volume injected superficially. Moreover, abscesses at the site of the local lesion are rare after superficial injections, but increase progressively in frequency with increasing depth of injection; and in this investigation nearly one-third of the children had associated subcutaneous abscesses when the vaccine was injected just below the skin into the subcutaneous tissues.

These findings bear directly on the question whether laymen should be employed as vaccinators for mass B.C.G. campaigns in countries with few professional personnel. So far as post-vaccination allergy is concerned, variations in the technique of giving intradermal injections apparently make so little difference that lay vaccinators could probably be employed with impunity. If, however, the success of a campaign depends on keeping to a minimum the frequency of large vaccination lesions and of complications, then all vaccinators must be intensively trained to inject the vaccine into the most superficial layers of the skin.

What bearing do these findings have on the search for more fundamental knowledge on the problem of tuberculosis immunization? We cannot answer this question, yet it may be permissible to speculate on certain possibilities. To regard a large, ugly vaccination lesion as a promise of strong and long-lasting immunity is, if not an obvious inference, at least a tempting idea to the vaccinator, and a consoling idea to the person vaccinated. But our findings provide little support for such an inference. Certainly a weak vaccine (such as the 1/100 dilution of standard strength used here) gave rise to extremely small lesions and rather weak allergy; but when standard strength vaccine was injected at different depths in the skin very considerable differences in the size of the lesions were not associated with differences in allergy. The inconstant correlation between the local reaction and the allergic response to B.C.G. does not justify the belief that both can be used as a true guide to immunity. A strong or a weak local reaction—a good or fair "take"—may reflect properties associated with immunization we do not know; yet from these studies it seems to depend to such an extent on the way the vaccinator did her work that size of the local lesion may well reflect factors that have nothing whatever to do with immunity.

Summary

An intracutaneous injection of B.C.G. vaccine may deviate from the recommended technique in two principal ways: more or less than 0.1 ml. may be given, and the vaccine may not be injected superficially into the skin. This paper reports the results of several studies, altogether comprising about 1,400 schoolchildren, in which volume and depth of injection of B.C.G. vaccine were deliberately varied within the limits likely to occur in ordinary mass vaccination campaigns, and the effect of such variations on the resulting post-vaccination tuberculin allergy and the local reactions at the site of vaccination.

Because of the fact that most syringes leak, the volume of vaccine injected is commonly gauged by estimating the size of the wheal formed on the skin rather than by reading from the graduated markings on the barrel of the syringe. In a study of about 950 children when different volumes of B.C.G. vaccine were injected very superficially into the skin, 0.1 ml. of vaccine produced wheals averaging 8 mm. in diameter. Doubling or halving the volume caused a difference averaging 1.5 mm.: a sixfold increase (from 0.05 to 0.3 ml.) caused a 4 mm. increase in the diameter. In a second study of over 400 children, depth of injection was varied but volume was kept constant: deep intracutaneous injection of 0.1 ml. gave wheals averaging 3.1 mm. smaller than the same volume injected superficially—a decrease as great as that from a fourfold reduction in volume given superficially.

Tuberculin allergy in schoolchildren, measured by the tuberculin reaction at intervals after vaccination, was practically unaffected by variations in intracutaneous technique likely to occur in the field. Volumes of 0·05–0.3 ml. of vaccine injected at depths ranging from very superficial in the skin to subcutaneous all gave about the same mean level of post-vaccination allergy.

A given volume of vaccine injected just beneath the skin into the subcutaneous tissue produced local lesions twice as large as those from the same volume injected superficially. The frequency of local subcutaneous abscesses increased with increasing depth of injection.

These studies show that the size of the reaction at the site of vaccination is influenced by the depth of injection of vaccine, but the level of B.C.G.-induced tuberculin allergy is not. If, therefore, post-vaccination allergy is to be taken as an index of immunity, the local vaccination reaction does not necessarily measure the same thing.

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MEASUREMENT OF LEAKAGE OF TUBERCULIN SYRINGES

BY

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Many of the syringes used for intracutaneous injections tend to leak between plunger and barrel, often to such an extent that reading from the graduated scale may be grossly misleading. In practice the quantity of liquid actually injected is therefore usually estimated by measuring the size of the wheal produced at the site of injection. For scientific work, however, where accurately measured dosage is required, unsatisfactory syringes must be detected and eliminated before use.

A simple and easily constructed apparatus for accurate and quantitative measurement of leakage in syringes is

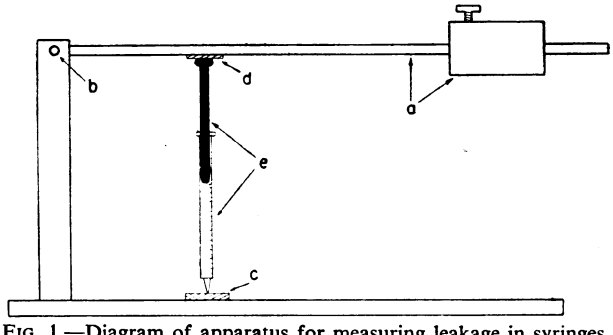

FIG. 1.—Diagram of apparatus for measuring leakage in syringes. a = lever with adjustable weight; b = joint for vertical rotation of lever; c and d = rubber disks; e = tuberculin syringe.

illustrated in Fig. 1. The syringe, when filled with distilled water, is placed vertically in the apparatus. The tip, with no needle attached, is pressed against the soft smooth rubber disk (c) and the lever with a certain weight (a) is brought down upon the plunger. This lever, while holding the syringe steady, subjects the plunger and consequently the contents of the syringe to a constant pressure. As the tip is embedded in the rubber disk, the contents can escape only by leaking out between plunger and barrel, and the rate of decrease of the contents thus becomes a measure of the rate of leakage. The pressure is correct only if the lever is nearly horizontal, so when testing short or partly filled syringes the distance between rubber disk and lever must be correspondingly reduced by placing the rubber disk on a box of appropriate height.

If the diameter of the plunger is about 4.5 mm. (as in most all-glass tuberculin syringes) an external pressure of about 1 kg. is used, corresponding to a pressure inside the syringe of 5 to 6 kg. per cm.^{2} or approximately 70 lb. per sq. in. (This pressure corresponds roughly to what was found in the following experiment. Several experienced testers were asked to exert pressure, by hand, on a tight air-filled syringe, corresponding to the estimated pressure used for an ordinary Mantoux test. By this procedure the air volume was reduced to between one-fifth and one-eighth, corresponding to an internal pressure of five to eight atmospheres.)

If the diameter of the plunger exceeds 4.5 mm. (as in most Record syringes) the external pressure must be correspondingly increased in order to keep the internal pressure per cm.^{2} constant.

Experiments

A series of experiments designed to provide data on the leakage rate of syringes—as measured by the apparatus—have been carried out. Twelve new all-glass 1-ml. tuberculin syringes of the type used for field studies by the Tuberculosis Research Office were filled with distilled water to a certain level and subjected to constant pressure in the apparatus.

Each syringe was tested for leakage at five different levels of filling—namely, filled to the 1, 0.8, 0.6, 0.4, and 0.2-ml. marks. Starting from each of these levels, the decrease in the contents of the syringe was measured by reading the scale every minute for a period of five minutes. All tests were repeated once, and then the syringes were boiled in distilled water for 30 minutes and tested again at two different levels of filling—1 ml. and 0.4 ml. When the tests were repeated the person reading the scale had no knowledge of previous results. The results were plotted on a graph, the time during which the fluid in the syringe was kept under pressure being indicated on the horizontal scale and the contents of the syringe during the testing period on the vertical scale. The slope of each curve thus gives the leakage rate, illustrated in Fig. 2 for four of the twelve syringes tested.

The leakage rate varied to a surprising extent from syringe to syringe, ranging from 0.06 to more than 0.4 ml. in five minutes. In some instances the leakage rate showed great variations from level to level in the same syringe. There was good agreement between the results of the first and the second series of tests; and boiling in distilled water for 30 minutes did not appear to alter the leakage rate.

Practical Application

Syringes to be used for research by our field teams are now pre-tested for a period of six minutes (not five) with an internal pressure of 5–6 kg. per cm.^{2} (70 lb. per sq. in.). As leakage may be different in different parts of the syringe, each syringe is tested for two different degrees of filling—namely, completely and two-fifths filled. The results are expressed in units of 0.01 ml. per six minutes (0.1 ml. per hour). When a syringe is said to have a leakage of 15, for example, it means that the leakage in six minutes (average of the two trials) amounts to 0.15 ml.

From such information it is also possible to estimate the percentage of fluid lost in injecting 0.1 ml. If it is assumed that the injection is made in five seconds, and that the internal pressure in the syringe is 5–8 atmospheres, a leakage rate of 10 corresponds with a loss of 2% or less of the 0.1-ml. dose. A leakage rate of 20 corresponds with a 3–4% loss, and a rate of 30 with a loss of 5–6%.

Fig. 3 summarizes the results of testing 387 new syringes of the particular brand we have found to be most satisfactory. The rate of leakage is plotted on the horizontal scale, percentage of syringes on the vertical scale, and each point on the line indicates the percentage of the total number of syringes which have a leakage rate below a specified value.

It may be seen from the figure that while the leakage rate ranged from 4 to 70 the syringes were not equally distributed over this range. About half of the total sample had

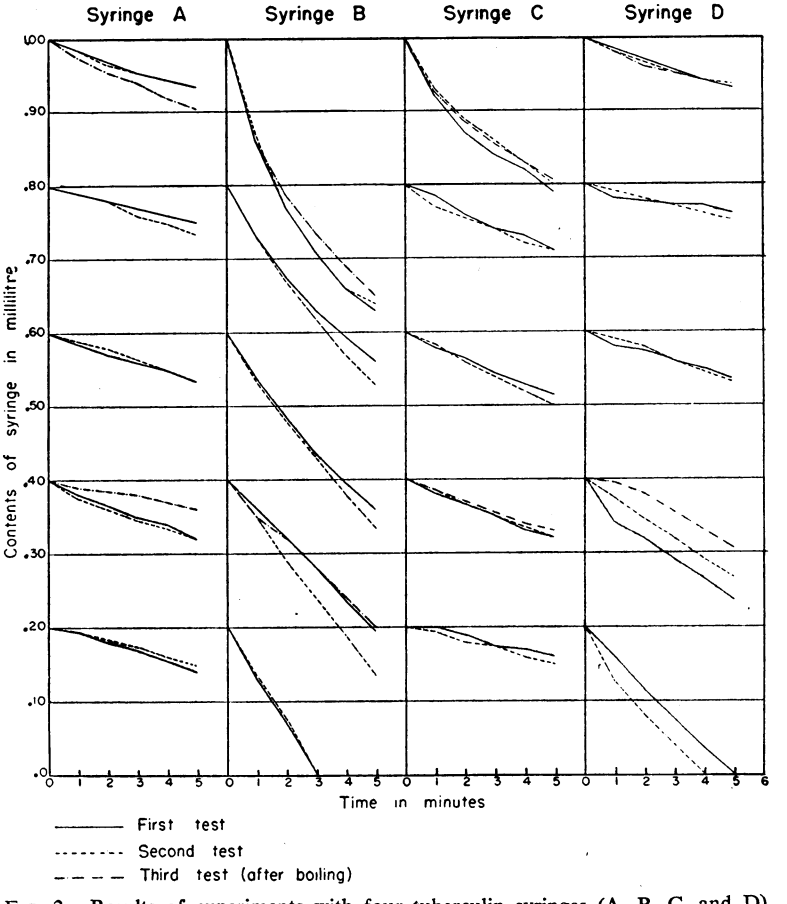

FIG. 2.—Results of experiments with four tuberculin syringes (A, B, C, and D). Each curve shows decrease in contents under pressure by time.

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

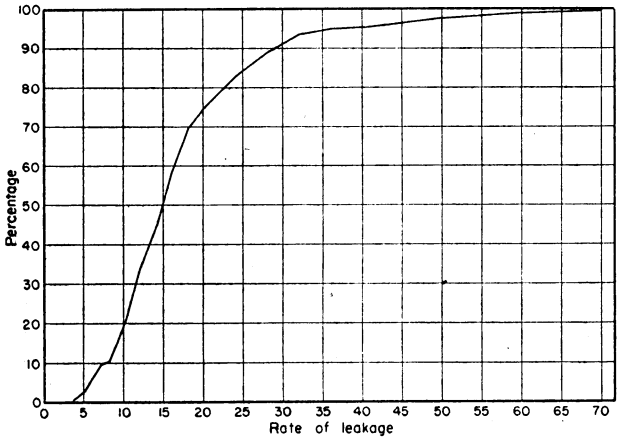

FIG. 3.—The cumulative distribution of the leakage in 387 new syringes. Leakage is measured in units of 0.01 ml. per six minutes under a pressure of approximately 5 kg. per cm.², and these units are marked on the horizontal scale of the figure. The vertical scale gives percentages of the 387 syringes 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 actually 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