FREEZE-DRIED B.C.G.

BY

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Freeze-dried B.C.G. of British manufacture became available for experimental work in 1955. The main reason for introducing it was the need for a vaccine which would retain its potency under conditions of prolonged storage. On reconstitution for injection the vaccine should produce a uniform suspension with minimal clumping, resulting in a uniform viable bacterial count. These properties mean that tests for potency and for sterility can be performed on samples of each batch before distribution, which will be more leisurely as only surface transport is required. Centres using B.C.G. can store stocks instead of depending on week to week supplies and finally, the vaccination lesions can be more uniform and predictable, if the viable count of the vaccines remains constant for anything up to a year or longer. Experimental work in the laboratory has shown that the vaccines made in this country have fulfilled all these requirements (Ungar, Farmer and Muggleton, 1956; Ungar, 1958; Medical Research Council, 1958).

It was essential to show that freeze-dried B.C.G. was safe, would produce acceptable vaccination lesions, a high degree of tuberculin conversion within eight to 12 weeks and a positive tuberculin reaction over prolonged periods in vaccinated subjects. The optimal number of viable organisms to be injected had to be determined in order to achieve a minimal vaccination lesion with practically 100% conversion rate. The most suitable subjects for the initial clinical trials were non-contacts, because failure of conversion or a rapid reversion would not result in serious inconvenience.

Clinical trials using the technique of intradermal injection were conducted in infants by Lorber, Hart, Farmer and Muggleton (1956); Lorber, Farmer, Muggleton, Hart and Menneer (1957); Griffiths and Gaisford (1956), and in children of various ages by Lorber and Menneer (1958) and Alston, Cowell and O'Meara (1958), and in school children (13-14 years) by the Medical Research Council (1958). These clinical trials established that there was a close correlation between the number of viable organisms injected and the size of the vaccination lesions, the incidence of regional lymph-node involvement and the degree of tuberculin sensitivity following vaccination. It was apparent that reconstituted vaccines containing 10×10^6 organisms per ml. or more were unnecessarily potent and that those containing less than 1×10^6 were not sufficiently antigenic for routine use. It was also shown on controlled trials that the immediate results obtained with freeze-dried vaccines were as good as with the standard Danish liquid vaccine (Lorber *et al.*, 1957; Medical Research Council, 1958) or with the Swedish liquid vaccine (Alston *et al.*, 1958). When the number of viable organisms injected was of similar order, the vaccination lesions and the conversion rates differed very little in subjects given the dry or the liquid vaccines. As the Danish liquid vaccine had proved to be excellent both with regard to the duration of tuberculin sensitivity after vaccination (Griffiths and Gaisford, 1956; Lorber and Menneer, 1959) and its protective value against tuberculous disease in children in intimate contact with infectious tuberculosis (Lorber and Menneer, 1959), it was hoped that the freeze-dried vaccine would eventually prove to be equally efficient in these respects.

The clinical trials quoted above were concerned with the demonstration of tuberculin conversion some weeks after vaccination and with the appearance of the lesions at this stage. Since the research suggested that the best results might be obtained by a vaccine containing between 1×10^6 and 10×10^6 viable organisms per ml. of reconstituted vaccine, the manufacturers marketed such a vaccine. The Ministry accepted this vaccine as suitable for the vaccination of contacts and others and it is estimated that at present this freeze-dried vaccine is used by about half the authorities using B.C.G. in this country.

TABLE

RESULTS OF VACCINATION WITH FREEZE DRIED B.C.G. VACCINE SIX TO 12 WEEKS AND ONE YEAR AFTER VACCINATION

Batch No.	Viability Count/ml.	Results Six to 12 Weeks after Vaccination: Method of Tuberculin Testing	No. Completing Tests	No. (%) Tuberculin Positive	Local Lesion (mm.) Range	Local Lesion (mm.) Average	Tuberculin Test One Year after Vaccination: Method	No. Completing Test	No. (%) Positive
Experimental Batches									
50	$0{\cdot}14 \times 10^6$	10 T.U. O.T.	92	52 (56·5)	1–15	6	10 T.U. O.T. 10 T.U. BCG(T)	83	83 (100)
77	23×10^6	10 T.U. O.T.	114	99 (86·8)	2–28	13	100 T.U. O.T.	111	109 (98)
93a	11×10^6	10 T.U. O.T. 10 T.U. BCG(T) 100 T.U. O.T.	315	312 (99)	2–28	10	Jelly Heaf 100 T.U. O.T.	240	240 (100)
Commercially produced batches: Annual tests completed									
143	8×10^6	Jelly Heaf 100 T.U. O.T.	40	40 (100)	—	15	,,	35	35 (100)
132 142 162 166 190	$2{\cdot}4$–5×10^6	,,	789	785 (99·5)	3–20	6	,,	536	536 (100)
Annual tests not yet completed									
193 200 240 253 263 270	4–5×10^6	,,	274	274 (100)	2–15	6			
		Total						1,005	1,003 (99·8)

BCG (T) = BCG Tuberculin (Lorber, 1957)

Present Study

The purpose of this paper is to report on the efficiency of this commercially available freeze-dried B.C.G. and to extend our previous observations by reporting on the follow-up of children who were re-examined one year after vaccination. Altogether 1,103 children (mostly contacts) had completed the vaccination procedure after the completion of our previous trials. These were all vaccinated with commercially available batches. In addition we recalled for examination and tuberculin tested 1,005 children one year after their vaccination. Of these, 434 were vaccinated earlier with experimental batches of widely differing viability counts ($0{\cdot}1 \times 10^6$ to 23×10^6 per ml.), and 571 with commercial batches of far more uniform viability counts ($2{\cdot}4 \times 10^6$ to $8{\cdot}0 \times 10^6$ per ml.) (see Table).

Vaccination and tuberculin testing technique. All subjects had been shown to be tuberculin negative a minimum of six weeks after exposure to tuberculous infection. They were then vaccinated with B.C.G. and were kept away from such exposure for a further period until tuberculin conversion was demonstrated six to eight weeks later. Freshly reconstituted vaccine, 0·1 ml. was injected intradermally at the level of the insertion of the left deltoid muscle.

The routine tuberculin testing consisted of the tuberculin jelly test, which was applied by the Health Visitors in the children's own home four days before they attended the clinic. This was carried out before vaccination and six to eight weeks after it. If the result was doubtful, or if for some reason the jelly test had not been performed, or if the result was negative after vaccination, the Heaf test was used and read 96 hours later. Those still negative were finally tested with 100 T.U. (Mantoux 1/100). The same procedure was used one year after vaccination.

The vaccination lesions. A small group of 40 children was vaccinated with a batch containing 8×10^6 viable organisms per ml. The lesions in these were unduly large, averaging 15 mm. in diameter and crusting frequently occurred. The remaining 1,063 children were vaccinated with batches containing $2{\cdot}4$-$5{\cdot}0 \times 10^6$ viable organisms per ml. The vaccination lesions in all these were very acceptable, with an average diameter of induration of 6 mm. No ulceration was seen in any and the regional lymph glands in the axilla did not become palpable. One year after vaccination there was only a shallow colourless depression.

Tuberculin tests. All but four of the 1,103 (99·6%) children vaccinated with these commercial

batches became tuberculin positive six to eight weeks after vaccination and only one in 10 of those who had a jelly test required a Heaf test to prove tuberculin conversion.

Of the 434 children who were vaccinated earlier with experimental batches, 432 (99·7%) were tuberculin positive a year after vaccination and the only two negative reactors have not been tested either with the Heaf test or with 100 T.U. intradermally. It is of great interest that 83 children who had been vaccinated with the weakest vaccine containing only $0{\cdot}14 \times 10^6$ viable organisms per ml., and whose post-vaccination conversion rate was only about 56%, all became tuberculin positive one year after vaccination.

Conclusion and Summary

Freeze-dried B.C.G. has been proved in the laboratory to be of uniform consistency with a viable bacterial count which, if kept in a cool place, persists unaltered in the dry state for a year or longer. It is safe and convenient to use. Suspensions, 0·1 ml., containing approximately $2\text{-}5 \times 10^6$ organisms per ml. were injected intradermally into over 1,000 children. The result was a papule, averaging 6 mm. in diameter. There was neither ulceration nor enlargement of the regional lymph-nodes. Tuberculin conversion was demonstrated in virtually 100% six to eight weeks after vaccination and a positive tuberculin reaction was maintained for up to at least one year after vaccination. In the case of others who were vaccinated with weaker vaccines the proportion of positive reactors increased with time, indicating the probability that prolonged tuberculin sensitivity will be produced with the freeze-dried vaccine in current use.

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References

Alston, P., Cowell, J. St. P. and O'Meara, R. A. Q. (1958). Comparison of freeze-dried and liquid BCG vaccines. *Irish J. med. Sci.*, 304.

Griffiths, M. I. and Gaisford, W. (1956). Freeze-dried B.C.G. Vaccination of newborn infants with a British vaccine. *Brit. med. J.*, **2**, 565.

Lorber, J. (1957). B.C.G. Tuberculin. *Arch. Dis. Childh.*, **32**, 441.

Lorber, J., Hart, C. B. S., Farmer, P. and Muggleton, P. W. (1956). British freeze dried BCG vaccine: Preliminary clinical trial. *Tubercle* (*Lond.*), **37**, 187.

——, Farmer, P., Muggleton, P. W., Hart, C. B. S. and Menneer, P. C. (1957). British freeze-dried B.C.G. vaccine: Further clinical trials. *Ibid.*, **38**, 227.

—— and Menneer, P. C. (1958). British freeze-dried B.C.G. A clinical trial in children. *Ibid.*, **39**, 7.

—— —— (1959). Long-term effectiveness of B.C.G. vaccination of infants in close contact with infectious tuberculosis. *Brit. med. J.*, **1**, 1430.

Medical Research Council (1958). Freeze-dried B.C.G. vaccine. Results of laboratory tests and of trials among schoolchildren in Middlesex. *Ibid.*, **1**, 79.

Ungar, J. (1958). Immunological response to freeze-dried B.C.G. *Proc. roy. Soc. Med.*, **51**, 380.

——, Farmer, P. and Muggleton, P. W. (1956). Freeze-dried B.C.G. vaccine. Methods adopted in preparation of a standard product. *Brit. med. J.*, **2**, 568.