The Ninth World Health Assembly in its resolution WHA9.60 requested the Director-General to submit to the Tenth World Health Assembly:

(1) a report on progress in the evaluation and production of typhoid, smallpox, and triple diphtheria-pertussis-tetanus vaccines; and

(2) a programme for further development in this field in 1958 and subsequent years.

This document is divided into three sections, one for each of the vaccines mentioned, and each section contains both a report on progress and an outline of future plans as far as these can be foreseen. However, since these programmes are long-term programmes necessarily extending over several years, the plans for future developments are a continuation or extension of existing programmes developed in the light of recent progress and are modifiable by intermediate results.

Typhoid Vaccine

Control of typhoid fever and other enteric infections by hygienic measures requiring heavy expenditure on environmental sanitation is inevitably a slow process. Some governments have therefore turned to vaccination as an interim method of control of typhoid fever. When the Government of Yugoslavia decided on this solution it appreciated the need for more reliable information as to what vaccine should be used, how its potency should be tested, and what results might be expected. Accordingly the Government approached WHO for advice as to how this information might be obtained. The result of these discussions was the decision to carry out the first strictly controlled field trial of typhoid vaccines some fifty years after their initial use. The final report of these trials has
now been received. A summary report is being published in the Bulletin of WHO¹ and a full report containing details of the extensive laboratory studies on the vaccines is being prepared for publication.

The field trial showed that of the two vaccines used one, a heat-killed phenol preserved vaccine, gave a degree of protection which appeared to be rather greater in children and adolescents than in adults. The other, an alcohol-killed and preserved vaccine could not be shown to give protection. The two vaccines were also tested in a number of different laboratories by several current methods of assaying typhoid vaccines. In most tests the two vaccines showed no significant difference, whereas in some the alcohol vaccine appeared to be the better, and no clear correlation could therefore be demonstrated between the laboratory and field results.

The present position is that it is known that typhoid vaccines can be prepared which will give a degree of protection against typhoid fever, but the best method of preparation is not known, nor is it known what type of laboratory test will predict which of two given vaccines will be the most effective prophylactic agent in man.

During the past year extensive consultations have been held with experts in this field to decide on the best way of solving these problems. These consultations have included discussions at the Expert Committee on Biological Standardization as well as discussions between consultants who have been co-operating in these studies. It has been agreed that eventually further field trials will be necessary but, because of the expense and difficulties involved they should be preceded by laboratory studies of the vaccines proposed, using current techniques and new techniques under development. This is necessary to ensure that the vaccines that will eventually be compared in the field shall have shown a sufficiently different performance in the laboratory so that a comparison between field and laboratory results will be possible. Large batches of these vaccines should be prepared so that if the results of the field studies warrant it sufficient quantities would be available for the establishment of an International Reference Preparation.

¹ Bull. Wld Hlth Org. 1957 (in press)
Further, because of the greater stability of a dried vaccine it was considered desirable that a quantity of acetone dried vaccine should be obtained and subjected to collaborative assay and eventually tested in the field. This work is being co-ordinated by the Statens Seruminstitut, Copenhagen.

Further information may be available at the time of the Assembly.

In anticipation that by the end of the year these preliminary studies will have reached a stage at which a field trial could be undertaken, consultations have taken place with a view to finding a place suitable for a trial starting in 1958. No commitments have yet been made but it appears likely that a suitable area might be found in Turkey and the Turkish Government has expressed its interest in principle.

**Dried Smallpox Vaccine**

The results of the field and laboratory studies sponsored by WHO were published in the *Bulletin*. These studies were initiated by WHO in 1952 to determine the reason for the variable results obtained with different dried vaccines, and to determine the most reliable method of preparation. It has been shown that it is possible to prepare a dried smallpox vaccine which is still capable of giving 100 per cent. successful primary vaccinations after exposure to 45°C for 2 years. Other batches of vaccine prepared in the same way have been shown to retain satisfactory potency for at least eight weeks at 45°C, and for at least three months at 37°C. These batches of vaccine were not tested for longer periods. The method of preparation of the vaccine is thus capable of producing consistently a stable product. These studies have also demonstrated the relationship between laboratory tests of potency and the results to be expected in man. It is now possible to determine a laboratory titre for a vaccine which permits the prediction of the percentage of successful vaccinations. A description of the method of production previously published by Collier* has been brought up to date with the co-operation of the Lister Institute of Preventive Medicine, Elstree, United Kingdom, and has been distributed to governments and interested laboratories in roneographed form through regional offices (WHO/Smallpox/7 annexed).

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1 *Bull. Wld Hlth Org.* 1957, 16, 63-77

2 Collier, L. E. (1955) *J. Hyg. (Lond.)* 53, 76-101
A report on the stability of this vaccine was presented to the Expert Committee on Biological Standardization in October 1956. The Committee agreed that its stability was such as to justify further investigations regarding its suitability for establishment as an International Reference Preparation. The Statens Serum Institut, Copenhagen, was therefore asked to arrange a collaborative assay of the material. This is now in progress.

In the course of these studies it was found that the character of the vaccine lesion produced by vaccines which had partially deteriorated differed from normal. Therefore field and laboratory studies have been continued with the aim of showing whether a vaccine that has partially deteriorated by exposure to heat gives as good protection after successful vaccination as a fully potent vaccine. The skin reaction to revaccination is being used as a measure of the protection against smallpox since direct measurement of the latter is at present impracticable. The results will be available later in 1957.

Plans in 1958 and subsequent years will be directed primarily to the successful introduction of this method of production in countries where the use of dried vaccine is imperative for climatic or other reasons, and to encourage adequate testing of the potency and stability of the product.

Pertussis vaccine and triple diphtheria-pertussis-tetanus vaccine

WHO gave financial support to the long series of field and laboratory studies on pertussis vaccine conducted by the Medical Research Council of the United Kingdom, the results of which were published in August 1956. These studies have revealed a clear correlation between the results of certain laboratory tests and the protection afforded to children so that it is now possible to predict with reasonable confidence whether or not a particular vaccine will give satisfactory protection. For the proper interpretation of these tests a reference preparation is necessary and for some years collaborative assays of a proposed preparation have been underway on the recommendation of the Expert Committee on Biological Standardization. These have now been completed and in October 1956 this Committee authorized the establishment of this material as the International Standard for Pertussis Vaccine.

1 Brit. med. J., 1956, ii, 454
As the result of certain animal experiments it has been suggested that when prophylactics are used in combination there may be interference between them and their protective effect may be different from that attained when they are used separately. As far as diphtheria and tetanus components of a triple vaccine are concerned it is generally accepted that the effectiveness of the product can be estimated from the antitoxin titres produced in children by vaccination. It appears that in a triple vaccine the pertussis vaccine may act as an adjuvant and increase the effectiveness of the toxoids if other adjuvants are absent. In the presence of other adjuvants pertussis vaccine does not appear to affect the immunogenic action of the toxoids. Until the results of the study of pertussis vaccine mentioned above became available it was only possible to obtain a reliable estimate of the effectiveness of a pertussis vaccine by means of a field trial. No satisfactory comparison in the field of the effectiveness of pertussis vaccine alone with its effectiveness in the combined form has yet been carried out. However, as a result of this study it now appears that there is a close correlation between agglutinin production in children and protection. It would therefore appear that the relative effectiveness of single and combined vaccines can be studied in terms of agglutinin production in children, a much simpler and cheaper method than a field trial. Consultations have therefore been commenced with a view to instituting such studies and earlier plans for encouraging field studies on this subject have been held in abeyance. Laboratory mouse protection tests with triple vaccine and single pertussis vaccine may yield supplementary information on this problem.

General

Although this report is concerned specifically with the three vaccines cited above, it is worthy of mention that the Organization is engaged in or is planning similar studies of the effectiveness, potency and safety testing, and biological standardization of other vaccines, such as rabies, yellow fever, poliomyelitis, influenza, and cholera. In 1957 a further step will be taken. A Study Group on Assay Methods and Minimum Requirements will meet for the first time in October, and will study their present status and consider the possibility of issuing Recommendations of Minimum Requirements which, if met, will permit a high degree of confidence in the protective power of a vaccine.
A STABLE DRIED SMALLPOX VACCINE

Summary of the method of preparation based on methods described by Collier

(Note: This vaccine is prepared from a partially purified suspension of vaccinia virus elementary bodies derived from sheep pulp in 5.5 per cent. peptone freeze-dried and sealed in vacuo. Repeated batches of the vaccine have been shown to retain satisfactory potency after exposure to 45°C for at least eight weeks, and 37°C for at least three months. For full details reference should be made to Collier's article. One batch has been exposed to 45°C for two years, after which time it still produced 100 per cent. successful primary vaccinations. In series production the conservative claim of retention of potency for one month at 37°C is made, but in practice this period may be expected to be considerably prolonged.)

Twenty five g of crude sheep pulp are ground in a mortar with 80 ml McIlvaine's phosphate-citric acid buffer, 0.004 M, PO₄ pH 7.2, and 1.0 g powdered neutral glass. The crude suspension is centrifuged at low speed (1000 g), the supernatant kept and the deposit re-extracted in buffer. This is repeated twice and the three supernatants are pooled. The virus is sedimented by centrifugation in an angle centrifuge. The speed and duration of centrifugation necessary to sediment the virus depend on the radius of rotation of the centrifuge head and the angle of inclination of the tubes (at 40° from vertical 2500 g for 60 minutes should be enough). The resulting deposit is resuspended in 15 ml of the same buffer, containing 0.5 per cent. phenol. This suspension is clarified by low-speed horizontal centrifugation for two minutes. The supernatant is saved, the deposit

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The description given here is modified from the original in Collier's paper in order to include modifications in procedure introduced since his paper was written.

The pressures given in Collier's paper were measured by McLeod gauge. Those given here are measured by Pirani gauge.
resuspended in a further 15 ml of buffer, and clarified again. The pooled supernatants constitute the final elementary body suspension (E.B.S.), which is then incubated at 22°C for 48 hours to reduce bacterial contamination. The E.B.S. is then plated to determine the bacterial count, and titrated in eggs for virus content. It is not used unless the bacterial count is less than 1000 organisms per ml and the virus titre more than $5 \times 10^9$ i.u./ml. After passing these tests, one vol. of E.B.S. is diluted 10 times with 5.5 per cent peptone, made up as follows:

A 5.5 per cent solution of bacteriological peptone is made in distilled water. The pH is adjusted to 8.0 with 40 per cent NaOH, after which the solution is heated to 90°C, and filtered while hot. The pH is then changed to 7.4 with 50 per cent HCl. The peptone solution is sterilized by autoclaving for 15 minutes at 15 lb pressure. The suspension is then ampouled in 0.25 ml amounts and dried in an Edwards centrifugal freeze-drier.  

The ampoules are closed with caps made of a layer of cotton wool between two layers of gauze. Such caps maintain sterility without interfering with the passage of water vapour.

**Primary drying.** The ampoules are placed in the primary chamber. The centrifuge is started and evacuation begun.

"Snap-freezing" occurs about 15 minutes later, when the vacuum has reached 1-2 mm Hg. The rotor is stopped shortly afterwards, and drying is allowed to proceed for about five hours at a vacuum of 0.05 mm Hg. During this time, heat is supplied to the drying heads, the total input of watts being approximately equal to the number of ml of material being dried. Drying can be satisfactorily carried out overnight, if necessary, without the application of heat.

**Constriction, secondary drying and sealing.** After primary desiccation, the ampoules are removed from the chamber, and constricted at the necks in a blow-lamp.

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&A# The centrifugal freeze-drier used is manufactured by Edwards High Vacuum Ltd., Crawley, Sussex, England.
flame to facilitate subsequent sealing. No ampoule is allowed to remain in contact with the atmosphere for more than two or three minutes during this process; those not actually being constricted are kept in glass desiccators over P₂O₅. They are then attached to the manifolds, and left for a further 18-20 hours at high vacuum over P₂O₅. They are sealed under a vacuum of 0.01-0.03 mm Hg.

Vacuum testing. The sealed ampoules are held at 4°C overnight, and are examined next day with a high-frequency tester for retention of vacuum, those failing to give a blue-green fluorescence being discarded.

Reconstitution. The dried material is reconstituted by adding 40 per cent. glycerol in buffer to the original volume.

REFERENCES