COMBINED ACTIVE AND PASSIVE IMMUNIZATION AGAINST DIPHTHERIA

I. STUDIES OF ANTITOXIN RESPONSE IN NORMAL STUDENTS

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Though in veterinary medicine the prophylactic treatment by combined active and passive immunization of animals has been in vogue since the beginning of the century, it is only within recent years that the method has been applied to the prevention of human disease. The reason for this is not far to seek. In the treatment of animals risks can be taken that are seldom justifiable with man. In such diseases as anthrax, swine fever, swine erysipelas, rinderpest, and African horse-sickness the vaccinating agent used is the living organism. When given along with a suitable dose of protective serum it leads to the more or less permanent protection of the animal after the initial temporary protection by the injected serum has worn off. The essence of the method consists in stimulating an active, under cover of a passive, immunity. In human medicine there are few infective diseases in which a powerful antiserum is available, and in the two outstanding examples, diphtheria and tetanus, no one has dared to attempt active immunization by injection of the living organisms. It was not, therefore, till a safe non-living and non-toxic vaccinating agent was made available by the discovery of formol toxoid (Glenny and Südmersen, 1921; Glenny and Hopkins, 1923; Ramon, 1923, 1928) that the possibility of combining active and passive immunization in the prophylactic or therapeutic treatment of these two diseases became practicable.

Review of Previous Work

Ramon and Laffaille (1925) studied the production of tetanus antitoxin in guinea-pigs. The animals were divided into four lots. Lot A received 1 c.cm. of anatoxin (formol toxoid) alone. Lot B received 1 c.cm. of anatoxin mixed with antitetanic serum containing 4,500 antitoxin units (A.U.). Lot C received 1 c.cm. of anatoxin and 4,500 A.U. of serum given simultaneously into different sites. Lot D received 4,500 A.U. of serum alone. Sixteen days later some of the animals in each lot were tested for their resistance to the injection of tetanus toxin. Lot A was found to withstand about 60 m.l.d., whereas none of the other lots withstood even 2 m.l.d. The conclusion was therefore drawn that the serum had interfered with the immunizing action of the anatoxin.

To see, however, whether it had also affected the sensitizing action of the anatoxin, the remaining animals in each lot were given 1 c.cm. of anatoxin 20 days after the first injection. Seven days later they were tested for their resistance to tetanus toxin. The animals of Lot A withstood several hundred m.l.d. The animals of Lot C withstood 10 to 50 m.l.d. The animals of Lots B and D proved almost as susceptible as non-immunized controls. This experiment showed that the simultaneous administration of serum to the animals of Lot C, though it had prevented their immediate antitoxin response, had not entirely destroyed the sensitizing effect of the anatoxin and the consequent ability of the animals to respond more rapidly to a second

dose. It also showed that the over-neutralized mixture given to the animals of Lot B was valueless in this respect. Not only did it fail to give rise to antitoxin production, but it likewise failed to sensitize the tissues and enable them to respond to a fresh injection of anatoxin. These experiments were repeated on rabbits with substantially the same results.

In 1928 Ramon described the general properties of anatoxin. Discussing combined active and passive immunization, he considered that the method should not be used except in cases of necessity, since it is less successful in producing active immunity than vaccination with anatoxin alone.

This conclusion was supported by observations on a small number of children described by Martin, Loiseau, and Laffaille (1928). In a school where diphtheria had broken out these workers inoculated 9 children with 0.5 c.cm. of anatoxin and 14 children with the same dose of anatoxin followed half an hour later by 1,000 units of diphtheria antitoxin. All received a second dose of 1 c.cm. of anatoxin 30 days later, and a third dose of 1 c.cm. after another fortnight. Three weeks after the last injection the children were Schick-tested. All the 9 children who were given anatoxin alone reacted negatively, whereas 2 of the 14 children who were given serum as well as anatoxin were Schick-positive.

In 1938 Ramon and his colleagues returned to the subject. Working this time with rabbits, they found that animals given 4 c.cm. of tetanus anatoxin and bled a fortnight later had an antitoxin titre of about 1/5 A.U. per c.cm., whereas those injected simultaneously but into different sites with 4 c.cm. of anatoxin and with serum containing 300 A.U. had a titre of less than 1/600 A.U. After a second dose of anatoxin, however, the titre of the first group was between 2 and 7.5 A.U., and of the second group between 7 and 8 A.U.

In another experiment animals receiving anatoxin alone had a titre 18 days later of 1 A.U. per c.cm., whereas those given both anatoxin and serum had a titre of less than 1/300 A.U.; but 14 days after a second injection of anatoxin the animals of both groups had a titre of about 7 A.U. It appears from these experiments, therefore, that serum delays or interferes with the primary response to anatoxin, but does not destroy the sensitizing power of the anatoxin and the consequent ability of the animal to respond rapidly to a second dose. This conclusion, however, was not drawn by Ramon and his colleagues.

In a later paper Ramon, Richou, and Maccolini (1939) made similar experiments on rabbits using diphtheria anatoxin and serum. Each lot contained 6 animals. Lot A received 3 inoculations, at five-day intervals, of 0.5, 1, and 2 c.cm. respectively of anatoxin. Lot B received in different sites on the same day 0.5 c.cm. of anatoxin and serum containing 3,250 A.U., followed at five-day intervals by injections of 1 and 2 c.cm. of anatoxin only. Lot C received both anatoxin and serum at each of the three injections, the anatoxin doses being the same as before, and the serum doses being each 325 A.U. The animals were bled 1, 4, 6, 8, and 12 weeks after injection, and then at intervals up to 15 months. The sera of the animals in each lot were pooled and titrated for antitoxin. In all three groups the maximum titre of 3 to 4 units was recorded after a week; it then fell gradually till after 15 months it was 1/20 to 1/10 A.U. per c.cm. It was therefore concluded that the

simultaneous inoculation of serum does not interfere at all with the development of active immunity following the administration of anatoxin.

This conclusion, which is very much less cautious than that reached in 1925, is seriously open to question. The number of animals used in Ramon's last experiment was small, and the administration of a very large dose of serum to the animals of Lot B and of three successive doses of serum to the animals of Lot C makes it difficult to interpret the antitoxin titres during the first few weeks following the injections. It is conceivable that the serum may have interfered with the active immunity response in the early stages, but that this interference was masked by the presence of circulating antitoxin remaining from the serum injections.

Other papers have been published by Ramon (1938, 1940), based partly on observations made on actual cases of diphtheria treated by the combined method, which have led him to conclude that serum therapy does not interfere at all with the development of active immunity. Since in patients suffering from diphtheria the issue is apt to be confused by the presence of toxin formed by the infecting bacilli themselves, it is hardly worth while abstracting these papers or those of his colleagues on the same lines.

Reviewing Ramon's work as a whole, it will be seen that his earlier experiments were better designed than his later ones to decide whether the administration of serum along with anatoxin interferes with the antibody response of the tissues. It is therefore understandable, though regrettable, that Ramon passed between 1925 and 1940 from an attitude of caution to one of dogmatic assertion.

The experience of other workers on combined active and passive immunization is conflicting. Glenny, Buttle, and Stevens (1931) found that if toxoid was inoculated subcutaneously into guinea-pigs most of the animals had a serum antitoxin content of between 1/100 and 1 A.U. per c.cm. 4 to 6 weeks later. But if antitoxin was given intravenously in an amount sufficient to neutralize all the toxoid injected, either at the time of inoculation of the toxoid or up to 3 days later, no antitoxin was formed. The longer the interval between the inoculation of toxoid and the inoculation of serum, the less was the degree of inhibition of antitoxin production. Even with a 21-day interval, however, antitoxin production was still not as good as in the controls that were given no antitoxin. It thus appears that neutralization of toxoid by antitoxin diminishes the antitoxin response, and that if combined active and passive immunization is to be successful an excess of toxoid must be present in the tissues.

Frey and Schmid (1939) injected 20 children with 1 c.cm. of alum-precipitated toxoid (A.P.T.) subcutaneously and 1,000 units of diphtheria antitoxin intramuscularly. The children were bled at intervals and the antitoxin content of their serum was titrated. All of them showed a fall in the initial value after injection, and none showed any development of fresh antitoxin. Most of the observations, however, were made within 4 weeks of the injection, and no second dose of A.P.T. was given. Since it is well known from the observations of Glenny and Südmersen (1921) and Glenny (1925, 1931) that the main rise in antitoxin does not occur till after the secondary stimulus has been given, the results obtained by Frey and Schmid are not surprising. Indeed, they would hardly be worth noticing were it not for the fact that they were made the basis of an editorial in the *Journal of the American Medical Association* (1939, 113, 1884), in which the following conclusion drawn from the work of Frey and Schmid and of Paschla (see later) was emphasized: "Combined active-passive immunization is not feasible in human medicine, the two types of immunization being incompatible with each other."

Much the same criticism applies to the observations, in Mexico, of Leon, Escarza, and Navarro (1939), who gave a single dose of 1 to 1.5 c.cm. of A.P.T. and 1,000 to 1,500 units of diphtheria antitoxin to 80 Schick-positive reactors. One month later, of 42 who were retested 6 were still Schick-positive. Two months later, of 38 who were retested 9 were Schick-positive. As in Frey and Schmid's work, only one dose of A.P.T. was given, and no comparison was made with a group of persons immunized by the active method alone.

Gundel and König (1938) made some very interesting observations on guinea-pigs. They injected them with various formalized and alum-precipitated toxoids or with toxoid-antitoxin floccules (T.A.F.), together with a dose of anti-diphtheria serum, and tested their immunity at intervals to infection with living diphtheria bacilli of the gravis and mitis types. In brief they found that (a) animals given serum alone—500 A.U.—were protected for less than a fortnight; (b) formol toxoids or T.A.F. alone produced partial or complete immunity within

a fortnight; (c) A.P.T. produced within a fortnight an immunity that was more complete and lasted longer than that obtained by injection of formol toxoids or T.A.F.; (d) the combination of formol toxoid and serum protected guinea-pigs for a week, but left them susceptible again within a fortnight; (e) the combination of A.P.T. and serum protected them for a week, left them susceptible during the 2-4-weeks stage, and then protected them again later. In other words, the passive immunity conferred by the serum wore off in a week or two and left the animals susceptible until active immunity resulting from the A.P.T. began to develop 4 to 6 weeks after injection. It is clear from Gundel and König's work that serum does interfere with the response of the body to an active immunizing agent. Unfortunately these workers did not give a second injection of toxoid. They concluded, however, that if combined active and passive immunization is to be satisfactory a dose of A.P.T. must be given which is sufficiently large to leave enough unneutralized toxoid at the injection site to stimulate the development of antibody after the serum has disappeared from the circulation.

Whether this conclusion is correct or not it is difficult to say, but, provided two injections of toxoid are given, neither of the doses need be very large. Gierthmühlen and Voges (1939), for example, in a nursery home where diphtheria had broken out, inoculated children with 0.3 c.cm. of A.P.T. along with 1,000 units of antitoxin. Three weeks later they gave a second dose of A.P.T. of the same amount. All children were Schick-tested six months later, and all were Schick-negative. It is interesting to note that 3 out of 19 children who had received only their first dose of A.P.T. developed diphtheria, and that of 43 who had received both first and second doses of A.P.T. two developed diphtheria within 21 to 46 days after the first inoculation. The disease, however, was of an extremely mild type. These observations support those made by Gundel and König in showing that there is probably a period of relative susceptibility between the time when the passive immunity due to the serum has worn off and the time when the active immunity due to the A.P.T. has developed. They do show, however, in addition, that, provided a second dose of A.P.T. is given, satisfactory immunity is produced at any rate within six months.

The observations of Paschla (1939) do not bear out Gundel and König's results. Paschla found that of 39 children with an initial titre of less than 0.005 A.U. per c.cm. who were given a single dose of 0.2, 0.5, or 1 c.cm. of A.P.T., 20 formed no antitoxin within 6 weeks, 11 reached a titre of about 0.03 A.U., and 8 reached a titre of between 0.5 and 20 A.U. When, in another series of 22 children, he gave one dose of 0.2, 0.5, or 1 c.cm. of A.P.T. along with 1,000 units of antidiphtheria serum, 21 completely failed to respond. Paschla therefore concludes that in children with no initial antitoxin in their blood the administration of serum hinders the response to A.P.T. In children who had already been exposed to diphtheria and whose blood contained some antitoxin, the administration of serum had no inhibiting effect. On the other hand, from the point of view of treatment, it had no advantage, since these children responded very rapidly to an injection of A.P.T. alone.

Fjord-Nielsen (1940) made some interesting observations, complementary to those of Glenny, Buttle, and Stevens (1931), on the effect of serum in delaying antitoxin formation. Working with guinea-pigs, she found that when A.P.T. and serum were given simultaneously no antitoxin was formed within 4 weeks, though the animals became sensitized and responded more readily to a second dose of A.P.T. If the first dose of A.P.T. was given 3 days after the serum, and a second dose of A.P.T. 4 weeks later, the antitoxin titre of the guinea-pigs' serum was higher than when the A.P.T. and serum were injected simultaneously. If the interval between the serum and the first dose of A.P.T. was lengthened to 7 days, then the final titre was higher still. If it was lengthened to 14 days a result was obtained that was as good as if no serum at all had been given. From these experiments it would appear that the administration of serum interferes with antitoxin production but does not destroy, though it may weaken, the sensitizing effect of the first dose of A.P.T.

It is not surprising that the results of different workers whose experiments varied in so many important particulars should have been conflicting and at times apparently contradictory. Looking at them, however, as a whole it is difficult to avoid the conclusion that the simultaneous administration of serum with toxoid interferes with the primary response of the tissues, but does not destroy, though it may weaken, the sensitizing power of the toxoid

which enables the tissues to respond to a second dose of toxoid by the rapid production of antitoxin. This is essentially the same conclusion as was reached, but subsequently abandoned, by Ramon and his colleagues.

Description of Experimental Observations

In view of the findings of previous workers it seemed important to choose (1) a dose of toxoid that was small enough to reveal any possible inhibitory action of the serum; (2) a dose of serum that was not so large as to conceal the active formation of antitoxin in the body as the result of the injection of toxoid; and (3) a toxoid in the form of the alum precipitate to ensure as little interference as possible by the action of the serum. The general plan of the experiments was therefore as follows.

Observations were made at Oxford, Cambridge, and Sheffield on third-year medical students, the majority of whom were between 20 and 22 years of age. In addition a small number of observations were made at Turner's Court Training Colony, near Oxford, on boys all but two of whom were between 14 and 19 years of age.

The subjects were first of all Schick-tested. About 6 to 8 c.cm. of blood was withdrawn from those giving a positive reaction, and sent to Mr. Glenny at the Wellcome Physiological Research Laboratories to be titrated for diphtheria antitoxin. The Schick-positive reactors were then divided into two groups, A and A+P. The subjects in Group A (active immunization) received 0.1 c.cm. (5 Lf) of Burroughs Wellcome's alum-precipitated toxoid (A.P.T.) into the left upper arm. Those in Group A+P (active plus passive immunization) received 0.1 c.cm. of A.P.T. into the left upper arm and 350 to 500 units of Burroughs Wellcome's refined diphtheria antitoxin (Pope, 1938, 1939) into the right upper arm. All injections were made into the deep subcutaneous tissue. Four weeks later the subjects were again bled, and were then given 0.3 c.cm. of A.P.T. into the right upper arm. Eight weeks after the second dose of A.P.T. the subjects were bled for the third time and were again Schick-tested. The results of the Schick test were read usually 3 to 5 days later, and any person who still reacted positively was given another dose of 0.3 c.cm. of A.P.T.

Though this was the general plan of the observations, departures were made from it at Cambridge and at Sheffield for various reasons. At Cambridge in one series of students the third bleeding was made 6 weeks instead of 8 weeks after the second dose of A.P.T. In another set of students only active immunization was given. In still further sets of students additional bleedings were made 12 to 23 weeks after the second dose of A.P.T. In these instances the final Schick test was not performed till after the last bleeding had been done. At Sheffield the intervals between the two immunizing doses, and between the second dose and the third bleeding, were rendered very irregular owing to enemy action.

The relevant facts relating to the different sets of observations are set out in Table I.

TABLE I.—Number and Type of Observations Made

Set	Place	Time	Original No. in Group		No. of Units of Antitoxin	Time Interval in Weeks between			
			A	A+P		1st and 2nd Bleeding	2nd and 3rd Bleeding	2nd and 4th Bleeding	2nd Bleeding and Schick Test
TC	Turner's Court	Michaelmas, 1939	5	9	350	5	8	—	8
O1	Oxford	Hilary, 1940	10	10	400	4	8	—	8
C1	Cambridge	Lent, 1940	27	27	400	4	6	12	12
O2	Oxford	Michaelmas, 1940	8	8	500	4	8	—	8
C2	Cambridge	" "	72	52	500	4	8	12 or 23	12 or 23
S	Sheffield	" "	26	25	500	4-10	8-10	—	8-10
Total ..			148	131					

A = Active immunization. A+P = Combined active and passive immunization.

The total number of subjects recorded in the table is 279. In fact rather more took part in the trials, but in analysing our figures we have excluded all initially Schick-positive reactors whose serum contained 1/1,000 unit per c.cm. or more of antitoxin. The figures recorded in this paper refer exclusively to subjects whose serum at the time of the first immunizing injection contained less than 1/1,000 unit per c.cm. Unfortunately a history of immunization was obtained only from the O2 and C2 sets of students. Of these, 7 in the A group and 3 in the A+P group had been immunized or Schick-tested pre-

viously. Since none of them contained as much as 1/1,000 unit per c.cm. of antitoxin in their blood they have been included in our figures.

Results

COMPARISON BETWEEN THE ANTITOXIN PRODUCTION OF GROUPS A AND A+P

In recording the results it will be convenient first of all to deal with those series of observations in which the original plan of the experiments was strictly followed.

In Table II are included the observations on Sets O1, O2, C1, and C2 of Oxford and Cambridge students who were bled 4 weeks after the first immunizing dose of A.P.T. or of A.P.T. plus serum. In this and in subsequent tables it has been thought wise, owing to the relatively small number of sera examined and the wide variations in the amounts of antitoxin contained in them, to report the results in simple frequency tables rather than to work out arithmetic mean titres. Here and throughout the paper all antitoxin figures are expressed per c.cm. of serum.

TABLE II.—Oxford and Cambridge Students: Sets O1, O2, C1, and C2. Antitoxin Titres 4 Weeks after First Immunizing Dose

Antitoxin Units per c.cm. of Serum	A		Total
	No.	A+P	
Good response : 1/50 or more ..	14 (12%)	3 (3%)	17
Fair response : 1/100 to 1/500 ..	7 (6%)	57 (61%)	64
Poor or no response : 1/1,000 or less ..	95 (82%)	34 (36%)	129
Total ..	116 (100%)	94 (100%)	210

A = Active immunization with 0.1 c.cm. of A.P.T.

A+P = Ditto, plus 400 to 500 units of antitoxin.

$\chi^2 = 73.527$. $P < 0.000001$.

Note.—The term "response" is hardly justifiable in the A+P group, but its use is convenient.

It will be observed that the proportion of sera containing 1/500 unit or more of antitoxin was far higher in the A+P than in the A group—64% as opposed to 18%. In the small class of sera containing 1/50 unit or more, however, the relation was reversed. The difference in antitoxin distribution between the two groups is seen by the χ^2 test to be far greater than is likely to have arisen by chance alone.

These facts are best explained on the assumption: (a) that the antitoxin in the A+P group of sera was mainly residual antitoxin—i.e., the remains of the antitoxin that was injected four weeks previously; and (b) that a single dose of 0.1 c.cm. of A.P.T. gives rise to little or no antitoxin response within four weeks except in a very few persons who have had previous experience of the diphtheria bacillus; in these it acts like a secondary stimulus (Glenny, 1925, 1931; Glenny and Südmersen, 1921), and gives rise rapidly to a considerable amount of antitoxin. Evidence in support of assumption (a) will be afforded at a later stage of this paper. In support of assumption (b) are the observations

of Paschla (see above) and of Fraser (1940), who found that of 50 children who had been immunized 5 years previously and whose serum antitoxin content had fallen to below 1/50 unit per c.cm. all responded rapidly to the reinjection of a single dose of 0.1 c.cm. of toxoid, 80% within 5 weeks having more than 1/2 unit per c.cm. It is also supported by examination of the records of students in the O2 and C2 groups who gave a history of previous immunization or of Schick-testing. Of 7 such students in the A group all but one had 1/10 unit or over 4 weeks after injection, and of 3 such students in the

A+P group 2 had 1/10 unit or over. Unfortunately a record of the immunization histories of the O1 and C1 sets of students was not available.

The antitoxin titres in Sets O1, O2, and C2 of Oxford and Cambridge students 8 weeks after the second immunizing dose of A.P.T. are recorded in Table III.

TABLE III.—*Oxford and Cambridge Students: Sets O1, O2, and C2. Antitoxin Titres 8 Weeks after Second Immunizing Dose*

Antitoxin Units per c.cm. of Serum	A		Total
	No.	A + P	
Good response : 1/50 or more ..	57 (65%)	22 (33%)	79
Fair response : 1/100 to 1/500 ..	20 (23%)	25 (38%)	45
Poor or no response : 1/1,000 or less ..	11 (12%)	19 (29%)	30
Total	88 (100%)	66 (100%)	154

A = Active immunization with 0.1 c.cm. and 0.3 c.cm. of A.P.T. at 4 weeks' interval.

A + P = Ditto, plus 400 to 500 units of antitoxin at time of first injection.

$\chi^2 = 15.366$. $P = 0.00047$.

The results are seen to be very different from those in Table II. Of those in Group A 65% had responded well by producing 1/50 unit or more of antitoxin, as against only 33% in Group A + P. The χ^2 test shows that such a difference in distribution can hardly have been due to chance.

It may therefore be concluded that, although 4 weeks after the first immunizing dose the average antitoxin titre is considerably higher in the A + P than in the A group, 8 weeks after the second injection the situation is reversed. From this the inference can be drawn that the administration of serum along with the first dose of A.P.T. leads to a lower antitoxin response, as determined by observations made 8 weeks after the second injection of A.P.T., than that due to A.P.T. alone.

We may now examine the results on all the subjects, including those that were not treated in strict accordance with the original plan. Table IV records the antitoxin titres on Sets TC, O1, O2, C1, C2, and S of students at Turner's Court, Oxford, Cambridge, and Sheffield, at an average of about 4½ weeks after the first immunizing dose.

TABLE IV.—*Turner's Court, Oxford, Cambridge, and Sheffield Students: Sets TC, O1, O2, C1, C2, and S. Antitoxin Titres 4 to 10 Weeks after First Immunizing Dose*

Antitoxin Units per c.cm. of Serum	A		Total
	No.	A + P	
Good response : 1/50 or more ..	20 (14%)	7 (6%)	27
Fair response : 1/100 to 1/500 ..	11 (7%)	67 (52%)	78
Poor or no response : 1/1,000 or less ..	116 (79%)	54 (42%)	170
Total	147 (100%)	128 (100%)	275

A = Active immunization with 0.1 c.cm. of A.P.T.

A + P = Ditto, plus 350 to 500 units of antitoxin.

$\chi^2 = 68.089$. $P < 0.000001$.

As in Table II, it is seen that the proportion of sera containing 1/500 unit or more of antitoxin was considerably higher in the A + P than in the A group—58% contrasted with 21%. And again as in Table II, more sera in the A group had a relatively higher antitoxin content than in the A + P group. The higher proportion, however, of sera in the A + P group containing antitoxin is shown to be significant by the χ^2 test.

In Table V are set out the antitoxin titres in Sets TC, O1, O2, C1, C2, and S at an average of 8½ weeks after the second dose of A.P.T. As in Table III, the proportion of sera in Group A containing 1/50 unit or more of antitoxin is higher than in Group A + P—65% as compared with 50%—but the difference is not so great, and the lower value of χ^2 renders this difference of far less significance.

Judged by the percentage values the difference between the two groups in Table IV is slightly less than in Table II; this may perhaps be due to the fact that the average time of bleeding of the students recorded in Table IV was later than that in Table II. This, in turn, might be expected (a) to lead to a

TABLE V.—*Turner's Court, Oxford, Cambridge, and Sheffield Students: Sets TC, O1, O2, C1, C2, and S. Antitoxin Titres 8 to 12 Weeks (average 8.7 Weeks) after Second Immunizing Dose*

Antitoxin Units per c.cm. of Serum	A		Total
	No.	A + P	
Good response : 1/50 or more ..	91 (65%)	59 (50%)	150
Fair response : 1/100 to 1/500 ..	36 (26%)	38 (32%)	74
Poor or no response : 1/1,000 or less ..	13 (9%)	21 (18%)	34
Total	140 (100%)	118 (100%)	258

A = Active immunization with 0.1 c.cm. and 0.3 c.cm. A.P.T. at 4 to 10 weeks' interval (average 4.4 weeks).

A + P = Ditto, plus 350 to 500 units of antitoxin at time of first injection.

$\chi^2 = 6.938$. $P = 0.031$.

greater excretion of the antitoxin inoculated into the A + P group, and (b), on the assumption that serum delays the antitoxin response, to reveal the beginning of antitoxin production resulting from the first dose of A.P.T. in Group A.

Again, the difference between the two groups in Table V is less than in Table III; this may be related to the greater length of time that elapsed between the second immunizing dose and the final bleeding of the students recorded in Table V. If it is assumed that the serum delayed but did not prevent the antitoxin response of subjects in the A + P group, this would have allowed time for the formation of antitoxin in an amount more nearly approaching that of subjects in Group A. These suggestions are admittedly hypothetical, but evidence will now be brought to show that they are not without foundation.

It may be noted that the results in Tables II to V are weighted to some extent in favour of the A group by the circumstance that 7 students in the A group are known to have been immunized previously, as against only 3 in the A + P group.

RATE OF DISAPPEARANCE OF INJECTED ANTITOXIN FROM THE CIRCULATION

At Cambridge 12 of the students in the A + P group, each of whom had received 0.1 c.cm. of A.P.T. and 500 units of antitoxin, and bled at weekly intervals for 4 weeks so as to determine the antitoxin content of their serum. The results are given in Table VI.

TABLE VI.—*Fall in Antitoxin Content of Serum within 4 Weeks of Inoculation of 0.1 c.cm. of A.P.T. + 500 Units of Antidiphtheria Serum. Part of Set C2*

Antitoxin Units per c.cm. of Serum	1 Week	2 Weeks	3 Weeks	4 Weeks
1/25 or greater	4	1	1	1
1/50	6	0	0	0
1/100	2	10	1	0
1/250	0	0	10	1
1/500	0	1	0	5
1/1,000	0	0	0	2
< 1/1,000	0	0	0	3
Total	12	12	12	12

It will be seen that, roughly speaking, after 1 week the commonest titre was about 1/50 unit, after 2 weeks about 1/100 unit, after three weeks about 1/250 unit, and after 4 weeks between 1/500 and 1/1,000 unit. Apart from one student who had been immunized three to four years previously and whose titre rose from 1/25 unit after 1 week to 1/5 unit after 2 weeks, it would appear that the antitoxin content of the serum fell progressively and that there was no obvious evidence of active formation of fresh antitoxin. At 4 weeks 75% still had 1/1,000 unit or more per c.cm. of serum.

These results may be usefully compared with those in Table II. After 4 weeks only 3 of the 94 subjects in the A + P group had an antitoxin titre of 1/50 unit or more, suggesting the formation of fresh antitoxin. The majority had a titre of between 1/100 and 1/1,000 unit, a total of 79%* having 1/1,000 unit or more. Since only 34%* of those in the A group had 1/1,000 unit or more, it is difficult to avoid the conclusion that the antitoxin present in the great majority of the A + P sera was residual antitoxin.

* These figures are obtained from the individual protocols.

This conclusion is further borne out by comparison of the results in Table VI with those of previous workers who have studied the rate of disappearance of injected antitoxin from the circulation. Most observations of this type appear to have been made on animals, but some, such as those by Ramon, Debré, and Bernard (1933) and E. Madsen (1936), were made on diphtheria patients who were injected with varying quantities of antitoxin for therapeutic purposes. Others, such as those by Frey and Schmid (1939), were carried out, like our own, on subjects who received active and passive immunization simultaneously. Smith (1907) seems to have been one of the few who made observations that were not complicated either by the presence of active disease in the subject or by simultaneous inoculation of an immunizing antigen; unfortunately his observations were limited to those on a single adult male.

Without discussing the results of these workers in detail, attention may be drawn to the work of T. Madsen (1902, 1923), who found that the rate of disappearance of antibody from the blood of passively immunized persons could be represented by an equation which was not unlike that of a monomolecular reaction. That is to say, a curve drawn by plotting the absolute values of antitoxin per c.cm. of blood against time fell rapidly to start with and then flattened out till it was almost parallel with the axis of abscissae. The rate of disappearance, however, varied not only with the amount of antitoxin injected but with the individual person studied. This second variable made it impossible to predict from the behaviour of one person what the rate of disappearance of antitoxin would be in another person. These conclusions of T. Madsen's were borne out by the work of E. Madsen (1936), and there is no reason to doubt their general validity.

The possession of a constant by each individual makes it difficult to compare the results of different workers with each other and with our own. When, in addition to this, other factors may have influenced the result, such as previous immunization against diphtheria by natural or artificial means, as in some of the subjects studied by Ramon and his colleagues, it is clearly impossible within a short compass to make any statistical comparison between the recorded figures of different workers. A general review of these figures, however, shows that when horse serum containing antitoxin is injected into the body a large proportion of it is excreted within the first two or three weeks, but that a small proportion generally persists for at least four weeks.

Perhaps the nearest parallel to our own figures are those given by Frey and Schmid (1939), who inoculated healthy children with 1,000 to 2,000 units of antitoxin intramuscularly together with 1 c.cm. of A.P.T. subcutaneously. All the children, it may be noted, had less than 0.0005 unit of antitoxin per c.cm. in their blood serum at the time of injection. In every child inoculated with antitoxic horse serum the titre fell from a maximum of 0.45 to 1.8 units after 3 days to a figure varying from 0.007 to 0.03 unit after 28 days. In none of them was there any evidence of a rise in the titre suggestive of active antibody formation.

Our own figures, it will be remembered, showed the presence of about 0.002 unit per c.cm. after 28 days. Considering that the amount of antitoxin inoculated into our students was only a half to a quarter of that used by Frey and Schmid, the rate of disappearance of antitoxin from the circulation appears to have been much the same in both groups. These figures are in general agreement with those obtained by Smith (1907) on a healthy adult, and by E. Madsen (1936) on diphtheria patients, whose curves were so regular as to exclude almost any possibility of active antitoxin formation within four weeks.

It may therefore be concluded that in healthy students having less than 1/1,000 unit of antitoxin per c.cm. of serum and inoculated with 0.1 c.cm. of A.P.T. and 500 units of anti-diphtheria serum, any antitoxin found in the blood during the succeeding four weeks is probably residual antitoxin. Occasional students who have had previous experience of the diphtheria bacillus, directly or indirectly, may respond rapidly by the formation of considerable amounts of antitoxin, but apart from these there is little evidence to suggest that when given along with 500 units of antitoxic serum 0.1 c.cm. of A.P.T. is sufficient to stimulate the formation within four weeks of more than minimal quantities of antibody.

RATE OF FORMATION OF ANTITOXIN IN THE A AND A+P GROUPS

From the figures just recorded it is evident that, with the immunizing doses used, fresh antitoxin, except in minimal quantities, is unlikely to be detected within 4 weeks either in those given active immunization alone or in those given combined active and passive immunization. A study of Table III, however, shows that 8 weeks after the second immunizing dose most of the subjects had formed fresh antitoxin. Of those in the A group 88% and of those in the A+P group 71% had produced more than 1/1,000 unit of antitoxin per c.cm. of serum. The question arises whether the poorer response of those in the A+P group was due to real inhibition of antitoxin formation as the result of the simultaneous injection of serum with the first dose of A.P.T., or merely to delay in its formation. To answer this question antitoxin titrations were made at Cambridge on the two groups of students at different times after the second dose of A.P.T.

In the first set of students, C1, the third bleeding was made 6 instead of 8 weeks after the second dose of A.P.T., and a fourth bleeding was made 12 weeks after the second dose of A.P.T. The summarized results are given in Table VII.

TABLE VII.—*Change in Antitoxin Content of Serum between 6 Weeks and 12 Weeks after Second Immunizing Dose. Part of Set C1*

Antitoxin Units per c.cm. of Serum	A		A+P		Total Observations
	6 Weeks	12 Weeks	6 Weeks	12 Weeks	
1/2 to 2	2	2	1	0	5
1/5 to 1/25	4	4	2	6	16
1/50 to 1/250	6	8	11	10	35
1/500 to 1/1,000	2	1	3	4	10
< 1/1,000	1	0	3	0	4
Total	15	15	20	20	70
Rise		7		14	
Fall		3		2	
No change		5		4	

A = Active immunization with 0.1 c.cm. and 0.3 c.cm. A.P.T. at 4 weeks' interval.

A+P = Ditto, plus 400 units of antitoxin at time of first injection.

The figures in the last three rows are obtained from the individual protocols.

It will be noticed that in the A group there was a slight increase in the titre of antitoxin at 12 weeks, 7 sera showing a rise, 3 a fall, and 5 no change. In the A+P group, however, 14 showed a rise, only 2 a fall, and 4 no change. The suggestion is therefore very strong that the rate of formation of antitoxin is slower in the A+P than in the A group. It looks also as if in the A group the peak of antitoxin production is probably reached somewhere between 6 and 12 weeks.

In a second set of students, part of C2, antitoxin titrations were made 8 and 12 weeks after the second dose of A.P.T. The results are shown in Table VIII.

TABLE VIII.—*Change in Antitoxin Content of Serum between 8 Weeks and 12 Weeks after Second Immunizing Dose. Part of Set C2*

Antitoxin Units per c.cm. of Serum	A		A+P		Total Observations
	8 Weeks	12 Weeks	8 Weeks	12 Weeks	
1/2 to 2	2	2	1	1	6
1/5 to 1/25	24	23	5	8	60
1/50 to 1/250	13	13	17	25	68
1/500 to 1/1,000	7	9	12	9	37
< 1/1,000	2	1	11	3	17
Total	48	48	46	46	188
Rise		8		28	
Fall		19		4	
No change		21		14	

A = Active immunization with 0.1 c.cm. and 0.3 c.cm. of A.P.T. at 4 weeks' interval.

A+P = Ditto, plus 500 units of antitoxin at time of first injection.

The figures in the last three rows are obtained from the individual protocols.

The difference between the two groups is even more striking than in Table VII; of the A group only 8 sera showed a rise, whereas 19 showed a fall and 21 no change. In the A+P group the corresponding figures were 28, 4, and 14. The two conclusions reached from a study of Table VII are therefore considerably strengthened.

In a third set of students, part of C2, antitoxin titrations were carried out 8 and 23 weeks after the second dose of A.P.T. Unfortunately the observations, which are recorded in Table IX, were restricted to members of the A group.

TABLE IX.—*Change in Antitoxin Content of Serum between 8 Weeks and 23 Weeks after Second Immunizing Dose. Part of Set C2*

Antitoxin Units per c.cm. of Serum	A		Total Observations
	8 Weeks	23 Weeks	
2 and over	1	0	1
1/2 to 1	0	1	1
1/5 to 1/25	12	6	18
1/50 to 1/250	1	8	9
1/500 to 1/1,000	5	4	9
< 1/1,000	2	2	4
Total	21	21	42
Rise		6	
Fall		10	
No change		5	

A=Active immunization with 0.1 c.cm. and 0.3 c.cm. of A.P.T. at 4 weeks' interval.
The figures in the last three rows are obtained from the individual protocols.

The number of students was small, but it will be seen that rather more showed a fall in the antitoxin content of their serum than a rise. The six subjects that did show a rise were mainly those whose titres after 8 weeks had been very low.

Combining the figures for the antitoxin values at 12 weeks, recorded in Tables VII and VIII, we may see whether the difference at this interval of time after the second dose of A.P.T. is as great as that recorded in Table III after 8 weeks (Table X).

TABLE X.—*Antitoxin Content of Serum 12 Weeks after Second Immunizing Dose. Set C1 and Part of C2*

Antitoxin Units per c.cm. of Serum	A	A + P	Total
	No.	No.	
Good response : 1/50 or more ..	46 (67%)	31 (44%)	77
Fair response : 1/100 to 1/500 ..	20 (29%)	30 (43%)	50
Poor or no response : 1/1,000 or less ..	3 (4%)	9 (13%)	12
Total	69 (100%)	70 (100%)	139

$$\chi^2 = 7.915. \quad P = 0.0193.$$

There is no question that the proportion of students in the A group having relatively high titres is greater than in the A + P group, but the difference is very much less than in Table III. Moreover, the value of χ^2 is considerably smaller. Eight weeks after the second dose the odds against obtaining by chance alone such a distribution as that figured in Table III were 2,128 to 1 against. Twelve weeks after the second dose, however, the odds against obtaining such a distribution as that figured in Table X are reduced to 52 to 1 against.

Taking together the results recorded in Tables VII, VIII, IX, and X, the following tentative conclusions may be drawn:

1. In Schick-positive students of the age and type that we are dealing with, doses of 0.1 and 0.3 c.cm. of A.P.T. at 4 weeks' interval give rise to antitoxin formation which, judged by the titre of the serum, reaches its height as a rule about 8 weeks after the second injection.

2. If an injection of 400 to 500 units of antitoxin is given with the first dose of A.P.T., antitoxin formation is considerably slower, and probably does not reach its maximum till 12 weeks or more after the second injection of A.P.T.

3. While no observations were made on students of the A + P group later than 12 weeks after the second injection of A.P.T., it would appear that up to this time, at any rate, the amount of antitoxin formed by members of the A group is rather greater than that by those of the A + P group. In other words, the simultaneous administration of serum with the first dose of A.P.T. not only delays the formation of antitoxin but probably inhibits it to some extent. The degree of inhibition, however, does not seem to be very great.

THE SCHICK-CONVERSION RATE AFTER IMMUNIZATION

Schick tests were carried out by inoculating 0.2 c.cm. of the toxin intradermally into the flexor surface of the left forearm and 0.2 c.cm. of the control toxin into the right forearm. The tests were usually read 3 to 5 days later, and the positive results

were classified into four groups on the basis mainly of the diameter of the erythematous area.

Before immunization all students had less than 1/1,000 unit of antitoxin per c.cm. and all were Schick-positive. After immunization the Schick tests were carried out at different times. If we examine the results of those students who were retested 8 or 8 to 10 weeks after the second dose of A.P.T. (i.e., TC, O1, O2, and S sets) and those who were retested 12 weeks after the second dose (i.e., C1 and part of C2) we obtain the following results (Table XI):

TABLE XI.—*Result of Final Schick Tests 8 to 12 Weeks after Second Immunizing Dose: Sets TC, O1, O2, C1, Part of C2, and S*

Groups	Time after 2nd Dose of A.P.T.	Group A			Group A + P		
		No. Tested	No. Pos.	% Schick Conversion Rate	No. Tested	No. Pos.	% Schick Conversion Rate
TC, O1, O2, and S	8-10 weeks	48	2	95.8	50	4	92.0
C1 and part of C2	12 "	67	3	95.5	66	7	89.4
Total ..	8-12 weeks	115	5	95.7	116	11	90.5

Observed difference between A and A + P = 5.2%. Standard error of this difference = 3.3%. Observed difference divided by S.E. of difference = 1.6.

No obvious difference is to be seen between the Schick-conversion rate at 8 to 10 weeks and at 12 weeks. At both intervals Group A is slightly superior to Group A + P, but the differences are clearly not significant. It may be noted that the Schick-conversion rate even in the A group is not as high as that obtained in school children with the same dosage of A.P.T.—namely, 98%. The reason for this will be discussed in another paper.

RELATION BETWEEN THE SCHICK REACTION AND THE ANTITOXIN CONTENT OF THE SERUM

For the sake of interest we have analysed the Schick reactions as observed 8 to 23 weeks after the second dose of A.P.T. in terms of the antitoxin content of the serum of the subjects tested. The results are set out in Table XII.

TABLE XII.—*Relation of Schick Reaction to Antitoxin Content of Serum 8 to 23 Weeks after Second Immunizing Dose. Sets TC, O1, O2, C1, C2, and S*

Antitoxin Units per c.cm. of Serum	Schick Reaction				Approximate Percentage Positive
	No. Tested	++	+	Total Positive	
1/10—>2	74	—	—	—	0
1/25	46	—	—	—	0
1/50	34	—	—	—	0
1/100	27	—	2	2	7
1/250	25	1	—	1	4
1/500	22	—	3	3	14
1/1000	12	1	4	5	42
< 1/1,000	9	3	4	7	78
Total	249	5	13	18	7

It will be seen that subjects whose serum contained 1/50 unit or more per c.cm. all reacted negatively to the Schick test, and that 2 out of 9 subjects whose serum contained less than 1/1,000 unit likewise gave a negative reaction. As previous observers have found (see Parish and Wright, 1938), the zone over which a positive or a negative Schick reaction may be registered is very wide, so that the Schick reaction affords no precise indication of the antitoxin content of the serum. It is true to say, however, that though a Schick-negative response may be obtained in a subject with less than 1/1,000 unit of antitoxin, it is uncommon for a Schick-positive response to be obtained in a subject with 1/100 unit or more, and very uncommon with 1/30 unit or more.

Discussion

These results require little discussion. On the whole they are in conformity with the earlier findings of Ramon and his colleagues working with tetanus anatoxin and antitoxin, and with those of Martin, Loiseau, and Laffaille (1928) and of Gierthmühlen and Voges (1939) working with

diphtheria toxoid and antitoxin. They show that the administration of antitoxic serum along with A.P.T. leads both to a delay and to some degree of inhibition in the formation of fresh antitoxin.

It is interesting to speculate on the cause of this effect. In view of the observations by Glenny, Buttle, and Stevens (1931) on guinea-pigs, the suggestion might be made that in our experiments the dose of A.P.T. used—0.1 c.cm.—at the first injection was so small that it was to a large extent neutralized by the simultaneously inoculated serum. That it was not all neutralized is clear from the fact that the great majority of the subjects in the A+P group were sensitized, and responded to a second injection of 0.3 c.cm. of A.P.T. The difference between the A and the A+P groups may therefore have been essentially a difference in the degree of sensitivity produced by the first dose. The members of the A group were highly sensitized, and responded to a second dose by rapid and fairly liberal production of antitoxin: members of the A+P group, on the other hand, were less highly sensitized, and responded by a slower and less liberal production of antitoxin. If this explanation is correct it follows that a larger dose of A.P.T. given at the first injection to members of the A+P group might lead to a higher degree of sensitization and therefore to a diminution or complete abolition of the difference in rate and amount of antitoxin production between members of the A and A+P groups. The truth of this hypothesis is happily open to trial, and it is proposed to test it in further experiments.

From the practical point of view it may be objected that the inhibitory effect of serum renders the method of combined active and passive immunization of doubtful value. If there occurs in the human being the same sort of intermediate stage of relative susceptibility between the disappearance of passive and the development of active immunity as Gundel and König (1938) observed in guinea-pigs, then it may be doubted whether the simultaneous injection of serum with the first dose of A.P.T. presents any considerable advantage over ordinary active immunization. The answer to this question is dealt with by Fulton and his colleagues in Part II of this paper—"Control of Epidemics in the Field"—in which it is shown that, provided the inoculated children are protected from the risk of infection for six weeks after the first dose of A.P.T. by the removal of carriers of the diphtheria bacillus, the chances of their developing diphtheria during the stage of passive immunity or during the subsequent stage of relative susceptibility before active immunity has developed are reduced to a minimum.

Summary and Conclusions

From a study of the literature there appears to be a considerable measure of disagreement between different workers on the value of combined active and passive immunization against diphtheria or tetanus. Much of this confusion has resulted from the use of different methods of experimental approach to the problem.

Our observations were made on a total of about 300 medical students of 20 to 22 years of age at Oxford, Cambridge, and Sheffield, and students of 14 to 19 years at a farm training college.

The subjects were first Schick-tested. All positive reactors were bled, and the antitoxin content of each serum was determined. Immediately after bleeding, the students were inoculated either with 0.1 c.cm. of A.P.T. alone (A group) or with 0.1 c.cm. of A.P.T. and 350 to 500 units of diphtheria antitoxin given simultaneously into opposite arms (A+P group). Four weeks later all students were again bled, and then received 0.3 c.cm. of A.P.T. A third bleeding and a final Schick test were carried out as a rule 8 weeks after the second injection, but this interval was varied somewhat for special purposes.

In analysing the results all students having 1/1,000 unit or more of antitoxin per c.cm. of blood serum at the time of the first inoculation were excluded. Altogether, therefore, observations were available for study on 148 subjects in Group A and 131 in Group A+P.

With the exception of a few in both groups who had presumably had previous experience of the diphtheria bacillus, and who responded rapidly by the production of considerable amounts of antitoxin, none of the students formed more than minimal quantities of antitoxin in the interval between their first and second injections. During this interval the average antitoxin content of the serum of the members of the A+P group fell from about 1/50 unit one week after injection to between about 1/500 and 1/1,000 unit per c.cm. 4 weeks after injection.

After the second injection of A.P.T., antitoxin was formed more rapidly and in rather greater quantity by the A than by the A+P group. The difference in antitoxin content between the two groups 8 weeks after the second injection was highly significant, but after 12 weeks it was far less striking.

The peak of antitoxin production was reached in the A group about 8 weeks after the second dose of A.P.T. In the A+P group antitoxin production was much slower, and probably did not reach its maximum for about 12 weeks.

The Schick-conversion rate 8 to 12 weeks after the second dose of A.P.T. was 95.7% in the A and 90.5% in the A+P group. This difference is not statistically significant.

So far as can be judged from the type of subject examined and the scale of dosage used in our observations, it may be concluded that the simultaneous administration of anti-diphtheria serum with the first dose of A.P.T. leads to a delay and to a slight degree of inhibition in the formation of antitoxin. The final degree of immunity, however, does not appear to be much inferior to that resulting from active immunization alone.

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[Part II, on Control of Epidemics in the Field, will appear next week.]

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