COMBINED ACTIVE AND PASSIVE IMMUNIZATION AGAINST DIPHTHERIA

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In a previous paper (Downie, Glenny, Parish, Smith & Wilson, 1941) the literature on combined active and passive immunization against diphtheria was briefly reviewed, and a description was given of our own observations on a total of about 300 students at Oxford, Cambridge and Sheffield. It was found that, when 0·1 and 0·3 ml. doses of alum-precipitated toxoid (A.P.T.) at 4 weeks' interval were used, the simultaneous injection into the opposite arm of 400–500 units of diphtheria antiserum with the first dose of A.P.T. led to a delay and to a slight degree of inhibition in the formation of antitoxin. The final degree of immunity, however, did not appear to be much inferior to that resulting from active immunization alone.

The purpose of the present paper is to record three additional sets of observations: (1) on increasing the dosage of A.P.T. from 0·1 and 0·3 ml. to 0·3 and 0·3–0·5 ml.; (2) on reducing the interval between the doses of A.P.T. from 4 to 2 weeks; and (3) on increasing the dose of diphtheria antiserum from 400–500 units to 5000 units.

The technique used was essentially that described in our previous paper. The subjects under investigation were third-year medical students, mostly of 20–22 years of age, who gave no history of diphtheria or of previous immunization against it. They were first of all bled and Schick-tested; further observations were made only on those who gave a positive Schick reaction and who had less than 1/1000 unit per ml. of antitoxin in their serum. The subjects were then divided alternately, without any selection, into two groups. The first group (A) was given active immunization only with A.P.T.; the second group (A + P) was given a single dose of diphtheria antiserum simultaneously with the first dose of A.P.T., the two injections being made into opposite arms. Four weeks after the first dose of A.P.T., all subjects were bled again, and given a second dose of A.P.T. They were bled for the third time 4 weeks, and for the fourth time either 6–8 or 10–14 weeks, after the second dose of A.P.T. At the time of the last bleeding, they were given a final Schick test. All Schick-test toxin, A.P.T. and diphtheria antiserum were supplied by Messrs Burroughs Wellcome. The results of the second bleeding, at the time of the second injection of A.P.T., will be omitted from the following tables, partly to economize space and partly because they do not contribute any important information to the comparison we wish to make.

EFFECT OF INCREASING THE DOSAGE OF A.P.T.

In the series of observations recorded by Downie *et al.* (1941), two doses of A.P.T., 0·1 and 0·3 ml., were given at 4 weeks' interval. In the present series these doses were increased to 0·3 and 0·3–0·5 ml. respectively, the total amount of A.P.T. injected being therefore 1½ to 2 times as great as formerly. The dose of antiserum given to the A + P students was 500 units. In Tables 1–3 the results in this series are compared with those in the previous series. Table 1 shows no appreciable difference between the two series in the antitoxin content of the blood serum 6–8 weeks after the second inoculation in either the A or the A + P group. Table 2 shows an insignificant difference in the antitoxin content of the blood serum 10–12 weeks after the second inoculation in the A group. In the A + P group there is a difference in favour of the students receiving the larger dosage of A.P.T., but, as the odds against observing such a difference by chance alone are only 15 to 1, it cannot be regarded as more than suggestive. The Schick-test results summarized in Table 3 again show an insignificant difference in the A group, but a very suggestive difference, on the border line of significance, in the A + P group.

As a whole, the results show that the effect of increasing the dosage of A.P.T. in the actively immunized group was practically negligible. Such differences as there were are slightly in favour of the larger dosage; but, as different batches of A.P.T. were used for the 1940 and the 1941–2 students, and as the comparison was made between students

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Table 1. *Comparison of antitoxin content of serum 6–8 weeks after second inoculation between students in 1940 inoculated with 0·1 and 0·3 ml. of A.P.T. and students in 1941–2 inoculated with 0·3 and 0·3–0·5 ml. of A.P.T.—both at 4 weeks' interval. Both the A + P groups had a dose of 400–500 units of antitoxin at the time of their first A.P.T. injection*

Antitoxin units per ml. of serum	1940 No.	1940 %	1941–2 No.	1941–2 %	Total
		Group A			
2–1/25	58	61·1	12	57·1	70
1/50–1/250	21	22·1	5	23·8	26
1/500–<1/1000	16	16·8	4	19·0	20
Total	95	100·0	21	99·9	116
	$\chi^2 = 0{\cdot}114$, $P = 0{\cdot}95$.				
		Group A + P			
2–1/25	21	28·8	6	28·6	27
1/50–1/250	24	32·9	7	33·3	31
1/500–<1/1000	28	38·4	8	38·1	36
Total	73	100·1	21	100·0	94
	$\chi^2 = 0{\cdot}002$, $P = 0{\cdot}999$.				

A = active immunization; A + P = combined active and passive immunization.

Table 2. *Comparison of antitoxin content of serum 10–12 weeks after second inoculation between students in 1940 inoculated with 0·1 and 0·3 ml. of A.P.T. and students in 1941–2 inoculated with 0·3 and 0·3–0·5 ml. of A.P.T.—both at 4 weeks' interval. Both the A + P groups had a dose of 400–500 units of antitoxin at the time of their first A.P.T. injection*

Antitoxin units per ml. of serum	1940 No.	1940 %	1941–2 No.	1941–2 %	Total
		Group A			
2–1/25	28	54·9	29	56·9	57
1/50–1/250	13	25·5	17	33·3	30
1/500–<1/1000	10	19·6	5	9·8	15
Total	51	100·0	51	100·0	102
	$\chi^2 = 2{\cdot}218$, $P = 0{\cdot}34$.				
		Group A + P			
2–1/25	12	23·5	20	40·8	32
1/50–1/250	26	51·0	24	49·0	50
1/500–<1/1000	13	25·5	5	10·2	18
Total	51	100·0	49	100·0	100
	$\chi^2 = 5{\cdot}597$, $P = 0{\cdot}064$.				

inoculated in different years, it is impossible to say whether the larger dosage of A.P.T. was really superior to the smaller dosage previously given.

In the combined actively and passively immunized group, on the other hand, the differences between students receiving the larger and those receiving the smaller dosage of A.P.T., though barely significant, are highly suggestive. They are rendered even more so when it is considered that some of the 1940 batches of students were injected with only 400 units of diphtheria antiserum, whereas all the A + P students in the 1941–2 batches were injected with 500 units. The results are in accord with the view

Table 3. *Comparison of final Schick tests 10–12 weeks after second inoculation between students in 1940 inoculated with 0·1 and 0·3 ml. of A.P.T. and students in 1941–2 inoculated with 0·3 and 0·3–0·5 ml. of A.P.T.—both at 4 weeks' interval. Both the A + P groups had a dose of 400–500 units of antitoxin at the time of their first A.P.T. injection*

Groups	Doses in ml. at 4 weeks' interval	No. tested	No. positive	% S.C.R.
		Group A		
1940	0·1 and 0·3	50	2	96·0
1941–2	0·3 and 0·3–0·5	48	0	100·0
		Group A + P		
1940	0·1 and 0·3	52	8	84·6
1941–2	0·3 and 0·3–0·5	47	1	97·9

S.C.R. = Schick-conversion rate.

Series 1940 and 1941–2. Observed difference between A groups = 4·0 %. S.E. of this difference = 2·86 %. Observed difference divided by S.E. of difference = 1·4.

Series 1940 and 1941–2. Observed difference between A + P groups = 13·3 %. S.E. of this difference = 5·79 %. Observed difference divided by S.E. of difference = 2·3.

that the effect of the antiserum is to neutralize to some extent the immunizing effect of the A.P.T., so that the antitoxic response of the subject is less than it would have been in the absence of an antiserum injection. If this is so, then the larger the initial dose of A.P.T. given, the greater should be the amount of unneutralized antigen, and the greater, therefore, the amount of antitoxin developed.

It seems probable that a proportion of the first dose of A.P.T. is absorbed while there is still sufficient circulating antitoxin to neutralize its antigenic effect. The result of this will be to diminish the total antigenic stimulus and to delay the time at which it comes into action. A person injected, therefore, with two doses of A.P.T. in the A + P

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group will behave as if a reduced dose had been given a short time before the second dose; provided his tissues had not been previously sensitized to diphtheria toxoid, such a person might then react virtually as if to a single dose. This view, which is put forward quite tentatively, would explain why the difference in antitoxin response of the two A + P groups recorded in Tables 1–2, did not become manifest till 10–12 weeks after the second inoculation. Further support is afforded by a comparison of the 1942–3 A + P group in Table 4 of this paper with that of the A group in Tables II and IV of our first paper (Downie *et al.* 1941), from which it is seen that in the A + P group, the secondary response, 4 weeks after the second injection, to two doses of 0·3 and 0·5 ml. of A.P.T. given at 2 weeks' interval was poorer than the primary response in the A group 4 weeks after the first immunizing dose of 0·1 ml. Before, however, accepting this view, it would be desirable to compare in non-sensitized persons the response to a first combined injection of A.P.T. and serum with the response to a single injection of A.P.T. in two groups of persons observed at intervals for a period of 3 months without any second injection.

Summary. The tentative conclusion we should draw from the experiment as a whole is that increasing the dosage of A.P.T. from 0·1 and 0·3 to 0·3–0·5 ml. conferred little, if any, advantage on the actively immunized group, but was probably of some value in the group receiving combined active and passive immunization.

EFFECT OF REDUCING THE INTERVAL BETWEEN THE DOSES OF A.P.T. FROM 4 TO 2 WEEKS

As was pointed out in our previous paper, the passive protection afforded by an injection of antiserum at the time of the first dose of A.P.T. wears off before active immunity is fully developed, resulting in an intermediate stage of relative susceptibility. It was thought possible that this might be avoided by giving the second dose of A.P.T. 2 weeks instead of 4 weeks after the first. Observations were therefore made on batches of students in 1942–3 given 0·3 and 0·3 ml. of A.P.T. at 2 weeks' interval, and the results compared with those in students in 1941–2 given 0·3 and 0·3–0·5 ml. of A.P.T. at 4 weeks' interval. All students in the A + P group received 500 units of antiserum at the time of the first dose of A.P.T. The figures given in Table 4 show a large and highly significant difference in both the A and the A + P groups, when the antitoxin content of the serum was measured 3–4 weeks after the second inoculation of A.P.T., the difference in each instance being in favour of the students receiving their injections at the longer interval.

It has already been pointed out that the response in the 1942–3 A + P group was poorer than the primary response to a single injection of 0·1 ml. A.P.T. in the A group of Tables II and IV of our first paper, suggesting that cutting down the interval between two doses of A.P.T. to a fortnight might be more or less equivalent to giving a single dose. Table 5, which sets out the antitoxin values 10–12 weeks after the second injection of A.P.T., shows a similar difference in the A group, but a considerably smaller difference, only verging on significance, in the A + P group. Table 6 summarizes the Schick-test results, and shows a significant difference in the A group, and a difference verging on significance in the A + P group, in favour of the subjects injected at the 4-week interval.

Table 4. *Oxford and Cambridge Students*, 1941–2 *and* 1942–3. *Comparison of antitoxin content of serum, 3–4 weeks after second inoculation between students in* 1941–2 *inoculated with* 0·3 *and* 0·3–0·5 *ml. of A.P.T. at* 4 *weeks' interval and students in* 1942–3 *inoculated with* 0·3 *and* 0·3 *ml. of A.P.T. at* 2 *weeks' interval. Both the A + P groups had a dose of* 500 *units of antitoxin at the time of their first A.P.T. injection*

Antitoxin units per ml. of serum	1941–2		1942–3	
	No.	%	No.	%
	Group A			
2–1/25	26	72·2	3	10·7
1/50–1/250	8	22·2	9	32·1
1/500–< 1/1000	2	5·6	16	57·1
Total	36	100·0	28	99·9

$\chi^2 = 24{\cdot}058$, $P = < 0{\cdot}001$ (exact value not obtainable).

	Group A + P			
2–1/25	9	28·1	0	0
1/50–1/250	9	28·1	3	10·3
1/500–< 1/1000	14	43·8	26	89·7
Total	32	100·0	29	100·0

$\chi^2 = 9{\cdot}568$, $P = 0{\cdot}002$.

Note. For calculation of χ^2, the figures have been regrouped as a four-fold table.

The results of this experiment are fairly clear; they leave no doubt that two doses of A.P.T. at 4 weeks' interval are much superior to the same doses at 2 weeks' interval. The difference is less striking in the A + P than in the A group, but nevertheless it is sufficient to cast doubt on the advisability of reducing the interval between the doses of A.P.T., in subjects receiving combined active and passive immunization, in the hope of

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accelerating the development of active immunity. If, for any reason, this should be resorted to in practice, it would be advisable to give a third dose of A.P.T. 2–4 weeks after the second in order to avoid leaving an appreciable proportion of the subjects Schick-positive.

Summary. Reducing the interval between the doses of A.P.T. from 4 to 2 weeks lowered the antitoxin response greatly in the actively immunized group, and to a considerable extent in the group receiving combined active and passive immunization. In neither group was satisfactory immunity attained, as judged by the Schick test, when the interval between the doses of A.P.T. was reduced to 2 weeks.

Table 5. *Oxford and Cambridge Students*, 1941–2 *and* 1942–3. *Comparison of antitoxin content of serum* 10–12 *weeks after second inoculation between students in* 1941–2 *inoculated with* 0·3 *and* 0·3–0·5 *ml. of A.P.T. at* 4 *weeks' interval and students in* 1942–3 *inoculated with* 0·3 *and* 0·3 *ml. of A.P.T. at* 2 *weeks' interval. Both the A+P groups had a dose of* 500 *units of antitoxin at the time of their first A.P.T. injection*

Antitoxin units per ml. of serum	1941–2		1942–3	
	No.	%	No.	%
Group A				
2–1/25	29	56·9	3	12·0
1/50–1/250	17	33·3	10	40·0
1/500–<1/1000	5	9·8	12	48·0
Total	51	100·0	25	100·0
$\chi^2=19{\cdot}171$, $P=0{\cdot}00007$.				
Group A+P				
2–1/25	20	40·8	7	21·9
1/50–1/250	24	49·0	15	46·9
1/500–<1/1000	5	10·2	10	31·2
Total	49	100·0	32	100·0
$\chi^2=6{\cdot}731$, $P=0{\cdot}035$.				

COMPARISON OF ANTITOXIN RESPONSE IN GROUPS RECEIVING ACTIVE AND THOSE RECEIVING COMBINED ACTIVE AND PASSIVE IMMUNIZATION. SUMMARIZED RESULTS

Before describing our third experiment, we think it would be useful to summarize the results reported here and in our previous paper so as to have a sufficient number of observations to justify us in drawing reliable conclusions on the effect of an injection of 400–500 units of diphtheria antiserum, given at the time of the first dose of A.P.T., on the subsequent development of immunity. Owing to the circumstance that bleedings were not always made at the same time-intervals, we have had to omit some of our results, but those recorded in Tables 7–9 contain all the observations which could justifiably be regarded as comparable. They refer to batches of students who were immunized with either 0·1 and 0·3 ml. and 0·3 and 0·3–0·5 ml. of A.P.T. at 4 weeks' interval, and of whom approximately one-half received a single injection of 400–500 units of antiserum at the time of the first dose of A.P.T. Table 7 summarizes the antitoxin content of the serum 6–8 weeks after the second injection of A.P.T. A considerable difference is noticeable between the two groups, the average level of antitoxin being considerably higher in the A than in the A+P group. Table 8, giving the figures for the antitoxin content of the blood serum 10–12 weeks after the second injection of A.P.T., likewise shows a higher average level of antitoxin in the A than in the A+P group, but the difference is considerably less though still highly significant. From Table 9 it will be seen that the difference in the Schick-conversion rate between the two groups is barely significant, though, taken in conjunction with the antitoxin content figures recorded in Tables 7 and 8,

Table 6. *Comparison between final Schick tests* 10–12 *weeks after second inoculation between students in* 1941–2 *inoculated with* 0·3 *and* 0·3–0·5 *ml. of A.P.T. at* 4 *weeks' interval and students in* 1942–3 *inoculated with* 0·3 *and* 0·3 *ml. of A.P.T. at* 2 *weeks' interval. Both the A+P groups had a dose of* 500 *units of antitoxin at the time of their first A.P.T. injection*

Series	Dose of A.P.T. in ml.	Interval between doses in weeks	No. tested	No. positive	% S.C.R.
		Group A			
1941–2	0·3 and 0·3–0·5	4	36	0	100·0
1942–3	0·3 and 0·3	2	25	6	76·0
		Group A+P			
1941–2	0·3 and 0·3–0·5	4	35	1	97·1
1942–3	0·3 and 0·3	2	31	5	83·9

S.C.R. = Schick-conversion rate.

Groups 1941–2 *and* 1942–3. Observed difference between A groups = 24 %. S.E. of this difference = 7·75 %. Observed difference divided by S.E. of difference = 3·1.

Groups 1941–2 *and* 1942–3. Observed difference between A+P groups = 13·2 %. S.E. of this difference = 7·09 %. Observed difference divided by S.E. of difference = 1·9.

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there can be little doubt that it is not attributable to chance alone.

Summary. A summarized comparison of observations made on batches of students during the years 1940–2 shows that the antitoxin content of the blood serum was considerably higher in those receiving active than in those receiving combined active and passive immunization, when the comparison was made 4 weeks after the second dose of A.P.T. At 10–12 weeks, however, after the second dose of A.P.T., the difference between the two groups, though significant, was much less striking; and the difference in the Schick-conversion rates was barely significant. The conclusion, therefore, is that the simultaneous injection of 400–500 units of diphtheria antiserum with 0·1–0·3 ml. of A.P.T., followed 4 weeks later by a second dose of 0·3–0·5 ml. of A.P.T., results in a delay and an inhibition in the formation of antitoxin. The final degree of immunity, however, is not greatly prejudiced, as indicated by a Schick-conversion rate of 91 % in the group receiving serum.

Table 7. *Summarized results for* 1940–2 *showing comparison of antitoxin content of serum at* 6–8 *weeks after second inoculation between A and A + P groups. Both groups received* 0·1–0·3 *and* 0·3–0·5 *ml. of A.P.T. at* 4 *weeks' interval. A + P group received, in addition,* 400–500 *units of antitoxin at time of first A.P.T. injection*

Antitoxin units per ml. of serum	A		A+P		
	No.	%	No.	%	Total
2–1/25	70	60·3	27	28·7	97
1/50–1/250	26	22·4	31	33·0	57
1/500–<1/1000	20	17·2	36	38·3	56
Total	116	99·9	94	100·0	210

$\chi^2 = 22{\cdot}003$, $P = 0{\cdot}000{,}017$.

Table 8. *Summarized results for* 1940–2 *showing comparison of antitoxin content of serum at* 10–12 *weeks after second inoculation between A and A + P groups. Both groups received* 0·1–0·3 *and* 0·3–0·5 *ml. of A.P.T. at* 4 *weeks' interval. A + P group received, in addition,* 400–500 *units of antitoxin at time of first A.P.T. injection*

Antitoxin units per ml. of serum	A		A+P		
	No.	%	No.	%	Total
2–1/25	57	55·9	32	32·0	89
1/50–1/250	30	29·4	50	50·0	80
1/500–<1/1000	15	14·7	18	18·0	33
Total	102	100·0	100	100·0	202

$\chi^2 = 12{\cdot}28$, $P = 0{\cdot}002$.

EFFECT OF INCREASING THE DOSE OF DIPHTHERIA ANTISERUM TO 5000 UNITS

Our third experiment was undertaken to find out whether the inhibiting effect on antitoxin production of a dose of 400 to 500 units of antiserum would be intensified by giving a much larger dose. As usual, two groups of students were studied, the A group receiving active immunization only with two doses of 0·3 and 0·3–0·5 ml. at 4 weeks' interval, the A + P group receiving in addition an injection of 5000 units of antiserum at the time of the first dose of A.P.T. The results were compared with those of previous years in which a dose of only 500 units of antiserum was given. Table 10 shows that 3–4 weeks after the second inoculation the antitoxin content in the actively immunized group is much greater than in the group receiving combined active and passive immunization. The difference, in fact, is so great that there is no need to subject it to a χ^2 test. Table 11 records the results 10–14 weeks after the second inoculation. Again the antitoxin content is much higher in Group A than in Group A + P, but the difference is not nearly so striking as after 3–4 weeks. Unfortunately, final Schick tests could not be

Table 9. *Summarized results for* 1940–2 *showing comparison of final Schick tests* 10–12 *weeks after second inoculation between A and A + P groups. Both groups received* 0·1–0·3 *and* 0·3–0·5 *ml. of A.P.T. at* 4 *weeks' interval. A + P group received, in addition,* 400–500 *units of antitoxin at time of first A.P.T. injection*

A			A+P		
No. tested	No. positive	% S.C.R.	No. tested	No. positive	% S.C.R.
98	2	98·0	99	9	90·9

S.C.R. = Schick-conversion rate.

Observed difference between A and A + P = 7·1 %. S.E. of this difference = 3·3 %. Observed difference divided by S.E. of difference = 2·2.

Table 10. *Comparison of antitoxin content of serum* 3–4 *weeks after second inoculation between students receiving active immunization alone with* 0·3 *and* 0·3 *ml. of A.P.T. at* 4 *weeks' interval and those receiving in addition* 5000 *units of antiserum at the time of the first inoculation (Series* 1943–4)

Antitoxin units per ml. of serum	A		A+P		
	No.	%	No.	%	Total
2–1/25	31	70·5	3	6·5	34
1/50–1/250	8	18·2	1	2·2	9
1/500–<1/1000	5	11·4	42	91·3	47
Total	44	100·1	46	100·0	90

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carried out at Cambridge, so that the figures given in Table 12 refer to the Oxford and Liverpool students only. The most striking feature of this table is the comparatively low Schick-conversion rate in the actively immunized group. The lowest previous Schick-conversion rate in this group during the whole 5 years of our experiments was 95·7 %, even when immunizing doses of only 0·1 and 0·3 ml. of A.P.T. were used. The present figure of 88·9 % is very much lower, and is not easy to explain, apart possibly from the small number of subjects tested. The Schick-conversion rate of 81·5 % in the A+P group is likewise less than the lowest previously met with, namely 83·9 %, but in view of the small number of subjects tested, little attention can be paid to this difference.

In Tables 13–15 a comparison is made between students in 1941–2 and in 1943–4 receiving combined active and passive immunization; the former group were injected with a dose of 500 units of diphtheria antiserum, the latter with a dose of 5000 units. Table 13 shows that 3–4 weeks after the second inoculation the antitoxin content of the

Table 11. *Comparison of antitoxin content of serum 10–14 weeks after second inoculation between students receiving active immunization alone with 0·3 and 0·3 ml. of A.P.T. at 4 weeks' interval and those receiving in addition 5000 units of antiserum at the time of the first inoculation (Series 1943–4)*

Antitoxin units per ml. of serum	A		A+P		
	No.	%	No.	%	Total
2–1/25	17	40·5	6	13·6	23
1/50–1/250	18	42·9	22	50·0	40
1/500–<1/1000	7	16·7	16	36·4	23
Total	42	100·1	44	100·0	86

$\chi^2 = 9{\cdot}140$, $P = 0{\cdot}01$.

Table 12. *Schick-test results 10–14 weeks after second inoculation in students receiving active immunization alone with 0·3 and 0·3 ml. of A.P.T. at 4 weeks' interval and those receiving in addition 5000 units of antiserum at the time of the first inoculation (Series 1943–4)*

Group	No. tested	No. positive	% positive	% S.C.R.
A	27	3	11·1	88·9
A+P	27	5	18·5	81·5
Total	54	8	14·8	85·2

S.C.R. = Schick-conversion rate.

Observed difference between A and A+P groups = 7·4 %. S.E. of this difference = 9·7 %. Observed difference divided by S.E. of difference = 0·8.

Table 13. *Comparison of antitoxin content of serum 3–4 weeks after second inoculation in 1941–2 students inoculated with 0·3 and 0·3–0·5 ml. of A.P.T. at 4 weeks' interval together with a single dose of 500 units of antitoxin, and 1943–4 students inoculated with 0·3 and 0·3 ml. of A.P.T. at 4 weeks' interval together with a single dose of 5000 units of antitoxin*

Antitoxin units per ml. of serum	1941–2		1943–4		
	No.	%	No.	%	Total
2–1/25	8	25·8	3	6·5	11
1/50–1/250	9	29·0	1	2·2	10
1/500–<1/1000	14	45·2	42	91·3	56
Total	31	100·0	46	100·0	77

$\chi^2 = 19{\cdot}877$, $P = <0{\cdot}001$ (exact value not obtainable).

Note. For calculation of χ^2, the figures have been regrouped as a four-fold table.

Table 14. *Comparison of antitoxin content of serum 10–14 weeks after second inoculation in 1941–2 students inoculated with 0·3 and 0·3–0·5 ml. of A.P.T. at 4 weeks' interval together with a single dose of 500 units of antitoxin, and 1943–4 students inoculated with 0·3 and 0·3 ml. of A.P.T. at 4 weeks' interval together with a single dose of 5000 units of antitoxin*

Antitoxin units per ml. of serum	1941–2		1943–4		
	No.	%	No.	%	Total
2–1/25	19	39·6	6	13·6	25
1/50–1/250	24	50·0	22	50·0	46
1/500–<1/1000	5	10·4	16	36·4	21
Total	48	100·0	44	100·0	92

$\chi^2 = 12{\cdot}46$, $P = 0{\cdot}002$.

Table 15. *Comparison between final Schick tests 10–14 weeks after second inoculation between students in 1941–2 inoculated with 0·3 and 0·3–0·5 ml. of A.P.T. at 4 weeks' interval together with a single dose of 500 units of antitoxin, and students in 1943–4 inoculated with 0·3 and 0·3 ml. of A.P.T. at 4 weeks' interval together with a single dose of 5000 units of antitoxin*

1941–2			1943–4		
No. tested	No. positive	% S.C.R.	No. tested	No. positive	% S.C.R.
34	1	97·1	27	5	81·5

S.C.R. = Schick-conversion rate.

Observed difference between 1941–2 and 1943–4 = 15·6 %. S.E. of this difference = 7·7 %. Observed difference divided by S.E. of difference = 2·0.

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1941–2 students was significantly higher than in the 1943–4 students. Table 14, giving the results 10–14 weeks after the second inoculation, similarly shows the superiority of the 1941–2 group, though the difference is considerably less. The comparison of the final Schick-test results in Table 15 shows a considerable difference between the two groups, though in view of the small numbers of students tested and the relatively low Schick-conversion rate even in the actively immunized 1943–4 group (see Table 12), it can hardly be regarded as significant.

Summary. The antitoxin and Schick-test results recorded in Tables 13–15 leave no doubt that a large dose of serum (5000 units) given at the time of the first A.P.T. injection leads to a greater inhibition of antitoxin production than a small dose (500 units). Even so, the inhibition caused by the larger dose is not so very great, as 80 % of the subjects treated became Schick-negative within about 12 weeks of the second dose of A.P.T.

SUMMARY AND CONCLUSIONS

Three sets of experiments were carried out on undergraduate medical students at Oxford, Cambridge and Liverpool during the years 1941–4 in order to supplement the information obtained previously (Downie *et al.* 1941) on the comparative antitoxin response of those given active immunization alone (Group A) and those given combined active and passive immunization (Group A + P). A summary of each of the experiments has already been given in the text, so that it is unnecessary here to do more than recapitulate briefly the main results.

1. The first experiment showed that in Group A the antitoxin response was not appreciably greater in students receiving doses of 0·3 and 0·3–0·5 ml. of A.P.T. at 4 weeks' interval than in those receiving doses of only 0·1 and 0·3 ml. In Group A + P no difference was noticed in the antitoxin content of the serum 6–8 weeks after the second injection of A.P.T., but 10–12 weeks after the second injection there was a difference in favour of the students receiving the larger doses of A.P.T., though it was below the conventional level of statistical significance.

2. The second experiment showed that when the doses of A.P.T. were spaced by 2 instead of by 4 weeks the antitoxin response was much less in both the A and the A + P groups, though the difference was less in the latter group, particularly when the measurements were made 10–12 weeks after the second inoculation of A.P.T.

3. The third experiment showed that a dose of 5000 units of diphtheria antiserum given at the time of the first injection of A.P.T. inhibited antitoxin production to a greater extent than a dose of 400–500 units, though the difference was much less when the measurements were made at 12 weeks after the second inoculation than at 4 weeks.

A compilation of the results obtained during 1940–2 in groups of students receiving active and those receiving active plus passive immunization shows that the antitoxin production in the first group (Group A) was much higher than in the second group (Group A + P) 6–8 weeks after the second injection of A.P.T., but that 10–12 weeks after the second inoculation the difference, though still significant, was considerably less. The final Schick-test results at 10–12 weeks gave a Schick-conversion rate of 98·0 % in Group A and of 90·9 % in Group A + P.

A review of the results obtained during the years 1939–44 on about 450 students at Oxford, Sheffield and Liverpool leads to the conclusion that the effect of giving diphtheria antiserum at the time of the first injection of A.P.T. is to cause a delay and some degree of inhibition in the antitoxin response of the subject. The larger the amount of antiserum given, the greater is this effect. With a dose of 500 units, though the delay in antitoxin formation is very obvious 4 weeks after the second injection of A.P.T., the final degree of immunity attained, as judged by the antitoxin concentration of the blood serum and by the Schick-conversion rate, is not greatly inferior to that resulting from active immunization alone; and even with a dose of 5000 units, the Schick-conversion rate reaches 81 % 12 weeks after the second injection of A.P.T. It is clear, therefore, that the antiserum, even when given in a dose as large as 5000 units, does not neutralize more than a small part of the antigenic activity of the first dose of A.P.T. Its main effect is apparently to diminish the rate of sensitization of the tissues, so that when a second dose of A.P.T. is given 4 weeks later, the rise in the antitoxin content of the blood serum is considerably delayed. Our experiments suggest that by increasing the size of the first dose of A.P.T., some of this delay may be avoided.

The partial neutralization of the first dose of A.P.T. will result in a decrease in the total antigenic stimulus and a delay in the time at which it comes into operation. It is suggested that, provided the tissues have not been previously sensitized to diphtheria toxin, the result may be that the two doses will act virtually as a single dose. Such an explanation, however, must remain unproven till further observations have been made (see p. 35).

The practical value of combined active and passive immunization, especially when joined with temporary segregation of healthy carriers, in combating outbreaks of diphtheria in schools and other institutions for children has been clearly shown by Fulton, Taylor, Wells & Wilson (1941). Our present experiments lead us to suggest that, when applying the method in practice, it would be wise to give an initial dose of 0·5 ml. of A.P.T., together with

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500 units of diphtheria antiserum injected at a different site, followed 6 weeks later by a second dose of 0·5 ml. A.P.T. It is probable that children treated in this way will develop approximately the same ultimate degree of immunity as those actively immunized with doses of 0·3 and 0·5 ml. of A.P.T. at 4 weeks' interval.

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