

## Tuberculin Skin Sensitivity Following BCG Vaccination with Vaccines of High and Low Viable Counts

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AS part of the tuberculosis control program in the Ontario counties of Victoria and Haldiburton, BCG vaccination of students leaving secondary school was begun in 1964 to increase the level of acquired resistance to tuberculosis in this relatively susceptible segment of the population.‡ This BCG vaccination program afforded an opportunity to observe the effects of the method of administration and the viable count of the vaccine on the tuberculin skin sensitivity induced by vaccination. The purpose of this paper is to present the results of comparative studies using BCG vaccines of high and low viable counts as defined below.

### MATERIALS

*Tuberculin purified protein derivative (PPD)*§: (a) for intradermal (Mantoux) test;<sup>2</sup> (b) containing 5, 10 and 100 tuberculin units (TU) per 1/10 c.c. dose.

*Freeze-Dried BCG Vaccines*§ (a) for the multiple-puncture method, containing after reconstitution 40 mg. BCG per c.c.; (b) for the intradermal method, containing after reconstitution 1 mg. BCG per c.c.

For the purpose of the present work, the viable count of a vaccine is defined as the number of viable units contained in 1 mg. of BCG, as determined by colony count on Loewenstein-Jensen medium.

The counts of vaccines designated as high-count vaccines ranged from 14.2 to 15 x 10<sup>6</sup> viable units; those of low-count vaccines, from 2.5 to 5.8 x 10<sup>6</sup> viable units per mg. BCG.

### METHODS

#### (a) *Prevaccination Tuberculin Testing*

Prevaccination tuberculin testing served two purposes—identifying those pupils eligible for vaccination and those who were already tuberculin-positive. Tuberculin PPD, 5 tuberculin units (TU), was used initially. All tests were read at 72 hours. A positive

reaction consisted of an area of induration 5 mm. or more in diameter. Pupils negative to the initial test were tested with 100 TU PPD intradermally, and if again negative, were vaccinated.

If either of the prevaccination tests was positive, the pupil was retested with old tuberculin and simultaneously with one of the atypical antigens, examined radiographically and, if indicated, was offered chemoprophylaxis.

#### (b) *Vaccination*

Vaccination was accomplished by one of two methods. Intradermal vaccination was given by injecting 1/10 of 1 c.c. of reconstituted vaccine into the superficial layers of the skin overlying the left deltoid muscle. Multiple-puncture vaccination (40 punctures) was effected by applying the 20-pronged multiple puncture apparatus\* twice to adjacent skin areas over the left deltoid, on which a drop of reconstituted freeze-dried multiple-puncture vaccine had been spread.

#### (c) *Post-vaccination Tuberculin Testing*

Tuberculin testing was repeated 6 to 12 weeks after vaccination was done, again using tuberculin PPD, 5 TU, initially. Students who were negative were tested further with 100 TU PPD.

The site of vaccination was also examined. In those vaccinated intradermally, the transverse diameter of the "take" was measured in millimetres. A rough description (O+ to 4+) of the multiple-puncture takes was noted according to the number of papules observed, their redness and induration.

### RESULTS

Whereas the determination of the relative value of the intradermal compared to the multiple puncture method is the subject of a separate publication,<sup>3</sup> the following account, reporting the results of a total of 804 vaccinations, deals essentially with the effect which the viable count of the vaccines may have upon the post-vaccination tuberculin allergy.

Conversion rates, although widely used to indicate the results of BCG vaccination, do not quantitatively measure the total allergic response. This total allergic response of a vaccinated group can best be expressed by the average (mean) diameter of all reactions, including the zero reactions. In consideration of this

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‡Details of the planning and implementation of this program may be obtained upon request from the Tuberculosis Prevention Branch, Ontario Department of Health.

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\*Modification of the Heaf apparatus by Allen & Hanburys Limited, London, E.2, England.

concept, first expressed by Edwards, Palmer and Magnus,<sup>4</sup> the results of vaccination trials to be presented include, besides conversion rates, the mean and the median diameters of induration, the median being used for statistical study.

#### (a) Intradermal Vaccination

Table I shows the effect of high and low viable counts of the vaccine on the resulting Mantoux tests. A group of 437 pupils was vaccinated intradermally, using three lots of freeze-dried vaccine, one of high count (14.2 million viable units per mg. BCG) and two of relatively low count (5.4 and 4.5 million). It should be emphasized, however, that these counts were all within

Three of the groups, I-A, I-B and I-C, were also tested with 10 TU, which in the case of groups I-B and I-C, vaccinated with low-count vaccines, produced appreciably higher conversion rates than the 5 TU tuberculin, whereas in group I-A, vaccinated with the high-count vaccine, the already high conversion rate (95.9%), based on 5 TU, was not increased by the use of 10 TU.

Measurement of the transverse diameter of the reaction at the site of vaccination revealed a median diameter of 5 mm. in group I-A vaccinated with high-count vaccine and one of 4 mm. in those vaccinated with low-count vaccines. This difference, although small, was

TABLE I.—INTRADERMAL VACCINATION WITH HIGH- AND LOW-COUNT BCG VACCINES, POST-VACCINATION MANTOUX TESTING AT 10 WEEKS

Group	Viable count of vaccine	Number vaccinated and retested	Positive to 5 TU PPD (Connaught)		Positive to 10 TU PPD (Connaught)		Negative to 5 TU PPD		Percent conversion achieved	Reaction to 5 TU		
			No.	%	No.	%	Positive to 100 TU	Negative to 100 TU		Mean (mm.)	Median (mm.)	Median take (mm.)
I-A.....	14.2 x 10 <sup>6</sup>	99	95	95.9*	95	95.9	4	0	100.0	12.30	12*	5*
I-B.....	5.4 x 10 <sup>6</sup>	84	67	79.7	72	85.7	17	0	100.0	9.00	10	4
I-C.....	4.5 x 10 <sup>6</sup>	39	29	74.3	32	82.0	10	0	100.0	8.55	10	4
I-D.....	4.5 x 10 <sup>6</sup>	39	30	76.9	—	—	8	1	97.2	8.52	10	4
			Positive to 5 TU (U.S.P.H.S.)									
			Number	%								
I-E.....	4.5 x 10 <sup>6</sup>	176	138	78.4	—	—	38	0	100.0	7.18	8**	4

\*Significantly higher than I-B to I-E.

\*\*Significantly higher than I-C and I-D.

acceptable limits as set by the manufacturer. It can be seen from Table I that group I-A, vaccinated intradermally with the high-count vaccine, had a conversion rate to 5 TU, significantly higher than the rates experienced by groups I-B, I-C, I-D and I-E, vaccinated by the same method but using low-count vaccines. In all groups, however, the 5 TU conversion rates were greater than 70%, and rates of total conversion (which include all reactions positive to 100 TU) approached or equalled 100%.

No significant difference was noted in this experiment between the Connaught and the U.S.P.H.S. 5 TU tuberculin as far as conversion rates were concerned (Table I, groups I-D and I-E). However, the median diameter of reaction to 5 TU was appreciably smaller for group I-E, tested with the U.S.P.H.S. tuberculin, being 8 mm., compared with 10 mm. recorded for groups I-C and I-D, which were vaccinated with the identical vaccine lot but retested with Connaught tuberculin.

In addition to recording the median diameters of the tuberculin reactions, Table I shows, for comparison, the corresponding arithmetic means. These indicate analogous differences between groups, as do the medians, group I-A showing the largest and group I-E the smallest mean diameter of induration.

judged to be statistically significant. The largest recorded reaction was 14 mm., the smallest 1 mm., indicating a reduction in size.

These intradermal reactions, examined at the time of post-vaccination tuberculin testing, had appearances ranging from that of small red papules to crusted lesions, which discharged and healed several times before clearing. No serious complications were encountered. When the vaccination sites were examined some months later, only tiny scars remained.

#### (b) Multiple-Puncture Vaccination

Table II shows the results obtained when high-count and low-count multiple-puncture vaccines were used. In contrast to the intradermal vaccinations, which were followed up uniformly by tuberculin testing six weeks later, the time interval between multiple-puncture vaccination and tuberculin retesting varied from 6 to 12 weeks. A comparison between high-count and low-count vaccines with respect to their effects on post-vaccination tuberculin allergy had, therefore, to be made in groups which underwent post-vaccination testing after similar time intervals.

Group II-A vaccinated with high-count vaccine and group II-B vaccinated with a low-count vaccine were tuberculin-tested six weeks after

TABLE II.—MULTIPLE-PUNCTURE VACCINATION WITH HIGH- AND LOW-COUNT BCG VACCINES, POST-VACCINATION MANTOUX TESTING AT 6-12 WEEKS

Group	Viable count of vaccine	Post-vaccination Mantoux testing at	Number vaccinated and retested	Positive to 5 TU PPD (Connaught)		Negative to 5 TU PPD		Percent conversion achieved	Reaction to 5 TU	
				No.	%	Positive to 100 TU	Negative to 100 TU		Mean (mm.)	Median (mm.)
II-A	15 x 10 <sup>6</sup>	6 weeks	37	34	91.8	3	0	100.0	11.38	12
II-B	5.8 x 10 <sup>6</sup>	6 weeks	38	25	65.7*	12	1	97.0	7.45	10
III	15 x 10 <sup>6</sup>	7-10 weeks	54	46	85.2	8	0	100.0	10.56	12
IV-A	15 x 10 <sup>6</sup>	10-12 weeks	23	16	69.6	7	0	100.0	8.20	8
				Positive to 5 TU PPD (U.S.P.H.S.)						
IV-B	15 x 10 <sup>6</sup>	10-12 weeks	111	55	49.5	55	1	99.0	4.85	3
IV-C	2.5 x 10 <sup>6</sup>	10-12 weeks	104	37	35.6	66	1	99.0	3.05	0

\*Significantly lower than Group II-A.

vaccination. The conversion rate using tuberculin PPD, 5 TU (Connaught), was significantly higher in group I-A (91.8%) than in group I-B (65.7%).

An analogous difference in conversion rates was observed in two groups tuberculin-tested 10 and 12 weeks after vaccination, using 5 TU tuberculin PPD, supplied by the United States Public Health Service (Table II). Of group IV-B vaccinated with high-count vaccine, 49.5% converted, whereas group IV-C, vaccinated with a low-count vaccine, showed a conversion rate of only 35.6%. Although this difference between conversion rates is not considered statistically significant, the impression is created that a low viable count of a BCG vaccine could result in a low tuberculin sensitivity.

In a third group, IV-A, also vaccinated with the high-count vaccine, the tuberculin testing was done 10 to 12 weeks later with Connaught PPD, 5 TU, resulting in a conversion rate of 69.6%. This is 20.1% higher than the conversion of 49.5% of group IV-B tested with the 5 TU tuberculin supplied by the United States Public Health Service. Although statistical significance of this difference cannot be proved because of the limited number of vaccinations involved, the results give the impression that Connaught 5 TU tuberculin PPD, under the conditions used, tends to give larger reactions than the corresponding product from the United States.

Finally, the conversion rate of 85.2%, obtained in group III, vaccinated with the high-count multiple-puncture vaccine and tested 7 to 10 weeks later with the 5 TU Connaught tuberculin PPD, confirms this particular vaccine's capacity to produce a relatively high level of tuberculin allergy.

#### DISCUSSION

Although it is recognized that the tuberculin allergy which develops following BCG vaccination is not a direct manifestation of immunity,

the tuberculin test can serve as a readily available yardstick for measuring the immediate effect of BCG vaccination in individuals or in groups.

The significance of the tuberculin skin allergy can be demonstrated by animal experiments. In guinea pigs vaccinated with BCG, the resulting increased resistance to tuberculous infection is regularly accompanied by the development of tuberculin skin allergy, although in long-term experiments this allergy tends to wane faster than the tuberculo-immunity.<sup>5</sup>

Significance of the post-vaccination tuberculin allergy can also be claimed on clinical grounds. In a survey of BCG vaccination field trials, which includes the extensive study by the Medical Research Council of Great Britain,<sup>6a-c</sup> Davies<sup>7</sup> concludes that vaccination programs showing a high tuberculin conversion rate result in a degree of protection of the order of 80%, whereas lower degrees of post-vaccination allergy result in considerably lower protection rates.

In the present report dealing with limited trials of freeze-dried BCG vaccine, the effectiveness of the vaccination is judged exclusively by the degree of post-vaccination tuberculin allergy. The results of intradermal vaccination (Table I) demonstrate a significant difference between high-count and low-count freeze-dried vaccines with regard to the allergic response they engender. This close dependence of post-vaccination tuberculin allergy upon dosage appears surprising in view of the wide dosage range of living BCG known to be effective in experimental animals. In guinea pigs a dose of 10<sup>-5</sup> mg. BCG, injected intradermally, is practically as effective as 10<sup>-1</sup> mg. in producing skin allergy and conferring a significant degree of immunity against the virulent tuberculous infection. This holds true for fresh fluid vaccine prepared with the Danish strain<sup>5</sup> as well as for the freeze-dried BCG vaccine produced with the Toronto strain.<sup>8</sup> Whatever the explanation of this apparent discrepancy between human trials and animal experiments may be, our results (Table I) are in

line with the observations made during the British M.R.C. field trials.<sup>6</sup> In that study tuberculin conversion rates (based on 3 TU), recorded three to five months after intradermal vaccination with the fluid Danish vaccine, proved to be closely dependent upon the viable counts of the respective vaccine lots used. The lowest conversion rate (76%) recorded in the British trial was produced by low-count vaccines which, however, still gave adequate protection in terms of a low incidence of tuberculosis during a five-year follow-up period.

Similar conversion rates (74 to 79.7% to 5 TU) were observed with low-count lots of intradermal Connaught vaccine (Table I, groups I-B, I-C) although these results are not strictly comparable with those of the British trial, for which a different BCG strain was used. But it is interesting to note that in the British trial, as well as the small one reported here, the tuberculin conversion rates based on 100 TU approached or equalled 100% for all vaccine lots used, while only the low-dosage tuberculin (3 and 5 TU respectively) clearly differentiated between the high-count and low-count vaccines.

Post-vaccination testing with 10 TU (Table I) resulted in conversion rates above 80%, which meets the minimum requirements for BCG as set by the United States Public Health Service.<sup>9</sup>

Although with multiple-puncture vaccination (Table II) the conversion rates, based on 5 TU PPD (Connaught), are higher than the rate reported for an earlier trial,<sup>10</sup> the results fall short of those recorded for the intradermal vaccination (Table I). Yet the superiority of the high-count over the low-count vaccine with regard to allergenic potency also applies to the multiple-puncture vaccines (Table II, groups II-A and II-B).

The observed advantage of producing higher conversion rates, which the intradermal method offers over the multiple-puncture vaccination, is in agreement with earlier reports.<sup>3, 11</sup>

The seemingly weaker allergic response observed when the U.S.P.H.S. tuberculin PPD, 5 TU, was used for post-vaccination testing (Table II) was likely due to partial adsorption of tuberculin to glass surfaces. The prevention of such adsorption by the addition of Tween 80 to the tuberculin solutions was demonstrated in the case of the Danish tuberculin by Magnusson *et al.*<sup>12</sup> and for the Connaught PPD by Landi *et al.*<sup>13</sup>

An analogous, though less marked, difference in potency between Connaught and U.S.P.H.S. 5 TU tuberculins was apparent in the results of the intradermal vaccinations (Table I, groups I-D and I-E), but there the difference showed

only in the diameters of the tuberculin reactions, without affecting the conversion rates.

**Summary** Comparative studies of tuberculin skin sensitivity following BCG vaccination using vaccines of high and low viable counts were carried out in tuberculin-negative students leaving secondary school. After intradermal vaccination using high-count BCG vaccine (14.2 million viable units per mg. BCG), a significantly higher degree of tuberculin skin sensitivity was found upon Mantoux testing with tuberculin PPD, 5 TU, than was found following intradermal vaccination with low-count vaccines (5.4 and 4.5 million viable units per mg. BCG).

In those vaccinated by the multiple-puncture method an indication of a similar difference in tuberculin skin sensitivity following vaccination with high-count and low-count vaccines was found. This requires further verification.

Vaccinees negative to 5 TU PPD were further tested with 100 TU PPD. Between 97 and 100% of those vaccinated were positive to one or other of these tests regardless of the method of vaccination or the viable count of the BCG vaccine used.

In no instance did the vaccinations produce any undesirable side effects.

**Résumé** Chez des jeunes gens tuberculino-négatifs qui venaient de quitter l'école, on a entrepris l'étude comparative de l'intradermo-réaction de Mantoux à la tuberculine après vaccination au BCG, au moyen de vaccins dont les titres viables étaient forts et faibles. L'injection intradermique d'un vaccin BCG de titre fort (14.2 millions d'unités viables par mg de BCG) a été suivie d'une intradermo-réaction à la tuberculine beaucoup plus forte lors du test de Mantoux à la tuberculine PPD (5 unités tuberculino-UT) qu'après la vaccination intradermique avec un vaccin à faible titre (5.4 et 4.5 millions d'unités viables par mg de BCG).

Cette même différence de degré de l'intradermo-réaction de Mantoux après vaccination avec des vaccins forts et faibles s'est retrouvée chez les sujets vaccinés par la méthode des piqûres multiples. Cette constatation demande à être vérifiée.

Les vaccinés qui étaient négatifs au titre de 5 unités de tuberculine PPD ont été par la suite soumis à l'épreuve de 100 unités de tuberculine PPD. De 97% à 100% des vaccinés étaient positifs suivant l'une ou l'autre de ces titres d'unités viables du vaccin BCG utilisé.

En aucun cas la vaccination n'a suscité des effets secondaires néfastes.

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## REVIEW ARTICLE

### Coronary Thrombosis and Myocardial Infarction

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SINCE the recognition of myocardial infarction as a clinical entity more than half a century ago, it has been assumed that the lesion is caused, in the vast majority of cases, by acute thrombotic occlusion of a coronary artery.

In the past few years some authors have challenged this interpretation and have concluded that the thrombosis is probably secondary to the necrosis, whereas other authors have supported the classic pathogenetic concept. For example, Ehrlich and Shinohara<sup>1</sup> conclude that ". . . it may be necessary to reassess traditional concepts of the significance of recent thrombi in the coronary arteries of hearts with recent infarcts. The term 'infarct' . . . may be an erroneous application to many lesions of the myocardium which possibly have resulted from as yet obscure mechanisms." On the other hand Harland and Holburn,<sup>2</sup> discussing the hypothesis that the coronary thrombosis could be the effect and not the cause of the infarction, say "It seems more reasonable to accept the traditional view that thrombosis causes the infarct" and that "future research must concentrate on the pathogenesis of coronary thrombosis". Baroldi<sup>3</sup> says ". . . it appears that in the so-called myocardial infarct . . . most of the cases develop independently of an acute occlusion and that it is incorrect to apply the term 'myocardial infarct' to the lesion". On the other hand Rona<sup>4</sup> finds that there is "an

interdependence between the grade of atherosclerosis, coronary thrombosis and myocardial infarction".

In view of these contradictory conclusions and because of the obvious importance that the clarification of this problem has for any rational approach to the therapy and prevention of myocardial infarction, in this paper I intend to review the incidence of coronary thrombosis reported in the literature and try to evaluate the evidence for and against the traditional view that myocardial infarction is caused by acute occlusion of a coronary artery.

#### INCIDENCE OF CORONARY THROMBOSIS IN CASES OF MYOCARDIAL INFARCTION

In the majority of the papers discussed here, the authors were not primarily interested in the incidence of coronary thrombosis in myocardial infarction—the relation of cause and effect between the two entities was considered a matter of course. Therefore in some cases data had to be collected that were disseminated throughout the text or distributed in several tables in a paper before the incidence of coronary occlusion could be calculated. For example, Schwartz and Mitchell<sup>5</sup> report 15 cases of large necroses and 29 cases of small disseminated necroses of the myocardium. Regarding the large lesions they clearly state that of 15 cases, 10 had coronary occlusion; however, the authors do not mention the incidence of coronary occlusion among the small lesions. The incidence had to be

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