There is as yet no proof that we have produced a stable substrain in which the characteristic isoniazid-resistance will be retained on repeated subcultivation. This property would be important if large-scale production of B.C.G. vaccine from the resistant culture were contemplated. The difficulty has, for the present, been avoided by the use of freeze-dried master cultures.

In guinea-pigs, tuberculin conversion after vaccination with the sensitive parent strain is strongly inhibited by concurrent isoniazid treatment—a finding that is consistent with the clinical observation that isoniazid treatment inhibits tuberculin conversion in children vaccinated with B.C.G. The vaccine from the isoniazid-resistant substrain was, however, fully effective in producing a positive tuberculin reaction with concurrent isoniazid therapy. It is therefore assumed that freeze-dried vaccine prepared from the isoniazid-resistant cultures should be satisfactory for the vaccination of tuberculin contacts while they are on prophylactic doses of isoniazid, and trials on children and adults with such a vaccine are described by Gaisford and Griffiths (1961).

Summary

An isoniazid-resistant substrain of B.C.G. has been produced by repeated subcultivation in the presence of isoniazid.

The resistant culture was unaltered in virulence, morphology, and staining properties, but its growth characteristics in some culture media were altered owing to its having become catalase-negative.

Batches of freeze-dried vaccine have been prepared from the substrain and a comparison made with batches from the isoniazid-sensitive parent strain.

Guinea-pigs, treated orally with isoniazid at 20 mg./kg./day, when vaccinated with even small doses of the isoniazid-resistant substrain became tuberculin-positive. With a control isoniazid-sensitive B.C.G. substrain, tuberculin conversion was inhibited by the isoniazid treatment.

The use of vaccine made from isoniazid-resistant B.C.G. in human tuberculosis contacts receiving prophylactic doses of isoniazid is suggested.

We thank Mr. J. Dudley, Miss Shirley Townsend, and Mrs. Audrey Oughton for technical help with the work reported here.

REFERENCES

- Beckett, L. G. (1954). In *Biological Applications of Freezing and Drying*, edited by R. J. C. Harris, p. 285. Academic Press, New York.
- Canetti, G. (1956). *Amer. Rev. Tuberc.*, **74**, No. 2, Part 2, p. 13.
- Gaisford, W., and Griffiths, M. I. (1951). *Brit. med. J.*, **1**, 1500.
- Greenberg, L., Eidus, L., and Diena, B. B. (1958). *Amer. Rev. Tuberc.*, **78**, 785.
- Jensen, K. A. (1946). *Acta tuberc. scand.*, **20**, 1.
- Lack, C. H. (1953). *J. Path. Bact.*, **65**, 278.
- Miles, A. A., Misra, S. S., and Irwin, J. O. (1938). *J. Hyg. (Camb.)*, **38**, 732.
- Muggleton, P. W. (1960). In *Recent Research in Freezing and Drying*, edited by A. S. Parkes and A. U. Smith, p. 229. Blackwell, Oxford.
- Ungar, J., Farmer, P., and Muggleton, P. W. (1956). *Brit. med. J.*, **2**, 568.

A FREEZE-DRIED VACCINE FROM ISONIAZID-RESISTANT B.C.G.

A CLINICAL INVESTIGATION

BY

WILFRID GAISFORD, M.D., M.Sc., F.R.C.P.

AND

MARGARET I. GRIFFITHS, M.D., M.R.C.P.
D.C.H.

The Department of Child Health, University of Manchester

The use of B.C.G. in the protection of contacts is well established and reaches its acme in the vaccination of newborn infants of tuberculous mothers or of infants born into tuberculous households. Unfortunately, in both these situations a period of segregation is necessary after vaccination until Mantoux conversion has occurred, whereafter protection may be assumed, and during this time, usually three to four weeks, the infants have to be nursed away from the risk of exposure to tuberculosis and from the risks of nosocomial infection. The abolition of this segregation period would be a major advance, and there are two possible ways in which it could be effected; the first, already tried successfully by Dormer and his colleagues (1959), of giving isoniazid from birth: the second, originally suggested by Canetti (1956), by giving an isoniazid-resistant vaccine at birth and concurrently administering isoniazid orally until the post-B.C.G. tuberculin test becomes positive, whereafter it may be discontinued. The obvious advantages of the latter proposal prompted us to undertake trials with such a vaccine. The preparation of the vaccine and its properties are described by Ungar *et al.*, (1961).

Preliminary trials in a few adults showed that the antigenicity of the first isoniazid-resistant vaccine (batch 192) was comparable with that of the ordinary freeze-dried vaccines used routinely in our B.C.G. clinic. The viable count of this batch was 8,800,000 per ampoule and the contents of each ampoule were dissolved in 2 ml. of saline. Each intradermal injection of 0.1 ml. therefore consisted of approximately 440,000 viable organisms. Alternate cases were given 650,000 viable organisms of an ordinary routine batch intradermally for comparison. The local reactions were similar—a fairly uniform 4–6 mm. papule—and the mean of the Mantoux response to 10 T.U. after three weeks was 12 mm. induration in each group.

Investigations were then undertaken in newborn infants: (1) to assess the effect of giving isoniazid at the same time as an ordinary freeze-dried vaccine, the treatment being continued for three to four weeks; (2) to assess the effect of giving isoniazid at the same time as the isoniazid-resistant vaccine; (3) to compare the resistant vaccine with an ordinary routine freeze-dried vaccine without giving isoniazid; and (4) to determine the degree to which post-vaccination conversion was maintained with the resistant preparation.

For these purposes three batches of freeze-dried vaccine were used: two were isoniazid-resistant-batch 192 (concentration 8,800,000 viable organisms per ampoule), which was prepared in March, 1958, and batch 331, prepared in December, 1959, with a concentration of 4,900,000 viable organisms per

During the year 1959–60 the East African Institute for Medical Research continued its work on body composition, energy expenditure, analysis of milk of African mothers, nutritional ophthalmology, filariasis, and schistosomiasis. (*East African Institute for Medical Research Report*, 1959–60.)

ampoule; the third, batch 258, was an ordinary freeze-dried vaccine with a concentration of 8,000,000 viable organisms per ampoule.

Three groups of newborn infants were studied; they all received 0.1 ml. of vaccine intradermally.

The first group consisted of 60 infants who were born in the maternity unit at the Chest Hospital, Baguley, their mothers being tuberculous. The infants were transferred to the isolation ward at the Royal Manchester Children's Hospital as soon as possible after birth, were vaccinated at once with one of the resistant vaccines, and isoniazid treatment was started immediately in doses of 10 mg./lb./day (22 mg./kg./day). Mantoux tests were performed at intervals from the end of the second week until conversion occurred, using 100 T.U. of O.T., and the isoniazid was discontinued when the test became positive.

The second group, consisting of 25 normal newborn infants born at St. Mary's Maternity Hospital, was given isoniazid in the same dosage as group 1, starting immediately after vaccination with an ordinary freeze-dried vaccine (batch 258). Isoniazid was similarly continued until tuberculin conversion, but if this did not occur after six weeks it was discontinued and re-vaccination performed.

The third group consisted of 306 newborn infants from the same hospital as those in group 2; they received equivalent doses of viable organisms of the three vaccines being studied—107 were given batch 192, 98 had batch 331, and 101 received batch 258. In none of these was isoniazid prescribed.

Results

Table I shows the development of Mantoux sensitivity after each batch when isoniazid was given. Three weeks after vaccination satisfactory Mantoux conversion was obtained with both batches of resistant vaccine. In one infant receiving batch 192 revaccination was necessary. The ordinary freeze-dried vaccine failed to produce conversion after six weeks in five of the infants, and these were revaccinated.

TABLE I.—Development of Mantoux Sensitivity to 100 T.U. With Concurrent Isoniazid Administration

Weeks after Vaccination	Isoniazid-resistant Vaccine				Ordinary Vaccine Batch 258†	
	Batch 192* (220,000 Viable Organisms per Dose)		Batch 331† (245,000 Viable Organisms per Dose)		(400,000 Viable Organisms per Dose)	
	% Positive	Mean Indn. (mm.)	% Positive	Mean Indn. (mm.)	% Positive	Mean Indn. (mm.)
2 or 3	90	6.25	80	6.0	31	1.4
4	90	8.0	100	10.0	35	2.0
More than 5 ..	90	8.0	100	10.0	70	5.3
Revaccinations necessary ..	1 (of 60)				5 (of 25)	

* Dilution 0.1 ml./4 ml. † Dilution 0.1 ml./2 ml.

TABLE II.—Development of Mantoux Sensitivity With and Without Isoniazid

	100 T.U. at 4 Weeks					
	Resistant Vaccine				Ordinary Vaccine Batch 258	
	Batch 192		Batch 331			
	% Positive	Mean Indn. (mm.)	% Positive	Mean Indn. (mm.)	% Positive	Mean Indn. (mm.)
With isoniazid ..	90	8.0	104	10.0	35	2.0
Without „ ..	96	13.4	100	14.7	95	11.2

In Table II the results of the tuberculin conversion produced by the three vaccines after four weeks, with and without isoniazid, are compared. The resistant vaccines both produced a slightly better conversion rate than the normal production batch, but the inhibitory effect of the concurrent administration of isoniazid was clearly evident in the infants receiving the non-resistant vaccine.

The tuberculin sensitivity to 10 T.U. one year after vaccination showed an average level of 15 mm. induration whichever type of vaccine had been used, and after two years those infants vaccinated with batch 192 showed that these levels were being well maintained.

Conclusions

The tuberculin sensitivity produced by a normal freeze-dried B.C.G. vaccine in newborn infants is seriously impaired when isoniazid is administered concurrently. Isoniazid-resistant vaccine, on the other hand, produces satisfactory tuberculin conversion even when isoniazid is prescribed in doses of 10 mg./lb./day (22 mg./kg./day). This vaccine is more antigenic than the ordinary freeze-dried vaccine, with which it was compared; it caused no complications, and the resultant allergy at one year and, in the few tested thus far, at two years is as satisfactory as with the routine vaccines. The concurrent administration of isoniazid had no evident effect on the conversion rate, and the vaccine may therefore be recommended for use in appropriate circumstances.

We are indebted to Dr. T. M. Wilson, superintendent of the Chest Hospital, to Dr. Sylvia Guthrie, paediatrician to the Chest Hospital, to our obstetric colleagues at St. Mary's Hospital for their co-operation, and to Dr. Elizabeth Wade-Evans for her clinical assistance. The vaccines were prepared by Glaxo Ltd., and we are grateful to them, and to Dr. J. Ungar for his help and for arranging supplies for us.

REFERENCES

- Canetti, G. (1956). *Amer. Rev. Tuberc.*, **74**, No. 2, Part 2, p. 13.
 Dormer, B. A., Harrison, I., Swart, J. A., and Vidor, S. R. (1959). *Lancet*, **2**, 902.
 Ungar, J., Thomas, Valerie, and Muggleton, P. W. (1961). *Brit. med. J.*, **1**, 1498.

"The history of the early medical services in Tanganyika is inseparable from an account of malaria, because more than half the total morbidity used to be due to that disease. No better summary of the attitude of the abler medical officers in the country at the turn of the century can be given than that of [Dr. Otto] Panse himself [appointed 1898]. In his usual military style he wrote: 'The European here, with all his modern equipment, has the same relationship to malaria that the victorious ruler has to the uncivilized country he has conquered; he is secure in his fortress but dares not leave it, unarmed, for fear of being attacked. An East Africa without malaria would resemble a pacified country through which a stranger could pass with only a walking-stick in hand. And it is not only the European who would benefit; I disagree with those who claim that the native should be left unprotected from malaria except by his immunity. We may not be able to do much about it yet, but the day will come when we can, and then he must be protected from a disease which causes him repeated illness whilst he is attempting to gain this immunity.'" ("Malaria Control in Tanganyika under the German Administration" by Dr. D. F. Clyde, *E. Afr. med. J.*, January, 1961.)