

Comparative Experimental Study of Sensitivity to Tuberculin and Lepromin in Guinea-pigs Previously Inoculated with BCG by Oral and Intradermal Routes^{1 2}

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Premunition by BCG for prophylaxis of leprosy and tuberculosis has been tried by several routes of inoculation. Regarding the intradermal route, Brown *et al.* (11) showed that BCG protects against leprosy, and D'Arcy Hart *et al.* (12) showed protection against tuberculosis.

In Brazil, oral BCG premunition has been carried out in several million children since 1927. The value of the oral route with respect to positive conversion of the tuberculin and lepromin tests has been presented in several publications either in the field of leprosy (4, 16, 18) or in the field of tuberculosis (1, 3, 15, 17).

There has been discussion concerning the immunologic and practical advantages of one route over others (9, 10). This problem is very important with respect to costs of administration and determination of the most practical use of BCG premunition in leprosy and tuberculosis in mass campaigns in underdeveloped countries.

A positive lepromin test is regarded as a sign of resistance against leprosy and a positive tuberculin test is an indirect sign indicating the presence of some resistance against tuberculosis despite recognition of the fact that tuberculin allergy and resistance against tuberculosis are different phenomena. Nevertheless, post-vaccination allergy is the only sign which permits evaluation, in children, of the efficiency of premunition. In elucidation of the problem of the optimum route of inoculation, an investigation of the lepromin and tubercu-

lin tests after oral and intradermal inoculation of BCG in guinea-pigs was undertaken. (A similar study is in progress in newborn infants and children. The results will be presented later.) This paper shows the first results of the experiment; the final results will be published after the verification of lepromin and tuberculin sensitization as evidenced by the tests done five months after BCG inoculations.

MATERIALS AND METHODS

Materials. One hundred guinea-pigs, three to four months old, weighing approximately 350 gm. each, were used. Vaccinations were made with either lyophilized BCG produced by Glaxo Laboratories, Greenford, England (Batch B818 J), or BCG Moreau (Rio de Janeiro strain), maintained alternatively in glycerine-potato and bile-potato media.

Tuberculin hypersensitivity was tested with Old Tuberculin (OT) and PPD by intradermal route. Lepromin hypersensitivity was tested with lepromin antigen, Hayashi-Mitsuda type (16×10^7 bacilli per cc.).

Technic of vaccination. All the 100 guinea-pigs were negative to an initial tuberculin test. They were divided in four groups (A, B, C and D), each having 25 guinea-pigs.

Group A—Control group.

Group B—Intradermal inoculation with 0.05 mg. of lyophilized BCG.

Group C—Intradermal inoculation with 0.05 mg. of BCG Moreau (Rio de Janeiro strain).

Group D—Oral inoculation with 100 mg. of BCG Moreau (Rio de Janeiro strain).

The inocula used were determined by analogy with those used in newborn infants

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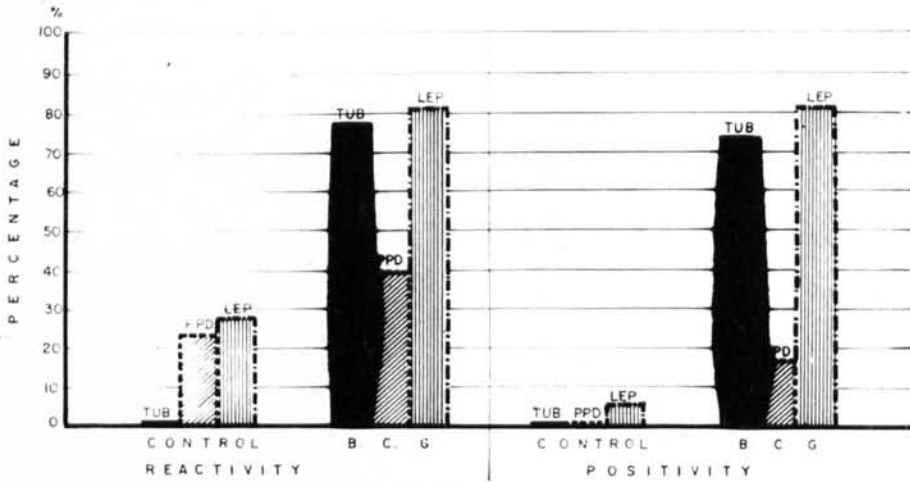


FIG. 1. Results of tests to tuberculin, PPD, and lepromin after vaccination with BCG. *Reactivity*: reactions having 1 or more mm. in diameter. *Positivity*: reactions having 2 or more mm. in diameter.

as recommended by Glaxo Laboratories and Viscondessa de Morais's Institute, Rio de Janeiro. Azulay *et al.* (5-8) showed that BCG oral vaccination in doses of 30 mg. per kg. of animal weight induces 87 per cent lepromin positivity in guinea-pigs. Amaral and Sorensen (2) obtained 90 per cent positive tuberculin test and in newborn guinea-pigs after administration of 20 mg. BCG by mouth (13, 14).

Thirty days after vaccination all guinea-pigs were tested with OT, diluted to 1/10 units of PPD (Rt 23-Copenhagen) and

TABLE 1. Results of tests to tuberculin, PPD and lepromin after vaccination with BCG.

Sensitivity	Reactivity ^a		Positivity ^b	
	Control %	BCG %	Control %	BCG %
Tuberculin	—	78.7	—	73.8
PPD	22.7	39.3	—	16.3
Lepromin	27.2	80.3	4.5	80.3
No. guinea-pigs	22	61	22	61

^a Reactivity = reactions having 1 or more mm. in diameter.

^b Positivity = reactions having 2 or more mm. in diameter.

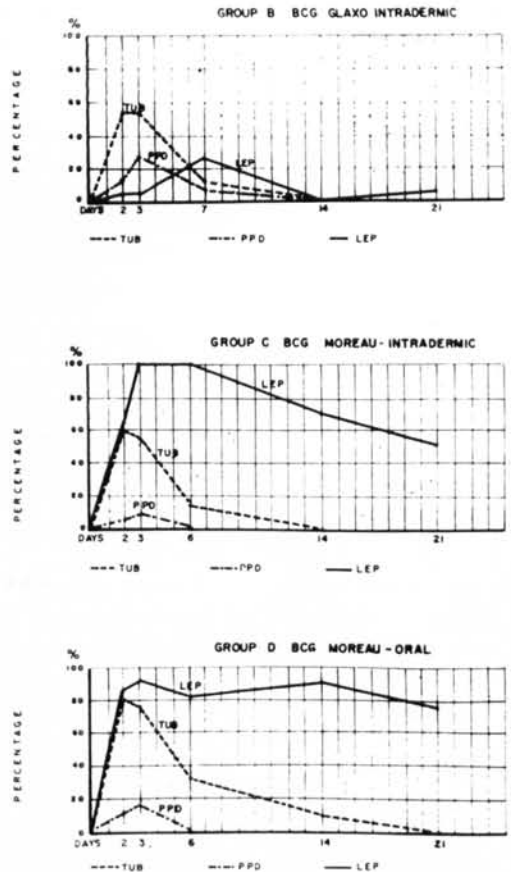


FIG. 2. Sensitivity to tuberculin, PPD, and lepromin in guinea-pigs previously inoculated with BCG.

TABLE 2. Sensitivity of guinea-pigs to lepromin, PPD, and tuberculin, after BCG vaccination by different routes.

		% Reactivity ^a						% Positivity ^b					
		Hours			Days			Hours			Days		
Control		48	72	7	14	21	48	72	7	14	21		
<i>Group A. Control</i>													
Tuberculin		—	—	—	—	—	—	—	—	—	—		
PPD		—	—	22.7	—	—	—	—	—	—	—		
Lepromin		—	—	27.2	—	—	—	—	4.5	—	—		
<i>Group B. BCG Glaxo intradermal</i>													
Tuberculin		63.6	59.1	13.6	—	—	54.5	54.5	13.6	—	—		
PPD		13.6	27.2	27.2	—	—	13.6	27.2	9.1	—	—		
Lepromin		4.5	9.1	27.2	—	9.1	4.5	4.5	27.2	—	4.5		
<i>Group C. BCG Moreau intradermal</i>													
Tuberculin		59.1	59.1	14.2	—	—	59.1	54.1	14.2	—	—		
PPD		27.2	27.2	—	—	—	4.5	9.1	—	—	—		
Lepromin		81.8	100.0	100.0	70.2	50.0	63.5	100.0	100.0	70.2	50.0		
<i>Group D. BCG Moreau oral</i>													
Tuberculin		85.0	76.1	33.3	9.5	—	80.0	76.1	33.3	9.5	—		
PPD		25.0	28.5	—	—	—	10.0	14.3	—	—	—		
Lepromin		85.7	90.4	85.7	90.4	76.1	85.0	90.4	80.7	90.4	76.1		

^a Reactivity = reaction having 1 or more mm. in diameter.^b Positivity = reaction having 2 or more mm. in diameter.

lepromin containing 16×10^7 bacilli/ml. by intradermal route. The readings were made at 48 hours, and at 7, 14 and 21 days.

Criteria for reading results. Positivity was determined by the frequency of the largest reactions measured in millimeters. A "reactive" test means any reaction independent of the size of it. The criteria of positivity were as follows:

1. *Positive tuberculin test:* reaction of 2 or more mm. at 48 hours.
2. *Positive PPD test:* reaction of 2 or more mm. at 72 hours.
3. *Positive lepromin test:* reaction of 2 or more mm. at seven days.

RESULTS

Group A (control)

1. Tuberculin test: absence of any reaction
2. PPD test: no positive test, but some discrete reactivity (1 mm.) was present in 22.7% at seven days.
3. Lepromin test: 4.5% positive test and 27.2% reactive test at seven days.

Group B (intradermal BCG-Glaxo).

1. Tuberculin test: 54.5% positive and 63.6% reactive test. It is interesting to observe that 13.6% of the positive tests were still evident at the seventh day. At the later readings all reactions had disappeared.
2. PPD test: 27.2% positive; 9.1% remained positive until the seventh day.
3. Lepromin test: 27.2% positive; 4.5% remained positive until the 21st day.

Group C (intradermal BCG-Moreau).

1. Tuberculin test: 59.1% positive; 14.2% remained positive until the seventh day; at later readings all reaction had disappeared.
2. PPD test: 9.1% positive, but the reactivity was three times higher (27.2%); absence of any reaction at later readings.
3. Lepromin test: 100% positive; this decreased to 70.2% and to 50% at the 14th and 21st day readings, respectively.

Group D (oral BCG-Moreau).

1. Tuberculin test: 80% positive; this decreased to 76.1%, 33.3% and 9.5% at the 72 hour, 7 day, and 14 day readings respectively. Absence of any reaction at 21st day reading.
2. PPD test: 14.3% positive but the reactivity was twice as high (28.5%). Absence of any reaction at 7, 14 and 21 days, respectively.
3. Lepromin test: 85.0% positive; this increased to 90.4% at the 14th day reading and decreased to 76.1% at the 21st day reading.

SUMMARY AND CONCLUSIONS

A definite sensitization of guinea-pigs to Old Tuberculin, PPD and lepromin was induced by the administration of BCG. The reactivity reactions were a little bit higher than the positivity reactions.

The highest points of the curves of positivity were: 48 hours for the tuberculin test, 72 hours for the PPD test and seven days for the lepromin test.

The lepromin reaction in guinea-pigs is of shorter duration than in human beings.

The induced sensitization of the guinea-pigs varied according to the type and route of administration of BCG.

Highest tuberculin sensitization was achieved in Group D (oral BCG-Moreau).

PPD sensitization was highest in Group B (intradermal BCG-Glaxo).

Lepromin sensitization was very low in Group B (intradermal BCG-Glaxo); it was very high in Group D (Oral BCG Moreau) and the highest in Group C (intradermal BCG-Moreau).

In Groups C and D, which received BCG-Moreau, the lepromin reactions were more persistent; in the Group D (oral BCG Moreau) the reactions were even more persistent than in the Group C (intradermal BCG-Moreau).

As the oral administration of BCG is easier and cheaper it should be recommended in the under developed countries, in the prophylaxis of leprosy and tuberculosis.

RESUMEN

La administración de BCG indujo en cobayos una sensibilización bien definida a tuberculina antigua, PPD y lepromina. Las reacciones de reactividad fueron ligeramente mayores que las reacciones de positividad.

Los puntos más altos de las curvas de positividad fueron: 48 horas para la prueba de tuberculina, 72 horas para la prueba con PPD y siete días para la prueba con lepromina.

La reacción de lepromina en cobayos es de menor duración que en seres humanos.

La sensibilización que se indujo en los cobayos varió de acuerdo con los tipos de BCG utilizados y vías de administración.

La mayor sensibilización contra la tuberculina se obtuvo en el Grupo D (BCG oral—Moreau).

La sensibilización contra PPD fué mayor en el Grupo B (BCG intradérmico—Glaxo).

La sensibilización contra la lepromina fué muy baja en el Grupo B (BCG intradérmico—Glaxo); muy alta en el Grupo D (BCG oral—Moreau) y mayor de todas en el Grupo C (BCG intradérmico—Moreau).

En los Grupos C y D, que recibieron BCG—Moreau, las reacciones de lepromina fueron más persistentes; en el Grupo D (BCG oral—Moreau) las reacciones fueron aún más persistentes que en el Grupo C (BCG intradérmico—Moreau).

Ya que la administración oral de BCG es más fácil y de menor costo, debe ser recomendada para países en desarrollo, para la profilaxis de la lepra y la tuberculosis.

RÉSUMÉ

L'administration de BCG a entraîné une sensibilisation indiscutable de cobayes à la Vieille Tuberculine, au PPD et à la lépromine. Les réactions de réactivation étaient légèrement plus prononcées que les réactions de positivité.

Les points les plus élevés des courbes de positivité obtenues étaient les suivants: 48 heures pour l'épreuve à la tuberculine, 72 heures pour l'épreuve au PPD et 7 jours pour l'épreuve à la lépromine.

La réaction à la lépromine chez les cobayes témoignait d'une durée plus courte que chez les êtres humains.

La sensibilisation ainsi induite chez les cobayes variait en fonction du type de BCG et de son mode d'administration.

La sensibilisation la plus forte à la tuberculine a été obtenue dans le groupe D (BCG Moreau Oral).

La sensibilisation par le PPD s'est révélée la plus élevée dans le groupe B (BCG intradérmique Glaxo).

La sensibilisation par la lépromine était très faible dans le groupe B (BCG intradérmique Glaxo); elle était très élevée dans le groupe D (BCG Oral Moreau) et la plus élevés dans le groupe C (BCG intradérmique Moreau).

Les réactions à la lépromine sont apparues les plus persistantes dans les groupes C et D qui avaient reçu du BCG Moreau; pour le groupe D (BCG oral Moreau); les réactions étaient même encore plus persistantes que dans le groupe C. (BCG intradérmique Moreau).

L'administration de BCG oral devrait être recommandée pour la prophylaxie de la lèpre et de la tuberculose dans les pays en voie de développement, vu qu'elle est plus facile et plus économique.

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