by two toxoids, of which one, the tetanus toxoid, is apparently a more potent antigen than the other. Utilizing an equation relating geometric mean titre to % S.C.R. (Holt, 1955), it is calculated that the addition of 20,000 million *H. pertussis* organisms to the 30 Lf of diphtheria toxoid increases the mean antitoxin response some 14 times and that the further addition of 5 Lf of tetanus toxoid reduces the increment to about fivefold. It may be that the answer to this difficulty will be to reduce the tetanus toxoid to 2 Lf per dose.

Discussion

Immunization against whooping-cough cannot reasonably be withheld in the very early months of life, because it is then that pertussis is most dangerous to the child, and this consideration certainly outweighs the very small risk of provoking paralytic poliomyelitis (Bousfield, 1951). The measure of the increase in risk entailed by adding fluid toxoid to the vaccine is still not by any means precisely known, but the long-term risk in a severe falling off in the immunization rate against diphtheria is one to be taken very seriously.

It would seem that in the case of very young infants the procedure which would incur the absolute minimum risk of causing post-inoculation poliomyelitis would be to inoculate them in one arm with plain pertussis vaccine and fluid toxoid in the other on each of three visits. This would keep to a minimum the number of clinic attendances and would very materially help to preserve a high immunization rate against diphtheria. The reduction in efficiency of immunization against diphtheria by adopting such a procedure would, in comparison with two doses of P.T.A.P., be marked, but a high percentage of the child population would be immunized well. Furthermore, there would seem to be no reason for not using mixed fluid tetanus and diphtheria toxoids in place of diphtheria toxoid only. In our opinion, every effort should be made to encourage immunization of children against tetanus in order to avoid the several disadvantages attending the use of tetanus antitoxin.

In view of the importance of primary immunization of infants against diphtheria, and the necessity for protection against whooping-cough during the first year of life, the only reason for withholding inoculations on the lines suggested would appear to be the existence of a local epidemic of poliomyelitis.

In respect of booster doses the problem is very much less complicated, in that there is no urgency, and one can choose the time of year. The period of minimum risk is, of course, during the winter, and, since the poliomyelitis provocation risk is then usually very low, some freedom in the choice of reagent is possible. Clinical experience lasting over several years and involving considerable numbers of children has shown that a subcutaneous injection of P.T.A.P. diluted 1 in 5 in 0.5-ml. dosage causes negligible allergic reactions, whatever may be the materials used for primary immunization.

The reason for the relative freedom from allergic reactions when using this diluted P.T.A.P. would seem to be that in P.T.A.P. the toxoid is adsorbed on to aluminium phosphate, and is only partly liberated after injection; when diphtheria toxoid is employed for boosting, all the toxoid is immediately available for the production of allergic manifestations in a sensitive subject. We have seen one or two most disturbing reactions following the use of diphtheria toxoid for boosting. The use of adrenaline has twice been most necessary. The 0.5-ml. dose of 1 in 5 P.T.A.P. (equal to 0.1 ml. of P.T.A.P.) has proved adequate to restore the Schick-negative state in every child of pre-school age who had relapsed to positive.

Although many may feel attracted by the idea of a multiple prophylactic incorporating diphtheria toxoid, pertussis vaccine, and poliovirus vaccine, with or without the addition of tetanus toxoid, it is clear from the findings in this present communication that precise exploratory field trials are necessary in order to determine the best balance of antigens in any new mixture to be used in man. The complete answer to the problem of post-inoculation paralysis would be to immunize against poliomyelitis before 3 months of age, provided such a procedure is shown to be efficient.

Summary

This study is concerned with the field efficiency of mixed antigens where the point of reference was the diphtheria response in children receiving one dose of 30 Lf of purified diphtheria toxoid measured one month after one inoculation.

It was found that the addition of *H. pertussis* vaccine to the 30 Lf of diphtheria toxoid enhanced the Schick conversion rate progressively with increase of dose of vaccine—from 50% conversion rate with no vaccine to 95% with 20,000 million *H. pertussis* microbes. There was no evidence of excess of vaccine although there is evidence that different batches of vaccine may have different toxoid-adjuvant properties.

The addition of purified tetanus toxoid to the constant amount of diphtheria toxoid also increased the S.C.R. up to an optimum of about 12 Lf of tetanus toxoid/30 Lf of diphtheria toxoid. The addition of 25 Lf of tetanus toxoid resulted, however, in a Schick conversion rate only equal to that from the diphtheria toxoid alone. The adjuvant action of the tetanus toxoid was less marked than that of *H. pertussis* vaccine.

The addition of 5 Lf of tetanus toxoid to a diphtheria toxoid/pertussis mixture resulted in a significantly lower S.C.R. than that obtained without the presence of the tetanus toxoid. It is calculated that the addition of *H. pertussis* vaccine (20,000 million organisms) increases the antitoxin response to the diphtheria toxoid some 14 times, and that the further addition of 5 Lf of tetanus toxoid to the mixture reduces that increment to about fivefold.

The inoculation of tetanus toxoid separately into one arm and the diphtheria-pertussis mixture into the other produced an S.C.R. identical to that obtained when the diphtheria-pertussis mixture was administered without any tetanus toxoid.

The order of mixing the components of the triple prophylactic produced no difference in the conversion rate.

The findings and their practical application are discussed; possible explanations of some of the phenomena observed are offered.

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