Tuberculin sensitivity and skin lesions in children after vaccination with 11 different BCG strains*

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In previously published studies, a number of BCG strains used in several production laboratories were compared in animal models. Liquid vaccines from the different strains were prepared in one laboratory with a uniform technique, the aim being to obtain uniform in vitro *properties. In the studies reported here, such vaccines were compared by means of vaccinating children in India and Denmark and then measuring their post-vaccination skin lesions and tuberculin sensitivity. One strain induced delayed hypersensitivity strikingly weaker than that induced by any of the others, although the vaccine was in no way inferior in terms of exhaustive* in vitro *tests. Differences among the other strains were slight, although sometimes statistically significant. The implications of such differences are discussed.*

The potency of a BCG vaccine is traditionally determined by measuring the tuberculin sensitivity induced by that vaccine in children who were tuberculin-negative before vaccination. If the vaccine under test induces tuberculin sensitivity lower than that induced by other vaccines, it is considered weak. The skin lesion (or—if it is examined later—the scar) at the site of intradermal vaccination is also measured and, if the lesion or scar is particularly large, the vaccine is considered too strong to be acceptable to the population.

Tuberculin sensitivity and skin lesion size follow simple dose–response functions. According to Edwards et al. (1), if the dose of vaccine is halved, both the tuberculin reaction and the scar may decrease by (very approximately) 1 millimetre. But other changes in the vaccine do not necessarily have the same effect. Edwards et al. (1) also found that the use of a vaccine of low viability (i.e., one with a large proportion of dead organisms) resulted in a relatively less steep decrease in scar size than in tuberculin sensitivity. Furthermore, if the dose is increased so as to compensate for the low viability, the result may be a combination of moderate tuberculin sensitivity and unacceptably large lesions or scars. A vaccine giving rise to moderately large lesions combined with weak tuberculin sensitivity may thus be suspected of low viability. Vaccines of low viability are considered undesirable because it is known that, in animals, the immunogenic potency of killed organisms is much lower than that of live BCG (2).

However, to draw an inference about viability from the response in human beings is to assume that the ability of a particular BCG strain to induce delayed hypersensitivity when given in a viable state is known. When vaccines prepared from different strains and differing in viability are compared, the possibility cannot be excluded that a particularly weak response to any one vaccine could be due to the antigenic structure of the strain used, rather than to a low viability of the vaccine. It is thus of con-

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siderable interest to know if strains differ in their ability to induce tuberculin sensitivity.

This paper reports on four studies carried out during the period 1966–68 in children in South India and in Copenhagen, Denmark.[a] All the vaccines used were prepared from a number of different strains in the BCG Department, Statens Seruminstitut, Copenhagen. Liquid vaccines prepared in one laboratory were preferred in the hope of obtaining vaccines of uniform *in vitro* properties.

MATERIAL AND METHODS

Strains

Four or five strains, always including the strain currently used in Madras and Copenhagen (seed lot 1331), were used in each study. The strains are largely identical with those used in animal studies previously published by Bunch-Christensen et al. (3, 4) and Ladefoged et al. (5). The strains are listed below.

Strain	Study 1 South India	Study 2 Copenhagen	Study 3 South India	Study 4 Copenhagen
Strain 1331	x	x	x	x
Former Danish	x	x		
Former Madras (809)	x	x		
Prague (725)	x	x		x
Tokyo (172)			x	
Paris (1173P2)			x	
Moscow (3522)			x	
Strain Y			x	
Rio de Janeiro				x
Gothenburg				x
London (Glaxo)				x

Vaccines

The strains were grown as surface cultures on Sauton medium and harvested after 10 days. Liquid vaccines were prepared according to the laboratory's routine technique. The growth characteristics were not the same for all strains. The Paris and former Madras strains grew faster than the others and showed pronounced pigmentation. The London strain included in study 4 grew more slowly than the other strains did and the morphology of the surface culture was distinctly different from that of the other strains.

Details of the various routine *in vitro* tests performed are given in the Appendix.[b] For the former Madras strain, 2 of the preparations in study 1 contained a lower proportion of live organisms, as evidenced by a lower oxygen uptake (see Appendix), but the other 2 preparations in study 1 and the 4 preparations in study 2 were satisfactory. The same phenomenon was found with the Paris strain used in study 3: two of the preparations showed a lower oxygen uptake and germination rate than the third preparation, which was satisfactory. The strain from Tokyo yielded vaccines with a very high colony count—a well-known feature of this strain. The proportion of live organisms in the vaccines prepared from the London strain was rather low for all four vaccine preparations, as evidenced by the oxygen uptake, germination rate, and viability count. Otherwise the vaccines from different strains were much alike in their *in vitro* properties.

The vaccines for the first three studies were all prepared in a strength of 0.75 g/litre; in the fourth study, the Prague strain was used in a strength of 1.50 g/litre, the other vaccines in a strength of 0.50 g/litre.

So as to avoid a reduction in viability, the vaccines were used without freeze-drying. Those used in India were sent by air in refrigerated containers. The great majority of the vaccinations were performed within 2 weeks of the preparation of the vaccine. In the case of the few exceptions, the last day of use of the vaccine was the 26th day after preparation.

Study population

The South Indian study population consisted of 1–15-year-old children in villages in Dodballapur Taluk, Bangalore District.

[a] A preliminary report on some of the data was contained in unpublished document WHO/BS/69.956.

[b] Space did not permit the inclusion of the Appendix. This has been deposited in the WHO library, and single copies may be obtained on request to: Chief Librarian, WHO, 1211 Geneva 27, Switzerland.

Children with strong tuberculin allergy were excluded from the study. Those included had not all received the same prevaccination tuberculin test. Various tuberculin studies were conducted in the same population with single and double tests, as part of the preparation for a major BCG trial. When two tuberculin tests of approximately equal strength were used in prevaccination tuberculin testing (e.g., 1 TU and 2 TU of RT 23 with Tween 80, or 3.2 IU and 5 IU of PPD-S), the vaccination criterion was a sum of both reactions of 15 mm or less. When only a single tuberculin test was given, the vaccination criterion was a reaction of 7 mm or less.

Both shoulders of each child included in the study were examined for a BCG scar as evidence of previous BCG vaccination. Only the data for children without a scar from previous BCG vaccination have been included in the present report.

The Copenhagen population consisted of schoolchildren about 7 years old who had neither a scar nor a history indicating previous BCG vaccination and who did not react to a prevaccination tuberculin test. In study 2, a Moro patch test was given; in study 4, an intradermal test with 2 TU of RT 23 with Tween. A reaction of 10 mm was considered as positive in the latter case. The numbers of children successfully followed up in the four studies were 699, 437, 888, and 488.

Vaccination

The design was approximately the same in all four studies. A small number of children (9–14, varying from study to study) were vaccinated in succession from each ampoule. Ampoules of different strains were used in random order, usually in a set with only one ampoule of each strain. A new set of ampoules, in a different order of strains, was used for the next group of children, and so on. In the two studies in South India, a new batch was received every month for the duration (3–4 months) of each study. All vaccinations were given in the left deltoid region, by intradermal injection of 0.1 ml.

Follow-up

To evaluate the tuberculin sensitivity induced by vaccination, an intradermal tuberculin test with 2 TU of RT 23 with Tween was given, always in a site different from that used for the prevaccination testing, 8–11 weeks later. The reaction was read after 3–4 days in terms of the transverse diameter of induration in millimetres. The skin lesion was also read, usually on the day of testing, in terms of the transverse diameter, in millimetres, of the tissue destruction (pustule, ulcer, scab, or scar). The readings were carried out " blindly ", without knowledge of the vaccine or even of the vaccine code used for each individual child.

Statistical analysis

The distributions of postvaccination allergy and vaccination lesions were computed and were found to be similar in nature to those usually obtained. They were all unimodal and nearly " normal ". These distributions are not presented here.

For studies 1–4, the mean responses (tuberculin reactions and vaccination lesions) are given, in that order, in Tables 1–4. For the studies in South India, the mean responses are given for separate batches (months). On the basis of these means and of their variances as expected from the distributions of individual reactions, an analysis of variance was carried out. For the studies in Copenhagen, each

Table 1. Means of tuberculin reactions and skin lesions, separately for monthly vaccine batches, 9–11 weeks after vaccination (Study 1, South India)

Strain	Mean size of post-vaccination reactions to 2 TU of tuberculin (induration in mm)				Mean size of skin lesions (in mm) 9–11 weeks after vaccination			
	time of vaccination				time of vaccination			
	March	April	May	June	March	April	May	June
1331	15.61	14.21	15.30	18.42	5.89	5.37	5.87	5.97
Former Danish	14.94	14.94	14.85	17.12	6.43	5.82	5.97	5.77
Former Madras	14.56	12.67	14.30	16.69	6.00	5.33	5.88	5.84
Prague	9.48	10.72	11.69	13.48	5.21	5.36	4.95	5.54

Table 2. Means of tuberculin reactions and skin lesions 8–10 weeks after vaccination (Study 2, Copenhagen)

Strain	Tuberculin reaction	Skin lesion
1331	19.48	5.91
Former Danish	20.01	6.22
Former Madras	18.35	5.53
Prague	13.69	4.16

Table 3. Means of tuberculin reactions and vaccination skin lesions, separately for monthly vaccine batches, 9–11 weeks after vaccination (Study 3, South India)

Strain	Mean size of post-vaccination reactions to 2 TU of tuberculin (induration in mm)			Mean size of skin lesions (in mm) 9–11 weeks after vaccination		
	time of vaccination			time of vaccination		
	January	February	March	January	February	March
1331	16.55	15.90	15.45	5.29	4.75	5.09
Moscow	16.27	17.38	16.69	5.67	5.64	5.50
Tokyo	17.56	16.59	17.56	5.95	5.27	5.54
Paris	14.42	16.94	16.45	4.96	5.13	5.50
Y	14.13	15.70	14.93	4.80	5.05	4.83

Table 4. Means of tuberculin reactions and vaccination skin lesions 8–10 weeks after vaccination (Study 4, Copenhagen)

Strain	Tuberculin reaction	Skin lesion
1331	17.69	6.29
Rio de Janeiro	18.44	5.98
Gothenburg	17.15	6.32
London	15.12	4.62
Prague [a]	12.98	5.67

[a] Increased dose, see page 493.

set of ampoules used in succession was considered as a randomized block, and the mean response for each ampoule was considered as a plot result, with the interaction of block and strain taken as the error term. The analysis of variance for each of the four studies is given in the Appendix.[a]

RESULTS

The results are summarized in scatter diagrams (Fig. 1) correlating the mean size of tuberculin reactions with that of skin lesions (scars).

[a] See footnote on page 490.

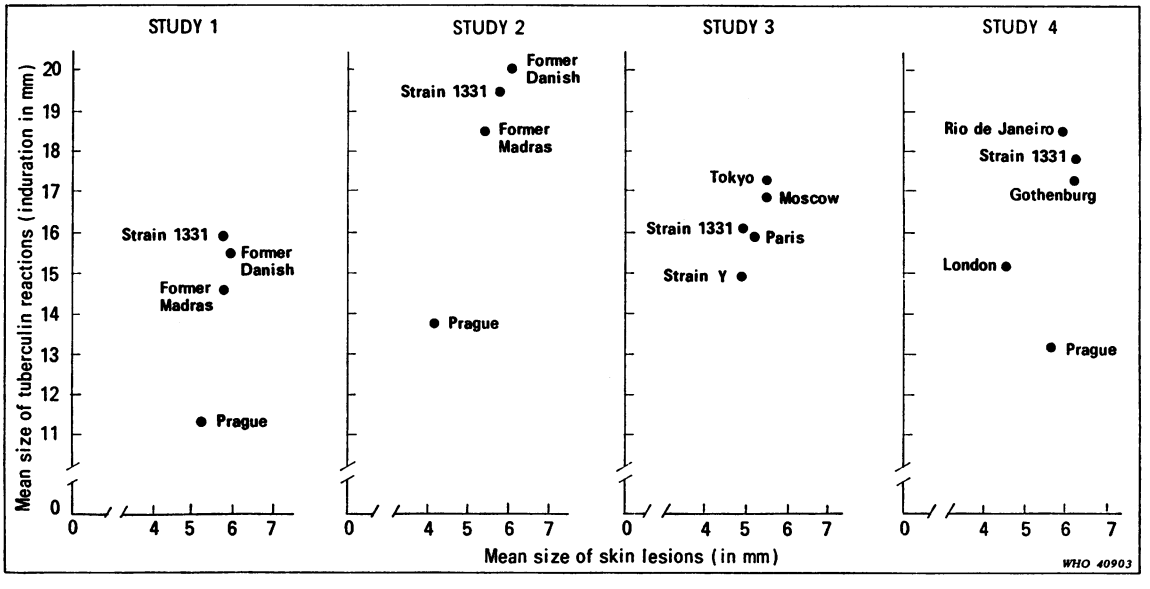

Fig. 1. Scatter diagrams showing the correlation between the mean of tuberculin reactions and the mean of skin lesions.

Tables 1 and 2, comparing the strains used in both South India and Copenhagen, are in mutual agreement. The tendency for higher estimates of tuberculin reactions in Copenhagen is not necessarily biologically significant. Indeed, for reasons unknown, results in different months differed systematically in South India (for all strains in parallel).

The Prague strain is strikingly different from the rest, with tuberculin reactions 5–6 mm smaller than those for the other vaccines, whereas the skin lesions were only 1–2 mm smaller. A similar tendency shown by the former Madras strain (as compared with the two strongest strains) was only slight, though statistically significant.

In study 3 (South India), the 5 strains examined differed only slightly (Table 3), although the analysis of variance indicated that the difference between strain Y and the average of the other 4 was significant at the 5% level (strain Y is an experimental strain not used for routine production).

In study 4 (Copenhagen), the Prague and London strains differed strikingly (Table 4) and highly significantly from the rest. In this case, the Prague strain was given in a dose three times as strong as that of the other four strains, resulting in skin lesions practically as large as those for the strongest strains. Yet the tuberculin sensitivity remained very weak, with reactions measuring about 5 mm less than those induced by the other strains. The London strain bore a simpler relation to the others, being weaker than the strongest vaccines in both respects. The vaccines prepared from this strain had consistently lower viability than almost all the other vaccines—possibly because the strain is not adapted to surface growth. This may explain part, but scarcely all, of the observed difference in tuberculin sensitivity.

The tendencies for the Rio de Janeiro strain to give stronger tuberculin sensitivity and at the same time smaller skin lesions than the two vaccines next in rank are not statistically significant.

DISCUSSION

In view of the large number of BCG strains currently in use for BCG production and the absence of reliable comparative data on their protective efficacy in man, the basis for educated guesses about the relative value of strains can only be a battery of other *in vivo* tests. A number of animal models have been developed—e.g., tests of BCG multiplication in the host and tests of induced resistance to virulent challenge. However, the induction of delayed hypersensitivity in man is of particular interest. As mentioned above, the degree of delayed hypersensitivity depends, for a given strain, on the dose of BCG—i.e., delayed hypersensitivity depends in a quantitative sense on the strength of the vaccine (1). It would therefore not be totally unreasonable to postulate that a strain that, other things being equal, induces a high degree of delayed hypersensitivity is a particularly potent strain. It was observed by Mackaness (6) that delayed hypersensitivity is a concomitant of protection, the two being mediated by the same class of host cell: the thymus-dependent lymphocyte. True, sensitivity to purified protein derivative denatured by heat may only incompletely overlap with delayed hypersensitivity to the live, virulent mycobacterium. Yet, in the absence of detailed knowledge about the latter, it would seem unreasonable to disregard existing knowledge about the former.

The present studies demonstrate, first of all, that strains differ in terms of allergenic potency in man. This would appear to be quite beyond doubt for the Prague strain. For the London strain the present evidence is less than conclusive, the vaccines prepared from this strain with the techniques chosen for this experiment being somewhat inferior *in vitro*. An increase in dose might conceivably have raised the level of tuberculin sensitivity to that of the other vaccines without an excessive increase in size of skin lesions. The evidence for the former Madras strain is more convincing. The strain yielded vaccines of high *in vitro* quality, yet induced less sensitivity than other strains by a significant, if slight, margin. According to Ladefoged et al. (5), this same strain protects bank voles very well, yet does not kill hamsters, as had been reported by Bunch-Christensen et al. (3). Its properties contrast curiously with those of the Tokyo strain, which induces a high degree of delayed hypersensitivity, yet is inferior in terms of minimum protective dose in the bank vole (5). Most probably, both strains protect man as well as any other; yet, it would seem prudent to prefer some strain that tends to be uniformly active in the entire battery of *in vivo* models.

RÉSUMÉ

SENSIBILITÉ TUBERCULINIQUE ET LÉSIONS CUTANÉES CHEZ DES ENFANTS APRÈS VACCINATION PAR 11 SOUCHES DIFFÉRENTES DE BCG

Lors d'études antérieures, on a comparé sur des modèles animaux un certain nombre de souches de BCG utilisées dans plusieurs laboratoires de production du vaccin.

Pour la présente recherche, des vaccins liquides ont été préparés à partir de différentes souches par un même laboratoire en employant une technique uniforme pour tenter d'obtenir des préparations possédant des propriétés identiques *in vitro*. Ces vaccins ont été comparés en procédant à la vaccination d'enfants en Inde et au Danemark et en évaluant les lésions cutanées et la sensibilité tuberculinique postvaccinales. On a constaté que l'une des souches induisait une hypersensibilité de type retardé beaucoup plus faible que celle provoquée par toutes les autres souches, bien que des épreuves *in vitro* très poussées n'aient pas montré une activité moindre de ce vaccin. Des différences, parfois statistiquement significatives, ont été également notées entre les autres souches, mais elles étaient d'une ampleur moindre. Les conséquences de ces différences d'activité entre souches de BCG sont examinées.

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