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IMMUNIZATION AGAINST DIPHTHERIA WITH ALUM-PRECIPITATED TOXOID

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DISCUSSION

Though the results obtained in this paper raise many subjects for discussion, space demands that we restrict ourselves to the main point at issue—namely, the optimal dosage of A.P.T. when given in two doses for the immunization of children.

The recommendation by the Ministry of Health of two doses of 0.1 and 0.3 c.cm. at not less than four weeks' interval has been subjected to severe criticism by Bousfield (1941 a, b, c, d) and Lewis (1941b) as being not only insufficient to bring about an adequate degree of immunity but as being positively harmful to the cause of diphtheria immunization as a whole. It is therefore interesting to review briefly the dosage of A.P.T. used by previous workers and to compare the efficacy of the different doses as judged by the Schick test performed at a suitable time after inoculation. The data are summarized in Table VIII.

TABLE VIII.—*Immunizing Effect, as judged by the Schick Test, of Two Doses of A.P.T.*

Observer	No. Tested	Doses in c.cm.	Interval between Doses	Interval between Second Dose and Schick Test	Result of Schick Test
Powell (1935) ..	9	0.1	1 mth.	1 mth.	100% neg.
" (1936) ..	100	0.1	2 wks.	?2-3 mths.	99% "
Parish (1936) ..	35	0.1	3 "	12 wks.	100% S.C.R.
" " ..	129	0.1	3 "	5 "	99.2% "
Sigurdsson (1936)	731	0.1	3 "	7-8 mths.	95.8% "
Anderson (1937) ..	52	0.1-0.2	1 wk.	3 "	82.7% neg.
Russell (1937) ..	159	0.1	2 wks.	?3 "	98.1% "
Bousfield (1938)*	611	0.1	2 "	3-5 "	98.7% S.C.R.
Chesney (1938) ..	1,555	0.1-0.2	4 "	2 "	99.6% neg.
Harder, Geiprin, and Cook (1939)	100ca	0.1	4 "	3 "	90.0% "
Lewis (1941a) ..	760	0.1-0.25	2-4 "	2-3 "	99.2% S.C.R.
" " ..	279	0.1	2-4 "	2-3 "	98.9% neg.
Bousfield (1941b)	187	0.1	4 "	3-5 "	92.5% S.C.R.
Fulton <i>et al.</i> (this paper)†	2,346	0.1	4-6 "	8-16 wks.	97.8% "

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

* Results obtained with one unsatisfactory product omitted.

† Results obtained with two unsatisfactory products omitted.

In the last column of this table the results are expressed in terms of the Schick-conversion rate (S.C.R.) if immunization was confined to known Schick-positive children, and in terms of the Schick-negative rate if a pre-immunization Schick test was not carried out.

It will be noted that most workers used an initial dose of 0.1 c.cm., and that the second dose generally varied from 0.3 to 0.5 c.cm. With the exception of Anderson (1937), who allowed only a week between the two doses, and of Bousfield (1941b), whose observations were made on a relatively small number of children, the Schick-negative or the Schick-conversion rates recorded varied between 97.8 and 100%. Apart from these, it is noteworthy that the two lowest rates—95.8 and 90%—were obtained by workers who injected two doses of 1.0 c.cm. each. It is difficult, therefore, to understand on what grounds the unexpectedly vehement opposition to two doses of 0.1 and 0.3 c.cm. is based.

Admittedly certain batches of A.P.T. are of low antigenic quality. Is an increase in the dosage, however, going to remedy this defect? With the B1 product referred to in Tables IV and V, which had an Lf value of only about 13 units per c.cm., the following results were obtained:

0.1 and 0.3 c.cm.	..	..	..	68.2% Schick-conversion rate
0.2 and 0.4 c.cm.	..	..	..	51.9% " " "
0.5 and 1.5 c.cm.	..	..	..	70.7% " " "

The number of children tested was not large, but it will be observed that even a fivefold increase in the total dosage failed to effect any appreciable improvement in the Schick-conversion rate. While we do not deny that with a product of moderate potency an increase in dosage may stimulate a better antibody response, it is clear that no practicable increase in dosage is going to remedy the antigenic deficiency of a poor product. The main conclusion, therefore, is that for adequate immunization a product of high antigenic potency is essential. With such a product dosage becomes of secondary importance.

The Antigenic Effect of A.P.T.

In trying to formulate a suitable system of dosage of A.P.T. for general use in children, two main factors must be considered: first, the antigenic effect; secondly, the degree of local and constitutional reaction likely to be caused. Unfortunately these two factors are antagonistic to each other. In the absence of properly documented evidence it may be assumed that, within limits (see below), the larger the dosage is, the better will be the antibody response but the worse will be the resulting tissue reactions; the smaller the dosage is, the poorer will be the antibody response but the less will be the trouble caused by severe reactions. There is clearly no perfect system of dosage; whatever system is adopted must be a compromise. Before deciding what compromise to recommend it is necessary to consider a little more closely the two main factors concerned.

A reasonably uniform antigenic stimulus could be assured if practicable methods for the assay of the potency of diphtheria prophylactic, similar in principle to those applied to other biological products, were available. It would then be possible to specify, either in units or by reference to a standard preparation, the quantity of A.P.T. which should be contained in each dose. A solution of the problem on these lines, however, presents great technical difficulties. Ramon (1925) maintained that the flocculation value of a formol toxoid was a good index of immunizing power. Experience, however, has shown that, though a formol toxoid with a high flocculation value is generally a better antigen than one with a low value, there are too many exceptions to this rule to permit of the flocculation value alone being taken as an accurate index of antigenic potency. In order to ensure that an adequate antigenic stimulus is provided, two essential requirements should be fulfilled in the preparation of A.P.T.: first, each preparation should contain an adequate quantity of antigen per unit volume; secondly, the antigen must be of high immunizing quality. Both of these desiderata are apparently met by the preparation of A.P.T. in the way described by Barr, Glenn, Pope, and Linggood (1941). This point will not be discussed further, as many of the samples used in the field trials described in this paper are being studied at the National Institute for Medical Research, Hampstead, one object of these investigations being to determine whether laboratory tests can be devised for the more efficient control of diphtheria prophylactic, A.P.T.

Some authors too readily assume that the antibody response to A.P.T. is in direct proportion to the dose given. Our information on this subject is deficient, but two observations deserve attention. Pharmacologists have found that in general the response to a drug varies not with the dose but with the logarithm of the dose. For example, doubling the dose does not double the response, but increases it to a considerably smaller extent. Again, immunologists have shown that the antibody response to an antigen varies with the point on the dose-response curve at which the antigen is given. At one range of the scale the curve rises steeply and the response to increase in dosage is maximal. Below or above this range the response falls off considerably. Taking the upper range, it is evident that above a certain critical level an increase in the dose will have relatively little effect on the production of antitoxin. If, for example, it can be shown that two doses of 0.1 and 0.3 c.cm. of A.P.T. produce on the average a given amount of antitoxin, there is no reason for supposing that two doses of 0.2 and 0.6 c.cm. will double the antitoxin production. Indeed, since there is reason to believe that with a high-quality product, such as that prepared by maker A, two doses of 0.1 and 0.3 c.cm. are near the upper limit of the maximal dose-response range, it is very doubtful whether raising the dose is likely to bring about any considerable increase in the production of antitoxin. These theoretical considerations are borne out by the observations of Saunders (1932), who found that a Schick-negative rate of 100% occurred as readily after a total dosage of 4.5 Lf units as after one of 22 or 30 Lf units, and by those of Park and Schroder (1932), who, using toxin-antitoxin mixture, obtained as good an immunizing effect after a 0.1 L+ dose as after 3 to 6 L+ doses. However, without making careful antitoxin titrations after injection of different quantities of A.P.T. it will be impossible to form any sound judgment on the relation of dose to antibody production.

On general grounds the greater the amount of antitoxin produced the longer is immunity likely to last. It is dangerous, however, to take the Schick-negative rate after any

long interval of time as a measure of the antitoxin generated, unless the children under observation are known not to have been exposed in the meantime to diphtheritic infection. In practice this can rarely be guaranteed, and for that reason a wide discrepancy is noticed between the reports of various observers on the duration of immunity. Latent infection augments the immunity conferred by inoculation, and the two effects cannot be disentangled. Some observers, such as Park (1922), working in densely populated cities, have had few Schick relapses over a period of many years, whereas others, working in areas where there has been little diphtheria infection, have had high relapse rates. Fraser and Brandon (1936), in Canada, for example, found that of children who had received three doses of formol toxoid five years previously and who had not been exposed to infection since, 34% had reverted to the Schick-positive state. Schwartz and Janney (1938), in the United States, recorded a Schick-relapse rate of 22% in children who had been inoculated six to seven years previously with formol toxoid and who had been living since in a neighbourhood with a low diphtheria incidence rate. Sigurjónsson (1939), in Iceland, likewise found that 26% of children who were Schick-negative seven to eight months after immunization had relapsed to the Schick-positive state four years later. It may be noted that Sigurjónsson gave two inoculations of 1.0 c.cm. of A.P.T. at three weeks' interval, the number of Lf units per c.cm. being not less than 50. His conclusion that immunity to diphtheria is transient unless it is maintained by latent infection deserves serious attention, confirming as it does one of the fundamental theses inherent in the work of Dudley and his colleagues (1934). In this connexion the following quotation from these authors is apposite: "The observations on 'diphtheria' in inoculated subjects show that, *provided it is known that the Schick test has once been negative*, relapses of Schick or antitoxin immunity are really of little consequence. The potential factors of 'immunizability' and 'durability' appear to remain permanently augmented by the same stimulus which produces the appearance of specific antibody, which is apparently a more variable and less permanent factor. Thus, although subjects may have lost their Schick or antitoxic immunity, they still possess a greatly augmented immunizability and rapidly regain Schick immunity on contact with T.C.D. (virulent diphtheria bacilli), generally latently and without symptoms."

The main conclusion, therefore, that emerges from this brief discussion on the antigenic effect of A.P.T. in relation to dosage is that the product used must contain an adequate amount of antigen of high immunizing quality, and that, provided such a product is used, there seems little advantage to be gained by increasing the dosage above that necessary to bring about an average Schick-conversion rate approaching 100%.

Local and Constitutional Reactions

Turning now to the local and constitutional reactions produced by A.P.T., there is a wide variation between the figures recorded for their frequency by such observers as Swyer (1932), McGinnes *et al.* (1934), Faragó (1935), McSweeney (1935), Patterson (1935), Underwood (1935a), Naughten *et al.* (1935), Harrison (1935), Sigurjónsson (1936), Hanly (1937), Jensen (1937), Paschlau (1939), Stott and Duke (1940), Bunch *et al.* (1940), and Lewis (1941a). There are probably several reasons for this. Certain batches of A.P.T. are known to excite more reaction than others; reactions are commoner in Schick-negative than in Schick-positive persons, and commoner in older than in younger children. In addition it is often difficult to obtain accurate information, in non-residential institutions, on the

degree of reaction or even on its occurrence. In our own experience, for example, we found that a certain proportion of children did not attend school for a day or two after inoculation. Possibly this may have been due to factors unconnected with the inoculation itself; on the other hand, their absence may have been accounted for by unusually severe reactions. If, therefore, the number and degree of reactions are assessed merely on the children who do attend school a false impression may easily be gained. There is little doubt, however, that a certain proportion of severe reactions do occur, and these constitute a serious drawback to the use of A.P.T. Unfortunately our information on the relation between the size of dose and the subsequent tissue reaction is very incomplete, and until more observations on this subject are recorded it is probably wiser to accept the assumption that, within limits, the smaller the dose the less will be the degree of reaction.

Dosage for Routine Use

In the light of these considerations it is now possible to discuss what dosage of A.P.T. should be recommended for general adoption in the immunization of school and pre-school children. Advocates of a large first dose, such as 0.5 c.cm., are apt to minimize the evil effect of occasional severe reactions, which in the experience of many observers (Patterson, 1935; Pickles, 1940; Stott and Duke, 1940) greatly prejudice the success of an immunization campaign. They argue that if the child does not return for its second dose it will at least develop some basal immunity to diphtheria toxin. Practically all workers with A.P.T., however, are now in favour of the two-dose method, and it is much better for a child to have a small first dose and return for a larger second dose than for it to experience such a severe reaction after a large first dose that its parents refuse to allow further inoculations to be made. Failures to attend for other reasons are more adequately dealt with by a proper follow-up system than by reliance on a large first dose.

A further argument sometimes used is that, even if a serious reaction does occur, it is of little practical importance, since it indicates that the child is already partly immune and does not require a second dose. The observations of Swyer (1932), Underwood (1935b), and Bunch *et al.* (1940), among others, show that there are too many exceptions to this rule for it to be generally applicable.

If a small first dose is the method selected, then it is necessary to choose between 0.1 and 0.2 c.cm. The smaller dose is quicker, cheaper, presumably excites less reaction, and, in our experience with A.P.T. containing an adequate amount of high-quality antigen, has resulted in a good Schick-conversion rate. Under certain conditions, however, it may have the disadvantage of approaching the borderline of satisfactory immunizing efficiency. For this reason, unless the inoculation is made in a properly calibrated syringe by plunging the needle up to the hilt through a valvular opening into the deep subcutaneous tissue, the full quantity may not be injected, or, if injected, may partly escape. Another error may be introduced by failure to ensure uniformity of the inoculum. This may happen either through neglect to shake the contents of the bottle thoroughly or because successive children are inoculated so slowly that partial flocculation of the alum toxoid occurs in the syringe. Mainly for practical reasons, therefore, a specified dose of 0.2 c.cm. is probably to be preferred to one of 0.1 c.cm., though there is no reason why the lower dose should not be given if circumstances point to its desirability.

On the whole, therefore, we are of the opinion that to minimize the risk of severe reactions and yet to provide a

sufficient antigenic stimulus the first dose should be 0.2 c.cm. Some workers, particularly Lewis (1941a), prefer to give a larger dose to children under 8 years of age on the ground (a) that they suffer from severe reactions less often than older children, and (b) that, owing to lack of any basal immunity established as the result of latent immunization, they require a greater antigenic stimulus than children over 8 years. The wisdom of this is open to question. There is no sharp transition in the frequency of severe reactions at the age of 8 years; and it is doubtful whether, except in a heavily infected residential institution such as that studied by Dudley and his colleagues (1934), latent immunization plays a sufficiently important part to justify a differentiation of dosage for older and younger children. Lewis himself found no significant disparity in the immunizability of children in schools with widely different incidence rates of diphtheria, and in our own experience analysis of the Schick-conversion rate in relation to ages failed even to suggest that up to the age of 18 years there was any appreciable difference in the immunizability of the children (see Table VI, p. 319). Variation of dosage with age merely complicates what should be a simple routine procedure, and has little to recommend it.

The size of the second dose can generally be increased with greater safety, since, even if a severe reaction does occur, the child will have completed its immunizing course, and need not return for some years. In clinics where the doctor talks to the mother and is inoculating perhaps only 20 to 30 children an hour, the size of the second dose can be graded according to the severity of the reaction after the first dose; but in school practice, where the mother does not attend, and where, as in our experience, one doctor with suitable clerical and technical assistance can inoculate 300 to 400 children an hour, this is impracticable. For general use, therefore, it seems wise to recommend a dose of 0.3 to 0.5 c.cm. for the second inoculation.

Bousfield (1941b) prefers a two-weeks interval between the doses. His advocacy, however, of this method is based on convenience rather than on immunological considerations, and there is now an increasing body of evidence to show that the interval should be at least four weeks, and probably even longer. For practical purposes, however, four weeks may be accepted as a suitable time. If it is exceeded, even by some weeks or months, there is no reason to fear that the antibody response to the second dose will be in any way diminished.

Summary and Conclusions

In the course of inoculating about 30,000 children against diphtheria during the past two years, we have put the efficacy of our method on trial by Schick-testing a proportion of the children before and after inoculation. Our practice was to give inoculations of 0.1 and 0.3 c.cm. of alum-precipitated toxoid (A.P.T.) at an interval of four to six weeks, to Schick-test eight to sixteen weeks after the second inoculation, and to calculate the Schick-conversion rate.

Our observations were made almost entirely on school children in small towns and villages. Apart from evacuees in some of the schools, few of the children had been inoculated before, and with one or two exceptions there had been little diphtheria in the district for some time previously. Of 2,751 boys Schick-tested before inoculation 74.5%, and of 2,150 girls 83.1%, reacted positively. The intensity of the Schick reactions did not appear to be affected by the age of the child. Pseudo-reactions were uncommon, and were noted in only 1.2% of subjects. It is doubtful whether the injection of the control toxin is worth making at the primary Schick tests in children under 10 years of age; the error caused by its omission in our series would have been less than 0.2%.

Observations on the local tissue reaction to A.P.T. were made in only a few schools. Approximately half the children we did inspect showed some degree of reaction after inoculation. In the majority of these the reaction was circumscribed, but occasionally the whole of the upper arm, and very occasionally the forearm also, was affected. Sterile abscesses were reported in a number of children inoculated with one particular batch of A.P.T. Local reactions were found to be commoner in Schick-negative than in Schick-positive reactors. We had no opportunity of observing the constitutional reaction that may have followed inoculation.

Seven different preparations of A.P.T. from six different makers were studied. One preparation, referred to as A, was used exclusively in twelve schools, and as a control to one or more of the other preparations in ten other schools. Different batches of A.P.T. from any given maker were generally used for different schools. In 1,786 children inoculated with preparation A the Schick-conversion rate was 97.8%. Four other preparations used on smaller numbers of children yielded Schick-conversion rates of 97.8%, 97.7%, 98.2%, and 99.4% respectively. Two preparations, B1 and F, proved thoroughly unsatisfactory, giving rates of only 68.2 and 74% respectively. One of these—B1—was tested in doses of 0.2 and 0.4 c.cm. and of 0.5 and 1.5 c.cm.; the results, however, were no better, the corresponding Schick-conversion rates being 51.9 and 70.7%.

To find out whether the immunity conferred by doses of 0.1 and 0.3 c.cm. was lasting, two schools that had been Schick-tested before and after inoculation were re-Schick-tested thirteen months after the second dose of A.P.T.; 97.7% of the children proved to be Schick-negative. Tests were then carried out on seven schools which had never been Schick-tested before. Of 966 children Schick-tested twelve months after their second inoculation of A.P.T., 91.3% were Schick-negative. Of the positive subjects, two-thirds gave trivial reactions, many of which would probably have been regarded as negative by less exacting observers. There had been no diphtheria in any of these schools after inoculation and practically none in the whole town. The effect of artificial immunization, therefore, can hardly have been augmented by that of latent infection, which has been shown by previous workers to be necessary for maintaining a high Schick-negative rate. For this reason the results we obtained may be regarded with satisfaction.

In the light of our findings, and of those of previous workers, the optimal dosage, of A.P.T. for the routine immunization of school children is discussed. Comparatively few exact observations have been reported on the two-dose method, and there is no general agreement on the best dosage to adopt. It is pointed out that any dosage recommended must be a compromise between the immunizing efficiency of the antigen and the tissue reaction to which it gives rise. Provided that in future only preparations of A.P.T. of guaranteed potency are issued, it is concluded that for general use two inoculations of 0.2 c.cm. and 0.3 to 0.5 c.cm. at an interval of not less than four weeks should provide an adequate degree of immunity against diphtheria without prejudicing the success of the immunization campaign by the occurrence of unduly severe tissue reactions. The increase of the first dose from 0.1 to 0.2 c.cm. is recommended mainly on practical considerations, since the observations recorded in this paper show that a primary dose of 0.1 c.cm., when properly administered and followed in not less than four weeks' time by a second dose of 0.3 c.cm., is capable of bringing about an adequate degree of immunity. The optional increase of the second

dose to 0.5 c.cm. is suggested mainly to widen the margin of safety whenever this is considered desirable. If, for instance, the A.P.T. has not been stored in the refrigerator or is nearing its expiry date, the larger dose might be preferable.

For routine purposes it is recommended that children should be immunized with two doses at the age of 1 year, and that they should receive a single dose of 0.3 to 0.5 c.cm. to stimulate their waning immunity on entering school at 5 years, and again at the age of 10 years. Except when comparing the immunizing efficiency of different preparations of A.P.T., pre-immunization Schick tests are rarely called for; but Schick tests carried out on a small sample of the children two to four months after inoculation serve as a useful control to the efficiency of the method employed.

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