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B C G Vaccination

Comparative Study of the Lepromin Response (Mitsuda-Test) to the Oral and Intradermal Routes of Administration of the Vaccine[1]

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On the basis of personal experience the senior author (1) of this paper proposed to the International Congress of Leprosy, Havana, 1948, the use of BCG vaccination in the prophylaxis of leprosy. The proposal was rejected. Later, in Buenos Aires, at the III Conferencia Pan-americana de la Lepra (2), 1951, BCG vaccination was recommended in the prophylaxis of leprosy. Since then all International Leprosy Congresses have recommended that measure. In spite of that, there still is no agreement among leprologists regarding the efficacy of that measure.

There is no doubt that lepromin reactors are resistant to leprosy infection. It is known also that lepromin conversion is obtained by BCG vaccination. In several papers (3–8) the senior author has demonstrated in newborns, in arrested leprosy patients, and in animals the influence of BCG vaccination in lepromin sensitivity.

In a recent experimental paper (9) we made a comparative study of the different routes (oral and intradermal) of BCG vaccination in guinea-pigs, and demonstrated that the two routes are equivalent in their action. This paper has the same aim regarding the newborns.

In developing countries the oral method of BCG vaccination is easier to perform, for these countries generally do not provide sufficient technical means and trained personnel for correct intradermal vaccination. This is true not only in the vast rural regions, where at least half of the Latin-American populations lives, but also in the urban areas. This fact is especially valid for the vaccination of newborn infants, whose protection has priority in almost all developing countries, on account of their epidemiologic situation.

The immunologic and allergic responses prove the effectiveness of a vaccination method independently of its purpose; i.e., whether the vaccination was intended to protect against leprosy or against tuberculosis. For this reason, in the following, all vaccination trials are considered as equivalent.

The vast experience in oral BCG vaccination in France and South America, especially in Brasil and Uruguay, shows that this method confers protection against tuberculosis. But the results described in these papers, almost all published in earlier years, are not in accord with modern statistical rules. For this reason, the efficiency of the oral method cannot be fully assessed.

The report made by WHO (16) to the Brasilian Government in 1966, the final conclusion (12) of the Seminario en el Centro Nacional de Lucha Antituberculosa, Argentina, and again the XVI Congreso Latinoamericano de Tuberculosis y Enfermedades del Aparato Respiratorio, Mexico, April 1969, agreed in the idea, expressed in No. 4 of the recommendations

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of the latter ([15]). "Taking into account the practical advantages of the oral dosage, the eventual evidence of this protective value, and the fact that it has been widely used for many years in several countries, it is worthwhile to promote a controlled, internationally supervised trial to elucidate the value of the oral vaccination in different age groups."

Such an investigation, performed by the Medical Research Council in Britain ([14]) confirmed the efficacy of BCG vaccination against tuberculosis in man by intradermal vaccination. In this study more than 50,000 participants were observed during 15 years, showing a protection of nearly 80 per cent for at least ten years.

For a similar trial to determine the degree of protective effect of the oral method, the previously mentioned WHO investigation suggested a study population of 200,000 to 600,000 subjects and an observation time of ten years. Such trial obviously could be performed only in a developing country, as the follow-up of the very fluctuant populations in vast territories is difficult, and, first of all, national or international funds for such an enormous trial are not available. So this kind of trial is impracticable.

A controlled trial of BCG vaccination against leprosy is under way in Uganda ([11]). More than 17,000 children are under observation. The follow-up during three and a half years showed a reduction of 87 per cent in leprosy incidence. Another trial is being made in Burma, under WHO control. A three year observation period report ([10]) shows no significant effect of BCG vaccine. A longer period of observation is necessary for final conclusions.

Common problems present in the practice of BCG vaccination are determination (1) if one batch of a specific vaccine confers the same protection as another, (2) if a strain has still the same qualities as before or the same as another, and (3) if one method or route of vaccination is as effective as others (intradermal introduction by needle, by jet-injector and by puncture). These questions can be answered in a strict scientific sense by trials in a statistically sufficient number of people and over a sufficiently long period to observe the morbidity in vaccinated and nonvaccinated groups. But such scientific long-term large trial is economically impracticable and its response (after ten or more years) is much delayed. For this reason short comparisons of known standard strains or known standard vaccination methods are made to determine the qualities of unknown batches, strains, or methods.

The basic idea is to compare, first in animals, the residual virulence, the vaccination lesions, the allergy conferred, and the resistance to virulent superinfection. These experiments are followed by observations made in people vaccinated in the same manner (the same strain and the same method as in animals). Vaccination lesions and the development of allergy observed in man are correlated with the findings in animals. If they correspond, the data collected permit conclusions about the protective value of strain or vaccination methods in man.

As the problem of the effectiveness of the oral method does not differ in any way from the problem mentioned, the same ways of investigation should be applied for the comparison of oral and intradermal vaccination. If the study population is formed by newborns, who are undoubtedly free from any leprosy, tuberculous, or other mycobacterial infections before the vaccination, the size of the population can be reduced. As tuberculous infection evolves rapidly to disease in newborns and small children, the morbidity can be studied in a short period. The same does not occur in leprosy, which has a very long incubation period.

The purpose of the present study was to show a practicable scheme to evaluate the effect of oral BCG vaccination in newborns. Animal experiments and vaccination of newborns were performed, using the same vaccines in corresponding groups (oral and intradermal vaccinated and controls). The results of the animal experiments have been published separately ([9]). The tuberculin allergy observed in the first five months after vaccination of newborns is described in a separate paper ([13]). The present study reports the influence of the two vaccination methods on lepromin con-

version. The study was made in the Hospital Universitário Antonio Pedro, Faculdade de Medicina da Universidade Federal in Niterói, Rio de Janeiro, Brasil. The maternity clinic was used only for a section of the poor population, living almost entirely in unfavorable epidemologic conditions. The groups of vaccines and controls were chosen at random.

MATERIALS

Vaccines. (a). For intradermal vaccination: Freeze-dried BCG vaccine, batch B 818j, manufactured October 1967 (expiration October 1968), by Glaxo Laboratories Ltd., Greenford, England. (b). For oral vaccination: Liquid BCG vaccine, prepared with the BCG-Moreau-Rio de Janeiro strain by the Instituto Viscondessa de Moraes of the Fundação Ataulpho de Paiva, Rio de Janeiro, Brasil. Seventeen different batches were used.

Antigens. (a). PPD/Rt23 received from the Statens Serum Institut, Copenhagen, and diluted to 2TU by the Serviço Nacional de Tuberculose, Rio de Janeiro. (b). Old Tuberculin produced by the Instituto Oswaldo Cruz of the Health Ministry, Rio de Janeiro, batch No. 41, manufactured October, 1967 and diluted to 1/100 by the Instituto Viscondessa de Moraes (c). Integral lepromin produced by the Serviço Nacional de Lepra, Rio de Janeiro.

Utensils. Syringes Microstat Tuberculin USA of 1 ml. capacity and needles 18-8 stainless UNICEF No. 26 were used for the intradermal vaccination and the postvaccination tests. The oral vaccine was given by nipples, adapted directly to the vaccine vials. Needles and nipples were used only once and sterilized before further use. Different syringes, needles and containers were used for each special purpose (intradermal vaccination, PPD test, OT test, lepromin test).

METHODS

The vaccinations were performed between 5 December 1967 and 8 May 1968 by two of the hospital's nurses (intradermal) and two vaccinators of the state's BCG service (oral). They were supervised by the authors. The freeze-dried vaccine was suspended in saline, produced by the Laboratório Granada, Rio de Janeiro. (1.0 ml. saline for each ampule, as prescribed by Glaxo). The dose recommended by Glaxo for newborns (0.05 ml.) was injected into the skin of the left deltoid region. The oral dose was a 5 ml. suspension containing 100 mgm. BCG, as generally recommended and used in Brasil. The newborns were vaccinated between the 24th and 48th hour of life. The selection for the three groups of newborns,[6] intradermal vaccination, oral vaccination, and unvaccinated controls, was at random and made in the following manner. The newborns, elegible on the first vaccination day, received intradermal BCG vaccine. The same number of children born next day were given oral BCG vaccination. The following identical number were left without vaccination. This scheme was modified, when the control group consisted of 249 children. Following April 6 no more newborns were left unvaccinated. The vaccinations then continued alternately via intradermal and via oral routes until 989 children[7] formed the study population, 366 in the intradermal group, 374 in the oral group, 249 in the control group.

All mothers were informed that their babies had been vaccinated, and asked to come to the hospital's outpatient clinic 14 days after the birth. On this occasion the existence and size of local vaccination lesions and lymphnodes were observed; this type of observation was repeated every time the children came to the clinic.

Postvaccination tests were made between February 2 and December 12, 1968. The nurse who worked during the first three weeks was no longer available. She was replaced by an attendant from the state's tuberculosis service, prepared for this work, so that (with exception of some of the first two months tests) all tests were executed and read by the same person. She

[6] Premature and sick infants and those who lived so far away from the hospital that follow-up observation was impossible, were excluded.

[7] The study population was formed originally by more than 1,000 newborns, but some cases were eliminated after their first visit (14 days after birth) when conditions were discovered, which would have excluded their participation from the beginning (distance of residence, contact or suspect of contact in the control-group, etc.).

was supervised by the authors. The PPD was injected intradermally on the superior third and the OT on the middle third of the inner side of the left forearm. Lepromin was injected intradermally on the superior-side of the right forearm.

Two months after birth the children were submitted to the first testing. Initially 0.1 ml. PPD/2 TU was injected. If induration was absent or measured only 1 to 4 mm. in diameter, a second test with 0.1 ml. OT-1/100 was performed. If induration was again absent or measured only 1-4 mm. in diameter, the child had to come back one month later (i.e., three months after birth). On this occasion it was submitted to the same tests as a month before. If both tests gave again no reactions or only 1 to 4 mm. indurations, the child had to repeat the same procedure two months later (i.e., five months after birth). The reactions were observed after 48 to 96 hours, usually after 72 hours. When the reactions showed absence of induration or indurations measuring only 1 to 4 mm. in diameter, five months after birth or later[8], the child was considered as a nonreactor.

Once a test with PPD/2 TU or with OT-1/100 provoked an induration which measured 5 mm or more, no other test was performed and the child was considered as a reactor, so that reactors resulted two months, three months and five months after birth or even later.

The same day on which the child received the first PPD/2 TU test, the lepromin also was injected (0.1 ml.). The reactions to lepromin were recorded after 48 to 96 hours, usually after 72 hours, and again after 21 days. The reactions were designated negative when, 21 days after injection, indurations measured 1 or 2 mm. Indurations of 3 mm. and more were considered as positive reactions. If the lepromin reaction was negative, the test was repeated one month later (three months after birth), and when again negative another test was made two months later (five months after birth.) A child was considered negative to lepromin only when the test applied five months after birth, or even later, resulted negatively in the 21 day reading. Once a test provoked an induration which measured 3 mm. or more in a 21 day reading, no other test was performed and the child was considered positive to lepromin.

As the children had to appear many times in the hospital (the non-reactors 13 times) a relatively small number finished the test series, so that from a population of 989 newborns the data could be evaluated only for the following cases:

	Lepromin test	Tuberculin test
Oral group	141	148
Intradermal group	157	154
Control group	92	95

The difference in the total number of cases in the lepromin test and the tuberculin test is explained by the fact that the two tests were not completed in all cases. The mothers were tested with the same antigens simultaneously.

RESULTS

Table 1 shows the size of reactions to lepromin (Mitsuda test) in the vaccinated groups, and Table 2 in the control group. Tables 3 and 4 continue these results for the vaccinated groups, representing children with infiltrations of 0 to 2 mm. as nonreactors and with 3 mm. and more as reactors. (Table 3: number of cases, table 4: percentages). Table 5 gives the same results in the control group. The lepromin test performed in 411 mothers gave 74.48 per cent of reactors.

All tables indicate also the time of conversion, after two, three, and five months for the reactors. Nonreactors are only children who still did not react five months after birth.

Table 6 and Figs. 1 and 2 illustrate the size of reactions, not considering the conversion time. Fig 3 illustrates the accumulated percentages of converters, two, three and five months after vaccination. The curves represent the reactors of the oral

[8] A few children could be tested only six to nine months after birth, because of their health condition or because they did not appear earlier.

TABLE 1. *Size of reactions to Mitsuda test in children vaccinated at birth with BCG.*

Diameter of infiltration in mm.	*Oral* route: Number of children completed at months				*Intradermal* route: Number of children completed at months			
	2	3	5	Total	2	3	5	Total
0	—	—	4	4	—	—	7	7
1	—	—	—	—	—	—	2	2
2	—	—	—	—	—	—	3	3
3	17	7	12	36	24	15	16	55
4	11	7	4	22	10	4	8	22
5	17	6	6	29	18	10	14	42
6	6	9	3	18	3	4	8	15
7	2	6	2	10	1	—	3	4
8	6	1	1	8	—	3	1	4
9	2	1	—	3	—	—	—	—
10	1	—	2	3	1	—	1	2
11	—	—	—	—	—	—	—	—
12	1	—	2	3	—	—	—	—
13	—	—	—	—	—	—	—	—
14	—	2	—	2	—	—	1	1
15	—	1	—	1	—	—	—	—
16	—	1	—	1	—	—	—	—
Total	63	41	37	141	57	36	64	157
Mean size			mm. 5.220				mm. 4.172	
Standard error			mm. 2.740				mm. 1.899	

TABLE 2. *Size of reactions to lepromin (Mitsuda test) in nonvaccinated children.*

Diameter of infiltration in mm.	*Control* group: Number of children completed at months			
	2	3	5	Total
0	—	—	31	31
1	—	—	7	7
2	—	—	2	2
3	5	10	24	39
4	—	—	—	—
5	1	1	7	9
6	—	—	2	2
7	—	—	—	—
8	2	—	—	2
Total	8	11	73	92
Mean size			mm. 2.185	
Standard error			mm. 1.956	

TABLE 3. *Time of conversion to lepromin (Mitsuda test) in children vaccinated with BCG at birth. Number of cases.*

Oral route

	Number of children completed at months			
	2	3	5	Total
Reactors				
At the first test performed	63	25	22	110
Tested before (at 2 and/or 3 months)	—	16	10	26
Subtotal	63	41	32	136
Nonreactors				
At the first test performed	—	—	3	3
Tested before (at 2 and/or 3 months)	—	—	2	2
Subtotal			5	5
Total				141

Intradermal route

	Number of children completed at months			
	2	3	5	Total
Reactors				
At the first test performed	57	23	35	115
Tested before (at 2 and/or 3 months)	—	13	17	30
Subtotal	57	36	52	145
Nonreactors				
At the first test performed	—	—	5	5
Tested before (at 2 and/or 3 months)	—	—	7	7
Subtotal			12	12
Total				157

TABLE 4. *Time of conversion to lepromin (Mitsuda test) in children vaccinated with BCG at birth. Percentages.*

Oral route

	Percentage of children completed at months			
	2	3	5	Total
Reactors				
At the first test performed	44.68	17.73	15.60	78.01
Tested before (at 2 and/or 3 months)	—	11.35	7.09	18.44
Subtotal	44.68	29.08	22.69	96.45
Nonreactors				
At the first test performed	—	—	2.13	2.13
Tested before (at 2 and/or 3 months)	—	—	1.42	1.42
Subtotal			3.55	3.55
Total				100%

Intradermal route

	Percentage of children completed at months			
	2	3	5	Total
Reactors				
At the first test performed	36.31	14.65	22.29	73.25
Tested before (at 2 and/or 3 months)	—	8.28	10.83	19.11
Subtotal	36.31	22.93	33.12	92.36
Nonreactors				
At the first test performed	—	—	3.18	3.18
Tested before (at 2 and/or 3 months)	—	—	4.46	4.46
Subtotal			7.64	7.64
Total				100%

TABLE 5. *Time of conversion to lepromin (Mitsuda test) in the nonvaccinated control group.*

Number of cases

	Number of children completed at months			
	2	3	5	Total
Reactors				
At the first test performed	8	1	20	29
Tested before (at 2 and/or 3 months)	—	10	13	23
Subtotal	8	11	33	52
Nonreactors				
At the first test performed	—	—	24	24
Tested before (at 2 and/or 3 months)	—	—	16	16
Subtotal			40	40
Total				92

Percentage of cases

	Percentage of children completed at months			
	2	3	5	Total
Reactors				
At the first test performed	8.70	1.09	21.74	31.53
Tested before (at 2 and/or 3 months)	—	10.86	14.13	24.99
Subtotal	8.70	11.95	35.87	56.52
Nonreactors				
At the first test performed	—	—	26.09	26.09
Tested before (at 2 and/or 3 months)	—	—	17.39	17.39
Subtotal			43.48	43.48
Total				100%

and the intradermal groups. These accumulated percentages are summarized in Table 7.

The collected data show that the lepromin and tuberculin conversions are higher in the vaccinated groups than in the unvaccinated controls, as shown in Table 8. The conversion rates to lepromin in both vaccinated groups are almost equal: 96.45 per cent (oral) and 92.36 per cent (intradermal). The conversion to lepromin obtained by the oral vaccination was a little earlier and somewhat more intense.

Statistical analysis of the data shows that

the differences between the results in both vaccinated groups in relation to the control group are significant. The same analysis applied to the difference between the two vaccinated groups shows no statistical significance for the results corresponding to two and five months. Significant differences are shown between the two groups in three months.

It must be emphasized that 74.48 per cent of the mothers (adults) were lepromin reactors, while the vaccinated babies (five months old) showed a higher percentage (96.45 per cent in the oral group and 92.36 per cent in the intradermal group). This demonstrates again the influence of BCG vaccination on lepromin reactivity.

TABLE 6. *Size of reactions to lepromin (Mitsuda test). Induration of 3 or more mm.*

Induration in mm.	*Oral* Cases	*Oral* Percentage	*Intradermal* Cases	*Intradermal* Percentage	*Control* Cases	*Control* Percentage
	141	100%	157	100%	92	100%
3	36	25.53	55	35.03	39	42.39
4	22	15.60	22	14.01	—	—
5	29	20.57	42	26.75	9	9.78
6	18	12.76	15	9.55	2	2.175
7	10	7.09	4	2.55	—	—
8	8	5.67	4	2.55	2	2.175
9	3	2.13	—	—	—	—
10	3	2.13	2	1.28	—	—
11	—	—	—	—	—	—
12	3	2.13	—	—	—	—
13	—	—	—	—	—	—
14	2	1.42	1	0.64	—	—
15	1	0.71	—	—	—	—
16	1	0.71	—	—	—	—
Total	136	96.45	145	92.36	52	56.52

TABLE 7. *Mitsuda Test. Time of conversion after BCG vaccination and accumulated percentages of converters.*

Time after vaccination	Oral group	Intradermal group
2 months	44.68	36.31
2 and 3 months	73.76	59.24
2, 3 and 5 months	96.45	92.36

DISCUSSION

The present study compared the lepromin conversion after routine oral BCG vaccination, as practiced in Brasil, with the conversion rate induced by an intradermal vaccine recognized as efficient. Simultaneous observation of a control group was considered necessary, in order to know the spontaneous conversion rate in the population studied. Table 9 shows the percentages of reactors in the control group during the first five months after birth.

The high spontaneous positivity to lepromin in this age group is surprising. The high percentage of reactors in the control group could raise the suspicion that the results might have been influenced by the repetitions of the lepromin test. Of 141 infants in the oral group only five remained nonreactors; three of them were tested once and only one twice and one three times. Ten of the reactors were tested once and only 26 twice or three times. Of the 157 infants of the intradermal group 12 remained nonreactors, five of them tested once and seven tested three times; 115 reactors were tested once and only 30 reactors twice or three times. Of the 92 infants in the control group, 40 remained nonreactors, 24 of them tested once and 16 tested twice or three times; 19 reactors were tested once and 23 reactors twice or three times.

These figures suggest that the repetition of the lepromin test had no influence on the results. The same conclusion is reached by comparison of the mean size of induration observed in the subgroups tested only once

TABLE 9. *Time conversion to lepromin in the control group.*

Time of conversion	Number	Percentage
At 2 months	8	8.70
At 3 months	11	11.95
At 5 months	33	35.87
Total	52	56.52

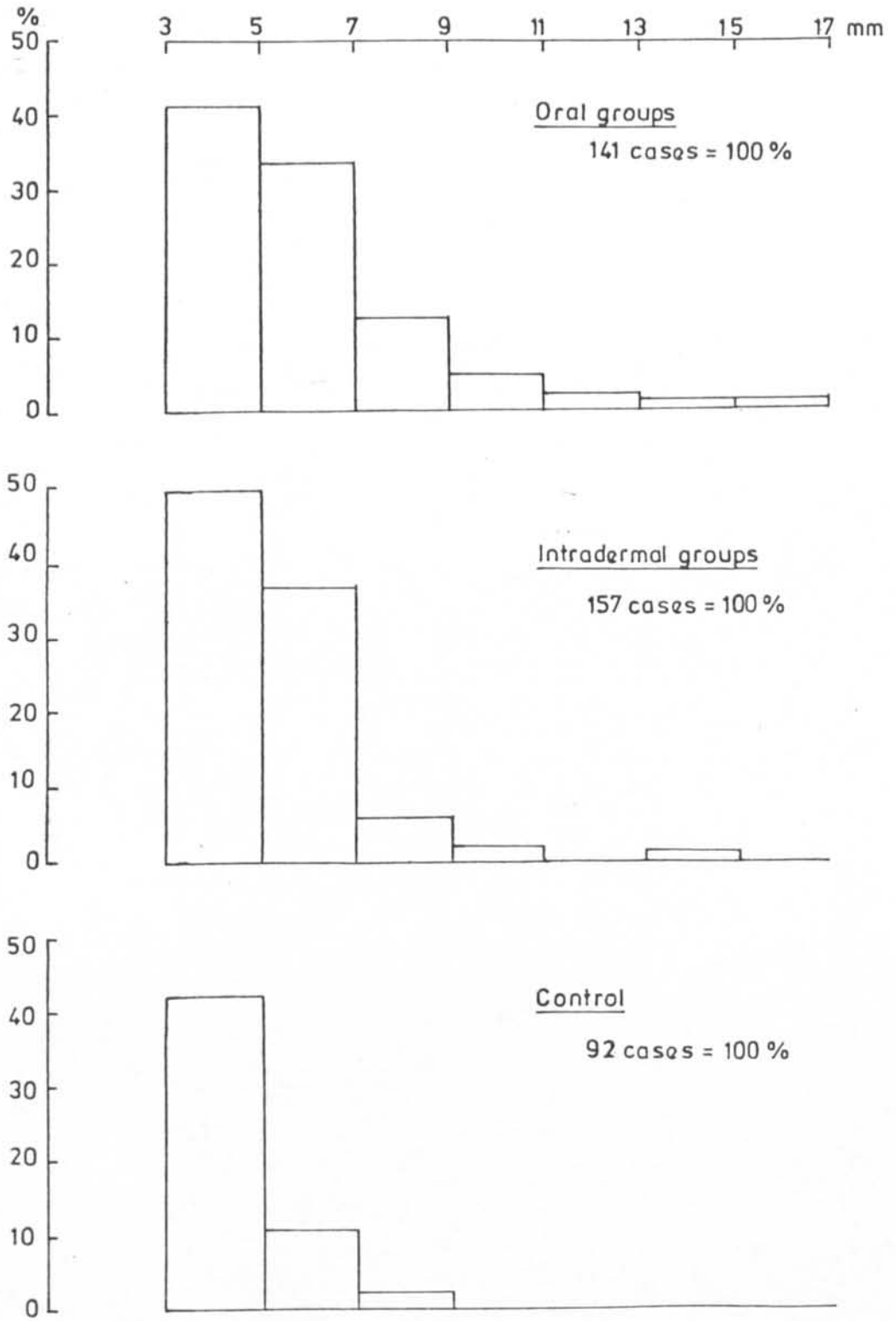

FIG. 1. Size of reactions to lepromin (Mitsuda test). Induration of 3mm and more.

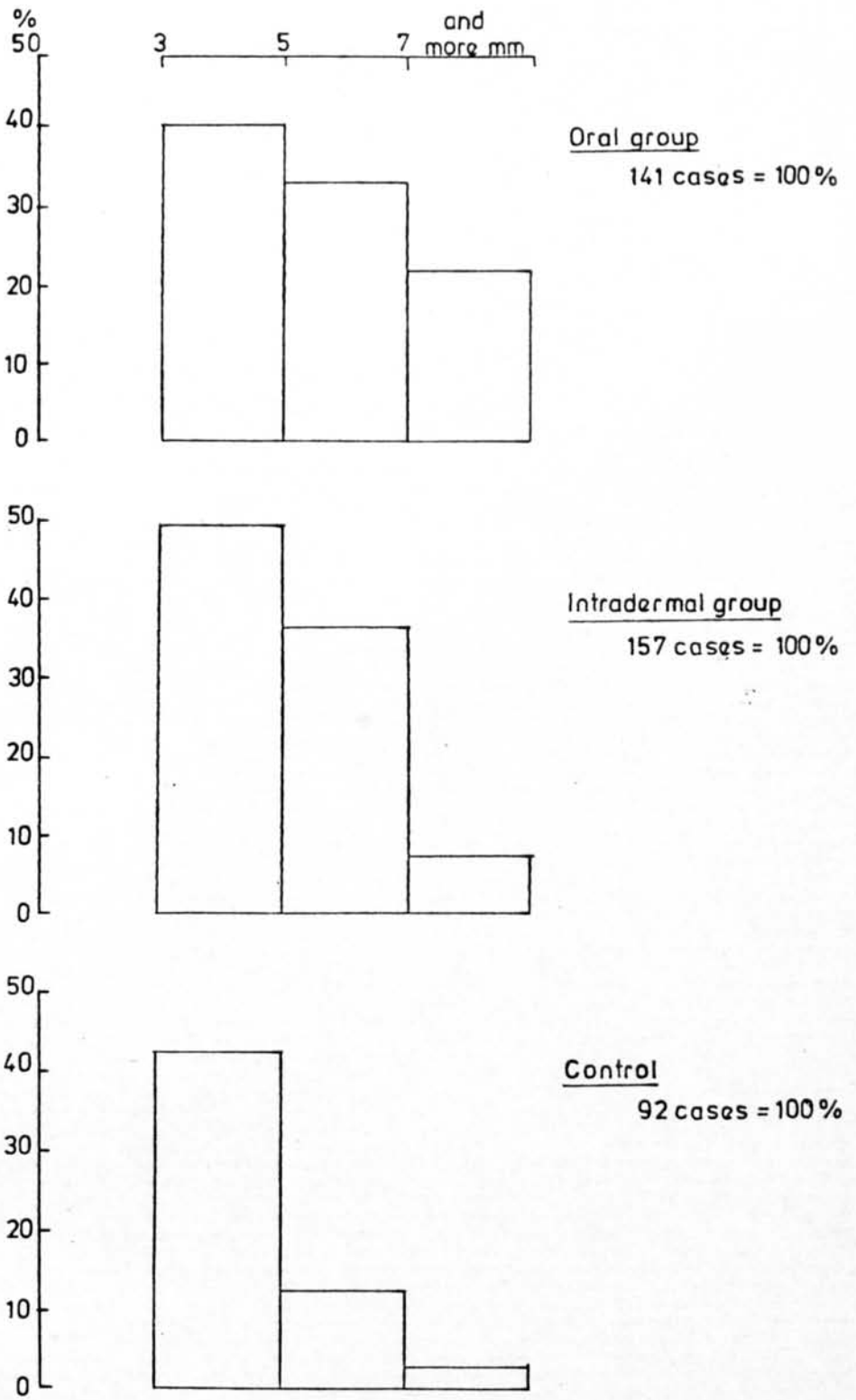

FIG. 2. Size of reactions to lepromin (Mitsuda test). Induration of 3mm and more.

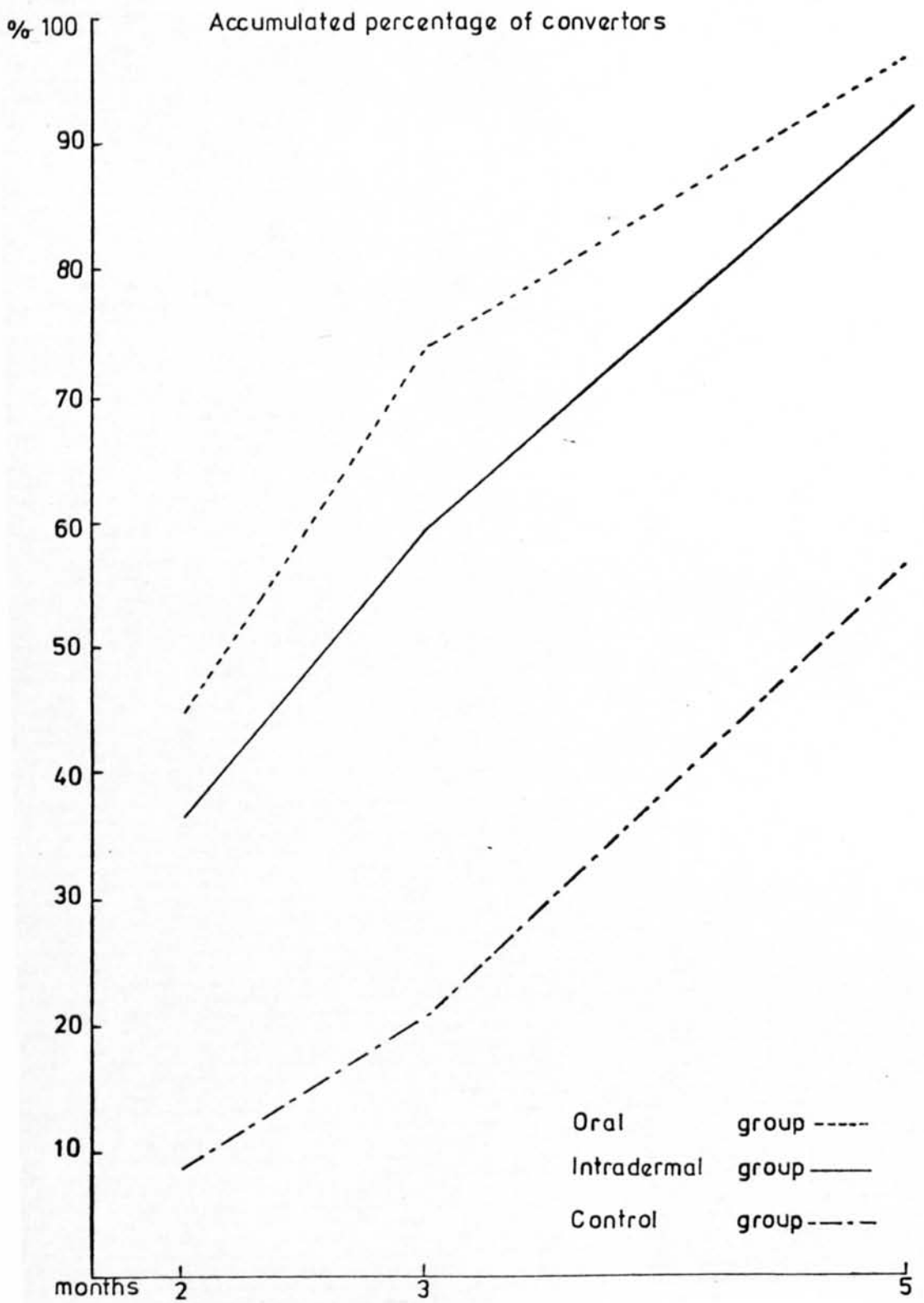

FIG. 3. Time of conversion (Mitsuda test).

TABLE 8. *Lepromin and tuberculin conversion after BCG vaccination by oral and intradermal routes.*

Groups	Tests	Total number	Number of converters	Percent of converters
Oral	Lepromin	141	136	96.45
	Tuberculin	148	121	81.76
Intradermal	Lepromin	157	145	92.36
	Tuberculin	154	117	75.97
Control	Lepromin	92	52	56.52
	Tuberculin	95	15	15.79

TABLE 10. *Comparison of mean size of lepromin reactions in all children (reactors and nonreactors) tested once and two or three times.*

Group	Tested once		Tested two or three times	
	Cases	Mean size	Cases	Mean size
Oral	113	5.425	28	4.393
Intradermal	120	4.375	37	3.514
Control	53	2.264	39	2.077

TABLE 11. *Comparison of mean size of lepromin reactions in children (reactors only) tested once and two or three times.*

Group	Tested once		Tested two or three times	
	Cases	Mean size	Cases	Mean size
Oral	110	5.572	26	4.612
Intradermal	115	4.539	32	4.219
Control	29	3.897	23	3.348

and the subgroups tested two or three times. Table 10 shows the mean size calculated for all infants (reactors and nonreactors).

Table 11 shows the mean size calculated only for the positive cases (infants who had 3 mm. or more induration in the lepromin test).

Both tables demonstrate the absence of any increase in the indurations due to repetition of tests, even showing mean sizes always smaller in the subgroups tested two or three times than in those tested only once. Therefore the high percentage of reactors to lepromin among control groups is probably due to natural infection either

with *Mycobacterium tuberculosis, Mycobacterium leprae* or other mycobacteria.

SUMMARY

In the maternity of the University's hospital in Niterói, Brasil, two groups of newborns were vaccinated with BCG, one by the oral, the other by the intradermal method, while a third group remained unvaccinated (control group).

Lepromin conversion was observed during the first five months of life. The results of the vaccination methods were equivalent: 96.45 per cent (136 cases) converters in the oral group and 92.36 per cent (145 cases) in the intradermal group. The control showed 56.51 per cent (52 cases) of conversion.

The mean sizes of the reactions were: 5.22 mm. for the oral group, 4.17 mm. for the intradermal group and 2.18 mm. for the control group.

The results of this experience show (1) that BCG vaccination induces lepromin-positivity, and (2) that both types of vaccination (oral and intradermal) are equivalent. This is very important for the developing countries because of the fact that the oral administration of BCG is easier and cheaper than the intradermal one.

This study confirms the South American experience with oral BCG vaccination, and suggests further experience and studies to obtain results which will permit the valuation of vaccination methods in a reasonably short time.

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