REACTIVITY OF CHILDREN TO LEPROMIN AND VARIOUS TUBERCULINS, AS AFFECTED BY RECENT AND OLDER BCG VACCINATION

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The development of reactivity to lepromin in a considerable proportion of children following vaccination with BCG, first reported by Fernandez (4) in 1939, has been amply confirmed by others and may be accepted as definitely established. At the time of the review of the subject by Souza Campos (10) in 1953, the literature was already extensive and it is much more so today. The observation has attained great practical importance because of the growing opinion that reactivity of an individual to lepromin, natural or acquired, is an indication of some degree of resistance, sufficient at least to protect against development of the lepromatous type of leprosy, and because of the consequent emphasis on BCG vaccination as a method of prophylaxis.

Retests with lepromin are usually made within a short time after vaccination. The possibility of subsequent reversion to negativity does not appear to have been studied adequately, chiefly because it is difficult to keep a considerable number of the vaccinated subjects under observation for a long period of time. Even if this were to be done, the observation would be subject to different interpretations unless a comparable group of unvaccinated controls was also studied. Reactivity to lepromin increases rapidly in childhood without the benefit of BCG vaccination.

PRESENT STUDY

In the course of an inquiry into the causes of natural reactivity to lepromin among schoolchildren in Cebu, Philippines (9) commenced in January, 1961, it was found that an appreciable number had been vaccinated intradermally with BCG in November 1960, less than four months on the average before they were tested in our study. These children had fresh BCG scars and some even showed persisting ulceration. It was also definitely established, from the presence of older BCG scars in all instances, supplemented by histories obtained from the teachers, some of the parents, and the children themselves, that other children had been vaccinated similarly from one and a half years to more than four years previously. All the vaccinations had been made with fresh BCG by the teams of the Department of Health of the Philippine government. It was therefore possible to divide the children of the study, for a comparison of reactivities to the same stock of lepromin and of each of various tuberculin preparations, into three groups: (*a*) those not vaccinated with BCG, they constituting the majority; (*b*) those with recent vaccinations, and (*c*) those with older vaccinations.

The test subjects numbered in total 917 children, 7 to 10 years of age, in Grades I and II of the two public elementary schools in the towns of Opon and Talisay, Cebu. The material and procedures of the study have already been described (9). It is necessary to

repeat here only that all the children were given lepromin tests, using a lepromin prepared by the Mitsuda-Hayashi method, and that approximately one-half were tested with each of three PPD-S tuberculins prepared, respectively, from a strain of *M. tuberculosis* of human origin, one of *M. avium,* and a third from an unclassified mycobacterium known as the "Battey" strain, also of human origin. All three tuberculins were given in the same dosage, 0.0001 mgm. (5 TU).

The injections of the tuberculin tests were given in the right forearm, and reactions were read after 72 hours. Lepromin test injections were given in the left forearm; early (Fernandez) reactions were measured at 24, 48 and 72 hours, and late (Mitsuda) reactions at 14, 21, 28, 32 and 43 days. Tuberculin reactions of 6 mm. or larger were regarded as positive; as were, respectively, early lepromin reactions of 10 mm. or more, and late (nodular) lepromin reactions larger than 4 mm. in size. All the tests and readings were made by two of us (RSG and MCM), in approximately equal proportions.

RESULTS

LEPROMIN TESTING

The lepromin reactivity of the children in relation to their vaccination status is shown in Table 1. The early reactions were relatively infrequent as compared to the late reactions, which has been the experience in all of our lepromin studies in Cebu, especially in children.

The highest frequency of early reactions occurred in the children who were recently vaccinated, 25.3 per cent, as compared with 16.9 per cent in those with older vaccinations and 14.7 per cent in those never vaccinated. The difference in the frequency of early lepromin reactions between the unvaccinated and the recently vaccinated was appreciable; that between the unvaccinated and those with older vac-

TABLE 1.—*Lepromin reactivity of Cebu children, 7 to 10 years of age, in relation to BCG vaccinations (none, recent or older).*[a]

Vaccination status	Number of children tested	Per cent positive to lepromin[a]	
		Early (Fernandez) reaction	Late (Mitsuda) reaction
No BCG vaccination, positive to same type of tuberculin[b]	235	28.2	87.0
No BCG vaccination, negative to same type of tuberculin[b]	342	5.4	63.7
No BCG vaccination	577	14.7	73.3
With old BCG vaccination (1½ or more years prior to present test)	194	16.9	77.8
With recent BCG vaccination (4 months prior to present test)	146	25.3	93.4
Total children in study	917	17.3	76.8

[a]All percentages have been adjusted to the age distribution by single years of age of the 917 children in the study.

[b]Children positive or negative to one or two of the following PPD preparations: human PPD, avian PPD and Battey PPD all in the same dose of 0.0001 mgm. or 5 TU.

cinations, however, was much smaller and not significant in the statistical sense.

The late lepromin (Mitsuda) reactions, as expected, were most frequent among the recently vaccinated children (93.4%). The frequency of late reactions in the children with older vaccinations (77.8%) was well below that in the newly vaccinated, but was above that in the group of unvaccinated children taken as a whole (73.3%).

The two groups of BCG vaccinated children were presumably tuberculin-negative at the time of vaccination. Tuberculin-positive children are more likely to be lepromin positive than those who are tuberculin negative. The vaccinated groups may therefore not be strictly comparable to the unvaccinated group as a whole.

When tested in our study the latter included about 25 per cent of children who were naturally tuberculin positive, and it must necessarily also have included a certain proportion of children (less than 25%) who were already tuberculin reactors at the time the other children were vaccinated. As shown in Table 1, however, when the tuberculin positives of the unvaccinated group are excluded and the comparison is made only with the lepromin reactivity of current tuberculin negatives, the situation is not greatly changed. The frequency of late lepromin reactors among these negatives was found to be 63.7 per cent, a percentage reduction of 9.6 per cent from the figure for the entire unvaccinated group.

Thus if it be assumed that BCG vaccination had effected the lepromin conversion of a high proportion of the vaccinated children, which is a fair assumption, it follows that reactivity to lepromin so induced was lost to an appreciable extent within a few years.

The children in the study were limited to those who were from 7 to 10 years of age. The vaccinated and unvaccinated groups were comparable in respect to age and sex except that the recently-vaccinated children on the average were somewhat younger than the others. The lepromin and tuberculin frequencies have been adjusted for this slight variation, using the distribution of all the children by single years of age as a standard. Thus they are not the actual or observed frequencies, but are what would have been expected if all the groups had the same proportions of children 7, 8, 9 and 10 years old, respectively.

Retesting of these children in the future would be desirable in order to learn whether there is actually a permanent gain in reactivity to lepromin attributable to BCG vaccination, or only an acceleration of the normal rate at which children naturally acquire such reactivity. Even the latter effect, however, may be advantageous; conceivably, the level of immunity is raised at a time when most leprosy infections take place.

TUBERCULIN TESTING

The tuberculin results are given in Table 2. The children were originally assigned at random to six groups for testing, respectively, with human, avian and Battey tuberculins, singly, and with combinations of two of these three PPD preparations. Thus, as noted above, about one-half of all the children in the study were tested with each preparation. Inspection of Table 2 shows that, with the exception of the somewhat larger number with old BCG vaccinations tested with Battey PPD (110), the number of recently vaccinated children and of those with older vaccinations did not vary greatly as between the groups given the different tuberculins.

TABLE 2.—*Reactivity of Cebu children, 7 to 10 years of age, to various tuberculins in relation to recent and older BCG vaccinations.*[a]

Vaccination status	Human PPD (0.0001 mgm.)		Avian PPD (0.0001 mgm.)		Battey PPD (0.0001 mgm.)	
	No. tested	Per cent positive	No. tested	Per cent positive	No. tested	Per cent positive
No BCG vaccination	293	24.7	287	37.3	286	45.4
With old BCG vaccination[b]	93	30.1	90	45.3	110	53.4
With recent BCG vaccination[c]	73	73.1	78	72.0	71	75.7
Total tested	459	32.3	455	45.1	467	51.5

[a]All percentages in this table have been adjusted to the age distribution by single years of age of the 917 children in the study.
[b]Vaccinated 1½ years or more before the present tests.
[c]Vaccinated 4 months before the present tests.

The unvaccinated children reacted with greater frequency to the Battey (45.4%) and avian (37.7%) than to the human (24.7%) tuberculin. Among those recently vaccinated with BCG, all three tuberculins showed significant gains in reactivity, i.e., 75.7 per cent for the Battey, 72.0 for avian, and 73.1 for human PPD. Thus the greatest relative increases were seen with the tuberculins prepared from the human and avian strains, especially the human one.

As with reactivity to lepromin, the proportions of the children with older BCG vaccinations who reacted to the three tuberculins were, in each case, appreciably lower than for those with more recent vaccinations, although the frequencies were still somewhat higher than for the unvaccinated children. This finding appears to confirm the prevailing opinion that reactivity to tuberculin induced by BCG tends to wane within a relatively short period.

SUMMARY

A comparison is made of the reactivity of Cebu (Philippines) schoolchildren, 7 to 10 years of age, to lepromin and to various tuber-

culins, as apparently affected by BCG vaccination. These vaccinated children were incidentally included in a study of the causes of natural reactivity to lepromin. All children completing the study, numbering 917, were distributed into three groups: (*a*) those never vaccinated with BCG; (*b*) those vaccinated from three to four months before the study, and (*c*) those vaccinated one and a half or more years previously.

Regarding lepromin, the frequencies of early (Fernandez) reactions were: 25.3 per cent for children with recent BCG vaccinations, 16.9 per cent for children with older BCG vaccinations and 14.7 per cent for those never vaccinated. The corresponding percentages of positive late lepromin (Mitsuda) reactions were: 93.4 for those with recent vaccinations, 77.8 for those with older vaccinations and 73.3 for those without any vaccination. Among the unvaccinated children, the frequency of late lepromin reactions was 87.0 per cent for natural tuberculin positives and 63.7 per cent for tuberculin negatives.

The tentative conclusion is reached that lepromin reactivity induced by a single BCG vaccination is lost to an appreciable extent within a few years. Even a temporary gain, however, may be beneficial if resistance can be increased during the critical ages of childhood.

Regarding the tuberculins, in the design of the original study about one-half of the children were selected at random for testing with each of the three PPD-S tuberculins, prepared respectively from a human strain of *M. tuberculosis,* a strain of *M. avium,* and the "Battey" strain. The recently-vaccinated children showed significantly higher percentages of reactors to each of these tuberculins than did the unvaccinated group. The most notable increase was seen in the reactions to the tuberculin from the human strain of *M. tuberculosis.* The children with old vaccinations showed only slightly higher frequencies than did the unvaccinated children. Thus our findings support the opinion that tuberculin conversions induced by BCG tend to wane within a few years.

RESUMEN

Se traza una comparación de la reactividad de los escolares de Cebú (Filipinas), de 7 a 10 años de edad, a la lepromina y a varias tuberculinas, según la afecta aparentemente la vacunación con BCG. Estos niños vacunados fueron incluídos incidentemente en un estudio de las causas de la reactividad normal a la lepromina. Todos los niños que completaron el estudio, ascendiendo a 917, fueron divididos en tres grupos: (*a*) los que jamás fueron vacunados con BCG; (*b*) los vacunados de tres a cuatro meses antes del estudio; y (*c*) los vacunados año y medio o más antes.

Con respecto a la lepromina, las frecuencias de las reacciones tempranas (Fernández) fueron: 25.3 por ciento para los niños con vacunaciones recientes con BCG, 16.9 por ciento para los niños con vacunaciones más antiguas con BCG y 14.7 por ciento para los jamás vacunados. Los porcentajes correspondientes de reacciones positivas tardías a la lepromina (Mitsuda) fueron: 93.4 por ciento para los vacunados recientemente, 77.8 por ciento para los que tenían vacunaciones más antiguas y 73.3 por ciento para los que jamás fueron

vacunados. Entre los niños no vacunados, la frecuencia de las reacciones tardías a la lepromina fué de 87.0 por ciento para los posittivos a la tuberculina natural y de 63.7 por ciento para los negativos.

Se alcanza la conclusión tentativa de que la reactividad a la lepromina inducida por una sola vacunación con BCG se pierde apreciablemente en término de pocos años. Sin embargo, hasta un aumento temporal puede resultar beneficioso si cabe acrecentar la resistencia durante edades críticas de la niñez.

Con respecto a las tuberculinas, en el plan del estudio primitivo se tomó al azar aproximadamente la mitad de los niños para comprobación con cada de las tres tubereulinas del PPD-S, preparados respectivamente de una cepa humana del *M. tuberculosis,* una cepa del *M. avium* y la cepa "Battey." Los niños recién vacunados mostraron porcentajes significativame te más altos de reactores a cada una de las tres tuberculinas que los del grupo no vacunado. El aumento más notable fué observado en las reacciones a la tuberculina derivada de la cepa humana de *M. tuberculosis.* Los niños con vacunaciones antiguas no monstraron más que frecuencias apenas más altas que las mostradas por los vacunados. Los hallazgos de los AA. apoyan, pues, la opinión de que los virajes de la reacción a la tuberculina inducidos por BCG tienden a menguar en término de pocos años.

RESUMÉ

Cette étude met en rapport la réactivité à la lépromine, à diverses tuberculines, et la vaccination par le B.C.G. chez des enfants des écoles de Cébu (Philippines) âgés de 7 à 10 ans. Ces enfants vaccinés furent à l'occasion incorporés dans une étude sur les causes de la réactivité naturelle à la lépromine. Tous les enfants envisagés à la fin de cette étude, soit 917 en tout, ont été distribués en trois groupes: a) ceux qui n'ont jamais été vaccinés par le B.C.G.; b) ceux qui ont été vaccinés trois à quatre mois avant l'étude; c) ceux dont la vaccination remonte à un an et demi ou plus.

En ce qui concerne la lépromine, la fréquence des réactions précoses (Fernandez) furent 16.9% pour les enfants ayant été vaccinés récemment, 16.9% pour les enfants dont les vaccinations par le B.C.G. datent de plus longtemps, et 14.7% pour ceux qui n'ont jamais été vaccinés. Les fréquences correspondantes de réactions tardives positives (Mitsuda) ont été: 93.4% pour ceux avec vaccination récente, 77.8% pour ceux avec vaccinations plus lointaines, et 73.3% pour ceux sans vaccination antérieure. Parmi les enfants non-vaccinés, la fréquence des réactions tardives à la lépromine fut 87.0% pour ceux réagissant spontanément à la tuberculine et 63.7% pour ceux qui ne réagissent pas à la tuberculine.

Les auteurs émettent l'hypothèse que la réactivité à lépromine survenue à la suite d'une vaccination unique par le B.C.G. se perd de façon notable en quelques années. Cependant,, même un accroissement temporaire de cette réactivité peur être avantageux s'il concourt à augmenter la résistance durant la période critique de l'enfance.

En ce qui concerne les tuberculines, la moitiè environ des enfants furent choisis au hasard, dans la première étude, pour être testés avec chacune des trois tuberculines PPD-S, préparées respectivement à partir d'une souche humaine de *M.tuberculosis,* d'une souche de *M.avium,* et d'une souche "Battey." Un pourcentage significativement plus élevé a réagi à chacune de ces tuberculines parmi les enfants récemment vaccinés que parmi les non-vaccinés. L'augmentation la plus notable fut constatée qour les réactions à la tuberculine provenant de la souche humaine de *M.tuberculosis.* Les enfants avec des vaccinations plus lointaines ont témoigné d'une fréquence de réactions qui n'etait que légèrement supérieure à celle montrée par les enfants non-vaccinés.

Dès lors, nos observations soutiennent l'hypothèse que le virage à la tuberculine induit par le B.C.G. tend à s'estomper en quelques années.

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