rates of between 4 and 15, while the other half were scattered between 15 and 70. Of incidental interest is the fact that if the arithmetic value of the leakage rate is replaced by its logarithm, the distribution is very nearly normal. It may also be seen from the figure that the maximum leakage may be lowered from 70 to 25 by rejecting 15% of the syringes. To have a maximum leakage of 20 means rejecting 25% of the syringes, and a maximum leakage of 15

FIG. 3.—The cumulative distribution of the leakage in 387 new syringes. Leakage is measured in units of 0.01 ml. per six minutes under a pressure of approximately 5 kg. per cm.², and these units are marked on the horizontal scale of the figure. The vertical scale gives percentages of the 387 syringes tested, and each point on the curve indicates the percentage of the total sample which have a leakage rate below the specified value.

means 'rejecting' half of the syringes. Information from a curve of this kind is thus a good guide when a compromise is sought between economy and low maximum leakage.

It should be understood, of course, that the particular curve shown here applies to a rather expensive brand of syringes selected as one of the best from a number of different products available in large quantities. As an example of the results obtained with other brands, we found leakage rates ranging from 19 to 144 with one sample of 24 new Record syringes of the type selected for a mass programme as having comparatively low leakage. Half of this sample of syringes had rates above 52.

New syringes with a leakage rate below 20 are seldom found to show any apparent leakage when first used in the field. Only the sides of the plunger become moist, evaporation evidently keeping pace with minimal leakage. After 1,000 to 1,500 injections, however, syringes usually begin to leak, one or two drops for every 10 to 20 injections. Both the syringe and the tester's hand then begin to get wet, and further increase in leakage (or leakage from between the needle and the tip of the syringe) can no longer be detected. Thus it is not difficult in the field to know when a syringe is leaking, but it is difficult to estimate how much fluid is lost.

In the Tuberculosis Research Office we have adopted the plan of testing all new syringes before they are sent to the field, rejecting those with leakage rates above 20. The moment a syringe begins to leak steadily in the field we ask that it be returned for retesting in the apparatus (to control gross error in the tester's judgment). Leakage rates between 20 and 30 are usually found; if above 20 they are rejected for further field use.

The cost of such strict criteria may seem prohibitive even for research work, but the cost of leakage cannot be disregarded. For example, the leakage rates for a sample of 55 syringes, discarded because of leakage in a mass B.C.G. campaign where "some" leakage was tolerated, was found to vary from 20 to 540, with half of the sample we tested having a rate above 90. A rate of 90 probably corresponds to a loss of 15 or perhaps 20% of the amount of fluid actu-

ally injected—a net loss of 15 ml. of tuberculin or vaccine for 1,000 injections, at a cost which cannot be considered negligible when compared with the cost of a new syringe. Workers from some of the mass campaigns maintain that occasionally they have used 1-ml. syringes yielding only one or two vaccinations for each refilling—a very costly procedure indeed, in terms of both time and money.

Summary

A home-made apparatus is described for measuring leakage between the plunger and barrel of tuberculin syringes. Even with the brand of syringe which we have found the most satisfactory, about one out of every four new syringes leaks to a greater extent than is compatible with accurate work. After being used for 1,000 to 1,500 intradermal injections, many syringes begin to show obvious signs of leakage, and, when retested, fail to meet the criteria we have adopted for acceptability. Unless new syringes are first tested before use and a plan for periodic retesting is followed, it is unreasonable to expect field workers to be able to measure accurately the amount of fluid given in intradermal injections or to avoid wasting rather large amounts of tuberculin and vaccine.

A NEW DIPHTHERIA PROPHYLACTIC (N.A.F.T.)

BY

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It has been known for some time that culture filtrates of toxigenic *Corynebacterium diphtheriae* may vary greatly in their antigenicity per Lf when tested in guinea-pigs and in horses (Barr and Glenny, 1949). Hartley (1935) suspected an undetermined factor in the original culture medium as a possible reason for these variations, but failed to isolate any substance having an adjuvant effect on a poorly antigenic sample of toxoid.

Experiments

During the past few years we have experienced the same phenomenon of marked variation of antigenicity of different samples of crude toxoid per Lf injected, particularly in respect of the hyperimmunization of horses. This was particularly clear-cut when the results from three types of culture medium were examined. The three media were: (a) veal-infusion-proteose-peptone, (b) pig-stomach autolysate, and (c) casein hydrolysate. The toxoid derived from the veal-infusion-proteose-peptone gave by far the best antitoxin responses. This observation was confirmed by carefully controlled experiments in guinea-pigs. Since the responses to the veal-infusion-proteose-peptone medium toxoid were so much better our attention was directed to a possible non-toxoid component of the crude toxoid possessing adjuvant properties. That the veal-infusion-proteose-peptone toxoid did contain such an adjuvant substance was revealed by the results from the following experiment.

The toxoid contents from each of the three culture media toxoids were removed by the addition of the optimal amounts of highly purified horse antitoxin. The T.A.F. floccules were centrifuged and the supernatants separated. Then a fixed amount of freeze-dried toxoid purified by the method of Levine *et al.* (1949) was added

to equal volumes of each of the three supernatants, using physiological saline as the control. Potency tests in guinea-pigs, tested four weeks after one dose of 1 Lf, showed that the veal-infusion-proteose-peptone toxoid supernatant greatly increased the antigenicity of the added toxoid, the casein hydrolysate supernatant decreased it, and the pig-stomach hydrolysate had no apparent effect compared with the controls.

Subsequent research in the veal-infusion-proteose-peptone crude toxoids showed that the adjuvant factor—previously described as the synergic factor (Lahiri, 1951)—was diffusible through “cellophane” (grade 600), then could be adsorbed on to activated charcoal and be eluted with N/50 sodium hydroxide. The method now adopted is to dialyse the crude toxoid, adsorb the adjuvant factor on to activated charcoal, and elute with diluted sodium hydroxide. The eluate is then neutralized and evaporated to dryness. Technical details of the method have already been described (Lahiri, 1952), and further details will be published later. The following experiment illustrates the adjuvant effect of the material obtained.

Freeze-dried purified toxoid was dissolved in (a) detoxoided crude toxoid, (b) the parent broth, (c) the neutralized charcoal eluate, (d) the residual fluid of the diffusate

after treatment with charcoal, and (e) saline—the control. The immunity-response results, using guinea-pigs, are recorded in Table I.

Subsequent experiments, using the same culture medium, failed to reveal the presence of the adjuvant factor in tetanus culture filtrates, and, conversely, the diphtheria toxoid adjuvant factor failed to augment the antigenicity of purified tetanus toxoid. This is illustrated by the following experiments: purified tetanus toxoid was dissolved in (a) a solution of the partially purified diphtheria toxoid adjuvant factor, (b) analogous material derived from tetanus toxoid diffusate, and (c) physiological saline. The results are recorded in Tables I and II.

Field Trials

These results were so promising, as judged by laboratory tests, that a series of field trials were carried out, employing 433 young children and 80 adults. Each of our groups of children (aged 1–8 years) were immunized with a different prophylactic: (1) with our purified diphtheria toxoid augmented with the charcoal eluate adjuvant factor Batch No. 1; (2) with a duplicated preparation, Batch No. 2; (3) with an A.P.T. of British manufacture; and (4) with A.P.T. made in the Haffkine Institute. The children were Schick-tested before their single dose of diphtheria prophylactic and post-Schick-tested four to eight weeks after inoculation; careful note was also made of the number and severity of the reactions encountered. The results obtained are summarized in Table III, and show (a) that the new prophylactic, termed N.A.F.T. (natural adjuvant factor toxoid) is as good an antigen as the A.P.T. of British manufacture or as that made in our Institute; and (b) that N.A.F.T. is much less prone to cause reactions than the two kinds of A.P.T.

In our experiments with 80 adults (aged 25–50), none of whom had previously received anti-diphtheria prophylactic, each of the subjects was bled before inoculation and again four weeks after inoculation. They were subdivided into six groups, each group receiving a different form of prophylactic. The results are analysed in Tables IV, V, and VI. The value of this experiment, however, was seriously diminished by the quite unexpected finding that nearly

TABLE I.—*Survival of Guinea-pigs Challenged with 20 C.L.D.* of Test Toxin, Administered Four Weeks After Immunization with 1 Lf of the Purified Diphtheria Toxoid, Dissolved in Different Fractions of Crude Veal-infusion-proteose-peptone Toxoid: Control in Saline*

Fraction into which the Purified Toxoid was Dissolved	Survival Rates in Three Animal Assays			
	1	2	3	Total
Detoxoided crude veal-infusion-proteose-peptone diphtheria toxoid ..	7/7	7/8	6/7	20/22
Neutralized charcoal eluate ..	8/8	7/8	14/15	29/31
Parent broth ..	1/8	0/8	2/8	3/24
Residue of toxoid diffusate after treatment with charcoal ..	2/8	1/8	3/16	6/32
Detoxoided crude tetanus-veal-infusion-bacto-peptone toxoid ..	2/8	1/7	2/8	5/23
Saline control ..	3/16	3/15	5/24	11/55

* 1 C.L.D. equals the smallest quantity of the test-toxin which kills all of four normal guinea-pigs within four days of injection.

TABLE II.—*Survival of Mice Challenged with 20 C.L.D. of Tetanus Test Toxin, Administered Four Weeks After Immunization with (a) 1 Lf, and (b) 2.5 Lf of Purified Tetanus Toxoid, each Dissolved in Different Toxoid Fractions*

Solvent for the Purified Tetanus Toxoids	Purified Tetanus Toxoids			
	Batch 1 Dose		Batch 2 Dose	
	1 Lf	2.5 Lf	1 Lf	2.5 Lf
Solution of diphtheria N.A.F. ..	4/30	10/28	7/30	12/29
Analogous material, derived from veal-infusion-bacto-peptone tetanus toxoid ..	6/30	9/30	5/30	10/30
Saline ..	5/29	14/30	7/28	10/29

TABLE IV.—*Pre-immunization Titre of Adult Volunteers Never Artificially Immunized Before. Titre Expressed in International Units of Antitoxin per ml. of Volunteers' Sera. The Figures Indicate the Number of Volunteers Whose Sera Showed a Titre Within the Stated Range*

Group	Ranges of Titre at which the Sera of the Volunteers were Tested							Total No. of Volunteers	Geometric Mean Titre (International Units) Approx.
	<0.01	0.01–0.025	0.025–0.05	0.05–0.2	0.2–0.5	0.5–1	1–2		
1	1	0	4	6	4	1	1	17	0.07
2	1	2	1	5	4	2	2	17	0.09
3	2	0	2	5	5	1	1	16	0.08
4	2	0	3	4	1	0	2	12	0.06
5	2	2	1	4	1	1	0	11	0.04
6	0	1	2	2	0	1	1	7	0.07

TABLE III.—*Reaction and Results of Schick Test in Children Immunized with Different Diphtheria Prophylactics. (The Figures Indicate the Number of Children of the Stated Category)*

Group	Total No. of Children	Immunized With	Results of Schick Test					Reactions		
			Before Immunization		After Immunization		Not Tested	Mild	Moderate	Severe
			Negative	Positive	Negative	Positive				
1	151	25 Lf/1 ml. N.A.F.T. batch 1 ..	Not tested		149	0	2	0	0	0
2	89	36 Lf/0.5 ml. A.P.T. of British manufacture ..	7	82	79	7	3	17	7	1
3	78	28 Lf/0.5 ml. A.P.T. of Haffkine Institute ..	9	69	69	8	1	18	5	2
4	115	25 Lf/0.5 ml. N.A.F.T. batch 2 ..	17	98	102	13	0	0	0	0

TABLE V.—Response of Adult Volunteers to Injection of Different Kinds of Diphtheria Prophylactic. Response is Shown by Approximate Titre of Antitoxin Developed per ml. of the Volunteers' Sera Four Weeks After Immunization. The Figures Indicate the Number of Volunteers Whose Sera Showed a Titre Within the Stated Range. Titre Expressed in International Units. Pre-immunization Titres of these Groups of Volunteers are Shown in Table IV

Group	Immunized With	Ranges of Titre at which Sera of Volunteers were Tested (I.U.)												Total Number of Volunteers	Geometric Mean Titre (International Units) Approximate
		0.01–0.025	0.2–0.5	0.5–1.0	5–10	10–25	25–50	50–75	75–100	100–150	150–200	200–250	250–300		
1	5 Lf of native toxoid precipitated with 0.2 mg./Lf alum.	0	0	0	0	1	4	9	2	0	1	0	0	17	43
2	5 Lf of native toxoid mixed with 0.2 mg./Lf N.A.F.	0	0	0	1	0	1	3	2	8	2	0	0	17	68
3	5 Lf of native toxoid alone	1	0	1	0	3	5	4	1	1	0	0	0	16	14
4	5 Lf of international ref. std. toxoid mixed with 0.2 mg./Lf N.A.F.	0	0	0	0	1	1	4	0	4	0	1	1	12	67
5	1 Lf of international ref. std. toxoid mixed with 0.2 mg./Lf N.A.F.	0	1	1	1	0	1	2	0	3	0	2	0	11	24
6	5 Lf of international ref. std. toxoid alone	0	0	1	0	2	2	1	1	0	0	0	0	7	14

TABLE VI.—Reaction of Adult Volunteers to Injection of Different Kinds of Diphtheria Prophylactic. The Figures Indicate the Number of Volunteers who Showed the Stated Variety of Reaction

Group	Total No. of Volunteers	Immunized With	Nature of Reactions Caused by the Prophylactic in the Volunteers			
			Immediate Allergic or Inflammatory			Delayed Allergic
			Mild	Moderate	Severe	
1	17	5 Lf of native toxoid precipitated with 0.2 mg./Lf alum.	5	3	1	0
2	17	5 Lf of native toxoid mixed with 0.2 mg./Lf N.A.F.	0	0	0	0
3	16	5 Lf of native toxoid alone	1	0	0	4
4	12	5 Lf of foreign toxoid mixed with 0.2 mg./Lf N.A.F.	1	0	0	0
5	11	1 Lf of foreign toxoid mixed with 0.2 mg./Lf N.A.F.	0	0	0	0
6	7	5 Lf of foreign toxoid alone	1	0	0	0

every adult tested gave evidence of having been in contact with toxigenic *C. diphtheriae*, as 90% possessed very significant amounts of antitoxin before receiving the toxoid. Even so, as a consequence of this experiment, it will be seen that purified toxoid augmented with our N.A.F. gave the best antitoxin response. It is realized that the antigenicity of a diphtheria prophylactic cannot reliably be assessed by its performance as a secondary stimulus. A factor of considerable importance, arising from this experiment using adults, is the almost complete absence of reactions in subjects receiving N.A.F. in the prophylactic used, and this confirmed our observations made in children. It must be recorded, however, that the crude veal-infusion-proteose-peptone diphtheria toxoid itself proved quite unsuited for human prophylaxis owing to the severity of the reactions produced. This is remarkable in view of the observation that the N.A.F. is derived from this very material.

Summary

As a consequence of an examination of the antigenicity of toxoids derived from three different types of culture media, it was found that those derived from our veal-infusion-proteose-peptone medium were outstandingly greater than the other two per Lf.

Research into the reason for the valuable difference showed that the adjuvant factor is diffusible through cellophane, and can be adsorbed on to activated charcoal and eluted by dilute sodium hydroxide. The addition of

this neutralized partially purified natural adjuvant factor—N.A.F.—to purified formol-toxoid greatly augmented its antigenicity when tested in guinea-pigs.

This factor did not augment the antigenicity of purified tetanus toxoid, and, conversely, an analogous preparation derived from crude tetanus toxoid made in the same medium failed to augment the antigenicity of purified diphtheria toxoid.

Field trials made in children showed that purified toxoid augmented with this natural adjuvant increased its antigenicity to a level at least equal to British-manufactured and our own make of A.P.T. Furthermore, the reactions encountered were nil, compared with a high incidence from control materials.

A detailed trial in adults revealed the unexpected fact that diphtheria is endemic in the region of Bombay, since 90% of the subjects tested possessed highly significant amounts of circulating antitoxin before inoculation. This fact considerably diminished the value of the experiment from the point of view of assessing the comparative antigenicity of the prophylactics under test, although the highest titres were obtained from those subjects receiving toxoid together with the purified natural adjuvant factor. A valuable observation, however, was the almost complete absence of reaction in those subjects receiving N.A.F. with the prophylactic, whereas without it the incidence was high.

This new prophylactic is a perfectly clear solution that can be sterilized by filtration and freeze-dried without damage to its antigenicity.

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The verbatim proceedings of the Third Commonwealth Health and Tuberculosis Conference, held in London in July, 1952, are now available in book form. This publication, price 21s., is obtainable from the National Association for the Prevention of Tuberculosis, Tavistock House North, Tavistock Square, London, W.C.1. Every speaker has revised his or her contribution, and the book contains, in addition to a full account of the proceedings, photographs of some of the speakers and overseas delegates at the conference.