Comparison of the risks of death from a second haemorrhage with those of carotid ligation in the first 2 weeks redounds greatly to the credit of carotid ligation.

Comment

It will be observed that when aneurysms causing subarachnoid haemorrhage are reviewed according to their anatomical site there is a consistently lower mortality rate in the groups dealt with conservatively. It should be pointed out that selection of cases has played little, if any, part; the control series was largely drawn from material acquired during a period of time when little good was thought likely to accrue from surgical methods of treatment. It is during the last few years that surgery has been attempted in almost every case. No attention has been paid to the presence of other disease processes present at the time of the patient's admission to hospital. Such factors are, of course, important and must be taken into consideration when larger numbers of case records dealing with specially sited aneurysms become available for study.

In assessing the state of survivors of treatment, whether medical or surgical, "slight" indicates the presence of abnormal neurological signs which do not, however, hamper the patient in leading his normal life. The term "severe" is self-explanatory.

Conclusions

We feel that a case has been made out for the very early investigation and treatment of cases of bleeding aneurysm, for a lower mortality has been shown to occur in cases handled in such a manner than is to be expected from the natural course of the disease, as evidenced by our conservatively treated cases and by the existing literature on the subject.

It would seem desirable that the subject of subarachnoid haemorrhage due to aneurysm should be dealt with not as a whole but in relation to the actual situation of the lesion, thus making for much easier comparison of results of differing treatment practised in a variety of centres. The time factor in relation to the haemorrhage and the physical condition of the patient must surely be very important details of which to be fully informed when attempting to assess the relative values of different lines of therapy.

It seems to us that little information of real value can be obtained from series of cases in which the situation and nature of the aneurysm have not been established by angiography; for we know, to take but one example, that about 10% of cases of subarachnoid haemorrhage are due to intracranial angiomas, which have a totally different natural history from that of aneurysms.

We feel convinced that surgical methods of treatment offer a definite lowering of the mortality rate when compared with purely conservative measures. For example, in medically treated patients with anterior communicating or middle cerebral aneurysms whose clinical state is so bad that they are included in category A we find the natural death rate to be over 90%. If even a few patients in this category can be restored to reasonable health by surgical measures the operative mortality rate should surely be assessed against the natural death rate of 90%, and so even a 50% operative mortality rate would be acceptable.

Summary

A review of 249 cases of proved intracranial aneurysm associated with subarachnoid haemorrhage is given.

These cases are considered in relation to the severity of the effects of the haemorrhage and the site of the aneurysms.

The results of various forms of surgical treatment in these cases are compared with the results in patients who had received medical treatment.

The frequency of associated intracerebral, intraventricular and subdural haemorrhage is given.

The frequency and time of occurrence of repeated haemorrhage is considered.

The operation of carotid ligation is discussed.

We should like to record our gratitude to Mr. Valentine Logue for permission to use many of his case records, particularly those relating to anterior cerebral/anterior communicating aneurysms. We also thank the Board of Governors of St. George's Hospital for the research grant made to one of us (L. W.), which has made the collection of this material possible.

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FREEZE-DRIED B.C.G.

VACCINATION OF NEWBORN INFANTS WITH A BRITISH VACCINE

BY

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The recent report of the Medical Research Council (1956) trials of B.C.G. and vole bacillus vaccines in school-leavers has confirmed the value of such vaccination as protection against tuberculosis for the children of this country at the present time. From the point of view of child health the most significant periods of risk of tuberculous infection are earliest infancy and puberty, but both of these groups were omitted in the Medical Research Council trial. One of the significant features of this investigation was the high percentage of children (40%) who had already had their primary infection. In girls the risk of genital tuberculosis following a flare-up of their primary infection at puberty makes it highly desirable that those not already infected should be protected *before the onset of puberty*, because genital involvement is a common cause of subsequent sterility. It would seem more logical, therefore, to vaccinate girls at an earlier age—10 or 11 years—rather than to wait until they are about to leave school, when many of them have already passed puberty.

Optimal Age for Vaccination

If the prophylactic value of B.C.G. in paediatrics is generally accepted, the question of the optimal age for vaccination should be seriously considered. The ideal procedure would surely be a single vaccination in infancy, using a vaccine of sufficient antigenicity to provide protection until after puberty. This may or may not prove possible. It is certainly generally agreed now that infants can be safely and successfully vaccinated with B.C.G., but the duration of the protection afforded is as yet undetermined. In a recent five-year follow-up we found that only 7 of more than 1,000 infants who had been vaccinated in the newborn period with Danish vaccine had reverted and required revaccination. Furthermore, the degree of sensitivity showed little, if any, diminution at the end of the fifth year (Fig. 1). It remains to be seen whether this state will persist for a further seven or eight years.

Although the duration of allergy depends on a variety of conditions, undoubtedly the most important single factor

is the vaccine used. This must be of adequate antigenic potency—not too potent, or complications will occur; and not too weak, or allergy will be slow in developing, insufficient in degree, and quick to fade.

The vole bacillus vaccine, for example, which proved very satisfactory in the school-leavers, is not suitable for use in infancy. We have found that even with very small doses, of the order of 1,250 viable organisms, which may fail to produce tuberculin conversion in some infants, complications may be produced in others; and, when a dose is used which will regularly cause conversion, complications are the rule rather than the exception (Fig. 2). Although these complications have not proved serious they persist for a long time and, what is more disturbing, they may be very slow to appear—even as long as two years after the vaccination. This appears to be a particular hazard of vole vaccination in infancy and does not occur in older age groups.

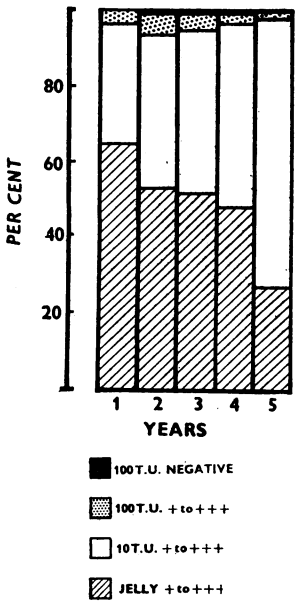

FIG. 1.—Maintenance of tuberculin sensitivity after vaccination of newborn infants with 0.075 to 0.15 mg. of Danish fresh B.C.G.

We have found, in contrast to the vole vaccine, that if early and strong conversion is desired the Swedish vaccine and French freeze-dried products are too weak. Complications with the former are almost non-existent but may occur with the freeze-dried preparations, despite its weak antigenicity, as a result of the clumping which occurs on suspending the vaccine; this may lead to excessive dosage or to subminimal doses of viable organisms together with a mass of dead ones.

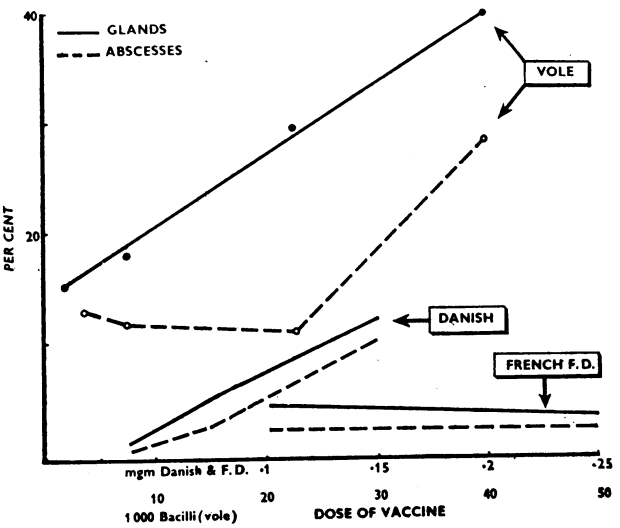

FIG. 2.—Incidence of regional adenitis and abscess formation in newborn infants according to the dose of three different vaccines. (Batches 52 and 62 of Glaxo freeze-dried vaccine produce a lower incidence of complications than any shown in the diagram.)

For the past three years we have regularly been using a dose of 0.0375 mg. of fresh Danish B.C.G. in infancy, and during this period satisfactory results have been consistently maintained. Mantoux conversion has developed quickly and adequately, and a high degree of sensitivity has continued throughout the period of observation. Complications have been minimal, of the order of less than 0.3%. Thus, apart from the practical disadvantages inherent in the use of any liquid vaccine, due to its vulnerability to outside conditions and the short life of the organisms, the Danish liquid preparation in suitable doses has now proved itself an excellent vaccine for use in infancy.

Nevertheless it is evident that an efficient dried vaccine would offer so many advantages over a liquid preparation that it would become the standard form for universal use. The difficulties encountered in the preparation of freeze-dried vaccines have been in conserving an adequate number of viable organisms *which will remain viable*, and in reconstituting the dried mass to produce a uniform suspension with minimal clumping. As the success of the vaccine depends largely on the number of viable organisms injected a reasonable estimate of this number is essential, but when clumping is excessive any accurate estimate is clearly impossible.

New Preparation

The problem of ensuring a consistently uniform dose with freeze-dried vaccine has now been overcome and the availability of a satisfactory preparation widens the scope and practicability of more extensive vaccination immeasurably. For the past eighteen months we have been using such a vaccine prepared in the Glaxo laboratories by the method described by Ungar, Farmer, and Muggleton (1956) (see page 568): 1,100 newborn infants have been vaccinated with various batches.

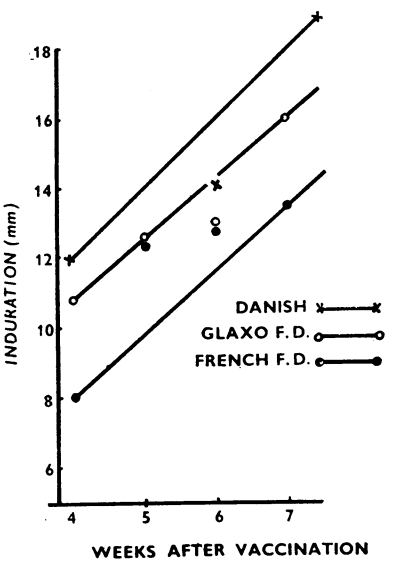

FIG. 3.—Rate of development of Mantoux sensitivity to 100 T.U. in newborn infants. Comparison between fresh Danish (0.0375 mg.), Glaxo freeze-dried (Batches 52 and 62), and French freeze-dried vaccines.

The vaccines were resuspended to contain approximately 10,000 (Batch 50), 120,000 (Batches 52 and 62), and 1,000,000 (Batch 77) viable organisms in 0.1 ml. and the injections were all made intradermally. Satisfactory conversion occurred with this freeze-dried vaccine if enough viable organisms were present. With such a vaccine the rate and extent of development of allergy, the size of the local reaction, and the response to tuberculin-testing a year after vaccination were all comparable with the results produced by injection of 0.0375 mg. of Danish fresh vaccine (Figs. 3, 4, and 5).

Of the four batches of Glaxo freeze-dried vaccine used, Batch 50 (containing approximately 10,000 viable bacilli per inoculating dose) was soon found to be antigenically inadequate and was discontinued. The recent paper by Lorber *et al.* (1956) similarly showed the results of using vaccines with an insufficient number of viable organisms, but their batches had been deliberately selected with low counts in order to ascertain the *minimal* number of viable organisms required to produce conversion. These were obviously going to prove unsatisfactory for use in contact children, as, indeed, they concluded. We, on the contrary, have been concerned with the *optimal* number of organisms because we have been aiming at providing the earliest possible protection in the newborn period and at the same time the longest-lasting protection with minimal complications.

Batch 77 (containing approximately 1,000,000 viable organisms per inoculating dose) was slightly more potent

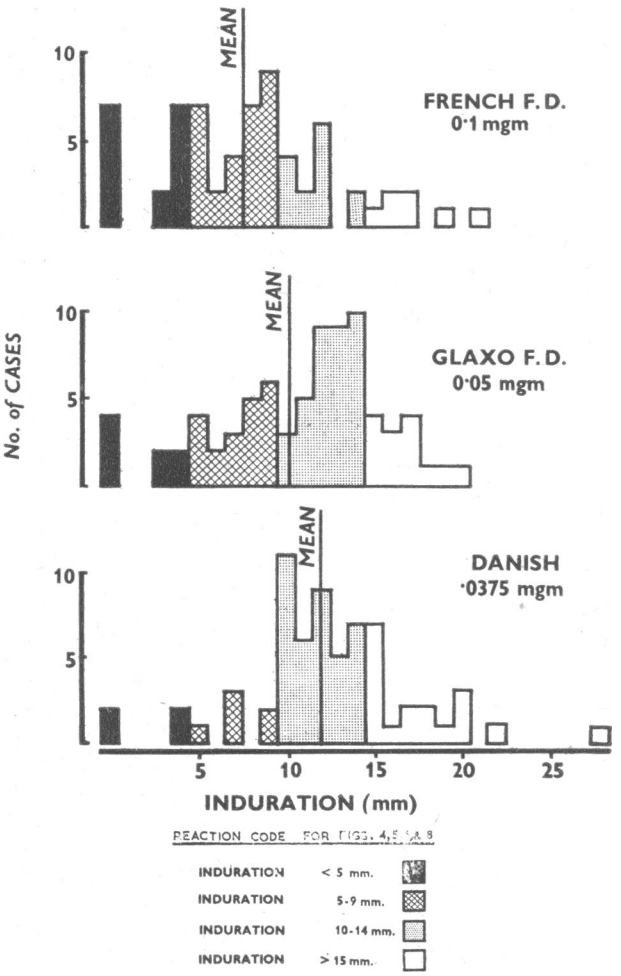

FIG. 4.—Degree of Mantoux sensitivity to 100 T.U. at four weeks in newborn infants. Comparison of fresh Danish (0.0375 mg.), Glaxo freeze-dried (Batches 52 and 62), and French freeze-dried vaccines.

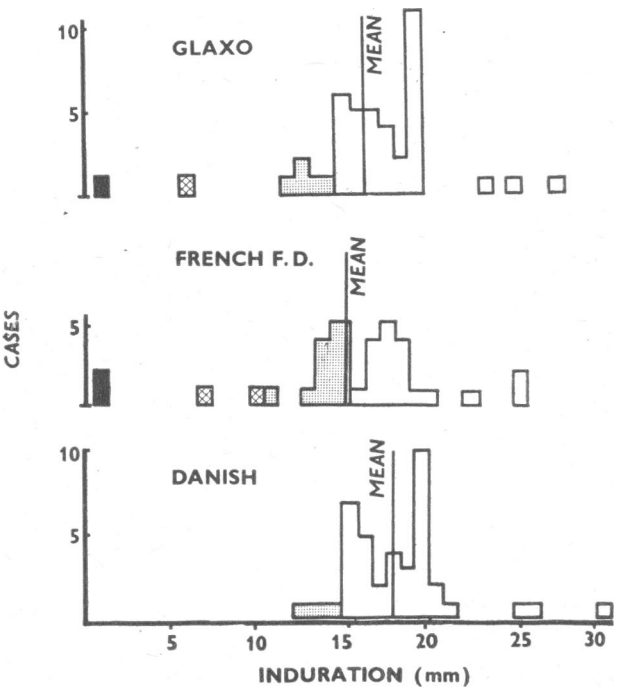

FIG. 5.—Degree of Mantoux sensitivity to 10 T.U. one year after vaccination of newborn infants with fresh Danish (0.0375 mg.), Glaxo freeze-dried (Batches 52 and 62), and French freeze-dried vaccines.

than the Danish liquid B.C.G. in allergy production, but as it produced large lesions at the site of inoculation and an increased tendency to glandular complications (5% of enlarged axillary nodes and 0.6% of nodal abscess formation) we consider that it is a little too potent for routine use. Batches 52 and 62 (containing 110,000–130,000 viable organisms per inoculating dose), although only very slightly less antigenic than the Danish vaccine, have produced no complications to date and appear to provide the optimal dose for which we are seeking (Figs. 6, 7, and 8).

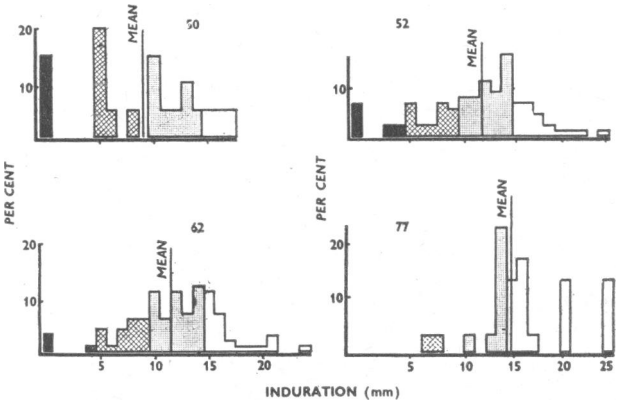

FIG. 6.—Degree of Mantoux sensitivity to 100 T.U. four weeks after vaccination of newborn infants. Comparison of different batches of Glaxo freeze-dried vaccines.

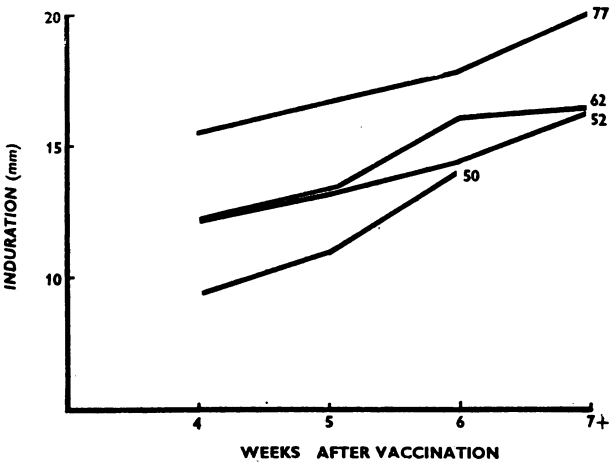

FIG. 7.—Rate of development of Mantoux sensitivity to 100 T.U. in newborn infants after injection of different batches of Glaxo freeze-dried B.C.G. vaccine.

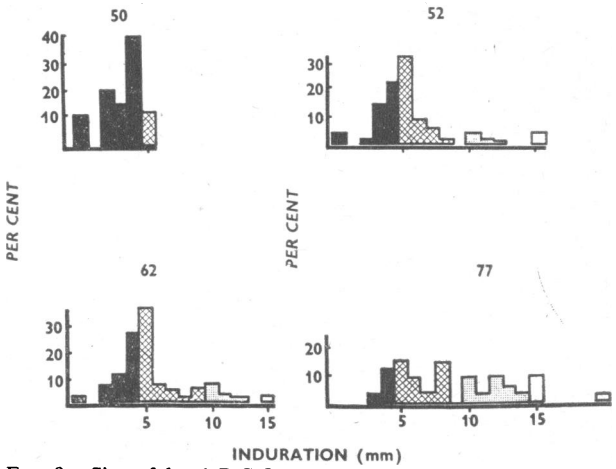

FIG. 8.—Size of local B.C.G. reaction four weeks after inoculation of newborn infants with different batches of Glaxo freeze-dried B.C.G. vaccine.

Conclusions

The Danish B.C.G. vaccine has proved uniformly satisfactory for use, in appropriate dosage, in newborn infants. It suffers, however, from the defects inherent in any living liquid vaccine and it produces complications of a minor, but nevertheless undesirable, nature in a small proportion of cases.

A new British freeze-dried vaccine has been found to be equally effective when given in suitable doses. The clinical effects of such a vaccine have been shown to vary according to the number of viable organisms injected. Since accuracy of bacillary dosage is now a practical possibility, it is probable that satisfactory free-dried vaccines with none of the disadvantages of the liquid preparations may soon replace them.

We are indebted to Dr. J. Ungar and Dr. P. W. Muggleton for their help and advice throughout these investigations.

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FREEZE-DRIED B.C.G. VACCINE

METHODS ADOPTED IN PREPARATION OF A STANDARD PRODUCT

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During the past thirty years B.C.G. vaccine has become established in many countries as an effective agent against tuberculosis. Liquid vaccines, which have been mainly used during this time, have had two major disadvantages —namely, a short life (fourteen days), with its attendant distribution difficulties, and consequent inadequacy of laboratory tests to prove that each batch contains effective antigen and is safe.

With the development of freeze-drying and the discovery that living cultures of bacteria could be freeze-dried without affecting their biological characteristics, including viability for long periods, attempts at producing freeze-dried vaccines from B.C.G. were a logical development. A number of such vaccines have been described (Ungar and Muggleton, 1948; Rosenthal, 1948; Ungar, 1949; van Deinse and Senechal, 1950; Obayashi, 1955).

An effective freeze-dried B.C.G. vaccine needs to satisfy a number of criteria: (1) it must contain a sufficient number of antigenic viable organisms; (2) it must not contain an excessive number of viable organisms producing marked reactions; (3) it must be stable when stored at normal temperatures; (4) it must be produced from a homogeneous suspension of cells containing the minimum of clumps and give an even suspension on reconstitution; (5) it should be pharmaceutically elegant, particularly in ease of reconstitution; and (6) it must be freeze-dried in a medium containing no substances whose subsequent injection would be liable to cause unpleasant reactions.

Almost all B.C.G. vaccines, whether liquid or freeze-dried, are prepared from strains grown as surface cultures; the pellicle collected from such cultures is milled by steel, glass, or agate balls to form a homogeneous suspension. Such suspensions may contain clumps of varying sizes, which can cause reactions to the vaccine injected intracutaneously and, moreover, make difficult the interpretation of viable counts on the vaccine. Further, the process of milling might affect the surface of the cells and render them vulnerable to certain chemical, physical, or thermal insults. In the preparation of freeze-dried B.C.G. vaccine the medium in which the cells are dried is of prime importance. Vaccines have been prepared in which serum, 50% glucose solution, 1% sucrose solution, various concentrations of lactose, sucrose solution with gelatin, and certain other solutions were used. Most of these have disadvantages of one kind or another, such as unsightly appearance, difficulties of reconstitution, unsuitability for injection into human patients, or "fluffy" consistency after freeze-drying so that part of the ampoule content is lost when the vacuum in the ampoule is released.

This paper describes the preparation and properties of a freeze-dried B.C.G. vaccine that has been designed to overcome some of the above-mentioned objections. It represents the result of prolonged and detailed studies for ten years on the numerous factors that had to be separately evaluated before it could reach the production stage.

Technical Details

1. Strain of B.C.G. Used for Vaccine Production.—The Copenhagen substrain of the B.C.G. strain has been used throughout our work. When relevant, comparative tests were carried out with the B.C.G. strain from the Pasteur Institute in Paris. Apart from certain minor differences in biological properties, the two strains behave in essentially the same way, such as growth requirements, sensitizing properties for guinea-pigs, and protective action. The strain was freeze-dried at the beginning of this work, regular recoveries from the freeze-dried master cultures being made on Löwenstein–Jensen medium at three-monthly intervals to give submaster cultures; they are subcultivated on the same medium once monthly until a fresh culture is started from the freeze-dried master culture.

2. Method of Preparation of Bacterial Suspension.—The submaster culture is carried through three transfers at seven-day intervals in Dubos liquid medium containing "tween 80" and 2% bovine albumin. The third culture invariably shows a homogeneous growth throughout the medium, and this, when seven days old, is used to inoculate the production medium, which consists of Sauton's medium with the addition of 1/4000 w/v of "triton WR1339," a non-ionic polyoxyethylene ether. This wetting agent is stable in the cultures and does not require the addition of albumin to protect the B.C.G. from the bacteriostatic action of hydrolysis products; it was chosen after investigating a large number of wetting agents. The cultures are prepared in "mould culture flasks" containing a shallow layer of medium (8 mm. depth). The depth of the culture medium has a profound effect on the quantity of growth obtained; the highest proportion of growth resulted from shallow cultures. The incubation temperature was also found to be critical, and variations, particularly above 38° C., resulted in a marked growth reduction. These cultures appear as a characteristic thin veil growing at the bottom of the medium on the glass surface. They are left in the incubator undisturbed at 37° C. for nine days. Gentle shaking of the grown culture produces a uniform turbidity in the medium; on microscopical examination this is seen to consist of single cells with a few small aggregates of up to 10 cells. No milling is necessary, and the cells are harvested by centri-