

## SENSITIZATION TO LEPROMIN INDUCED EXPERIMENTALLY WITH BCG<sup>1</sup>

NORBERTO OLMOS CASTRO, M.D.  
*Chief, Division of Dermatology and  
Chief, Dermatological Dispensary  
Tucuman, Argentina*

Since the first work in this field by Fernandez, beginning in 1939, (4, 5), various investigators—Gines and Poletti (7), Chaussinand (2, 3), Azulay (1), Rosemberg, Souza Campos and Aun (9), and Floch and Destombes (6)—have reported experiences which demonstrated that BCG vaccination of lepromin-negative individuals is capable of making them reactive to lepromin. Chaussinand (3) reported that a lot of animals, negative to the lepromin test, responded positively after BCG vaccination.

In June 1951, I presented to the Institute of Pathological Anatomy of the National University of Tucumán a thesis, based on experimental study, on the early reaction of Fernandez induced by BCG vaccination (8). Lepromin-negative guinea-pigs had been found to respond positively after vaccination, giving early but not persistent reactions after 24 and 48 hours. The tests were performed at intervals varying from 12 to 30 days after the vaccination. It was evident that that phenomenon was the manifestation of a state of cosensitization.

In the present article are reported the results of a new investigation of this state of cosensitization, dealing with the responses to lepromin at various periods after BCG vaccination. Only the clinical study of the reactions obtained is presented, the histopathological study being left for another communication.

### EXPERIMENTAL

In this experiment 6 guinea-pigs were used which had given negative results to intracutaneous injection of tuberculin, and also to integral lepromin.

The antigens used were: (a) crude tuberculin of Koch;<sup>2</sup> (b) BCG, 1 cc. equaling 1.5 mgm.;<sup>2</sup> and (c) integral lepromin prepared by Mitsuda-Hayashi technique.

<sup>1</sup> Read at the Third Pan-American Leprosy Conference, Buenos Aires, December 1951. Translated from the Spanish.

<sup>2</sup> Prepared by the Malbrán Institute of the Ministry of Public Health of the Republic.

All of the guinea-pigs were inoculated intraperitoneally, some with variable doses of BCG, the others with varying mixtures of BCG and integral lepromin.

After the vaccinations the reactional state of the animals to lepromin was investigated by intradermal injections of 0.1 cc. of that antigen, the tests being made on the 7th, 14th, 24th and 56th days after the vaccination.

The results were observed beginning 24 hours after the injections, to determine the early reactional phenomena that might occur, as well as the late reactions.

#### OBSERVATIONS

The following are the detailed observations of the results of the lepromin tests. The vaccinations and first injections of lepromin were all made on April 9, 1951.

*Guinea-pig No. 1.*—Vaccination with 1.5 mgm. First injection of lepromin: Negative for 28 days, when there was observed a small nodule which remained visible for 8 days (remote positivization?). Second lepromin test, 4/16: Erythematous, slightly infiltrated reaction in 24 hours, diminishing in 48 hours and disappearing in 72 hours; negative thereafter to the end of the experiment. Third test, 4/23: Erythematous papule in 24 hours, diminishing in 48 hours and disappearing in 72 hours. On the 10th day a small nodule was observed which persisted for 5 days (remote positivization?). Fourth test, 5/3: After 24 hours an erythematous papule, easily palpable and visible, which remained unmodified for 4 days and then decreased to become negative on the 8th day. Fifth test, 6/4: In 24 hours an erythematous papule which became more marked during the next two days, becoming on the 4th day an erythematous nodule; this persisted until 7/4, when it was seen to be receding.

*Guinea-pig No. 2.*—Vaccination with 1.5 mgm. of BCG and 1 cc. of lepromin. First injection of lepromin: Negative for 28 days, when a small nodule was observed which remained visible for 8 days. Second lepromin test, 4/16: Erythematous, slightly infiltrated reaction in 24 hours, stronger after 48 hours, diminishing thereafter and disappearing by the 4th day. On the 21st day (5/7) a small nodule was observed which remained visible until 5/15. Third test, 4/23: Negative after 24 hours, slight erythematous reaction after 48 hours, disappearing thereafter. On 5/3 there was a small nodule which persisted until 5/15. Fourth test, 5/3: Erythema and infiltration in 24 hours which continued until 5/15. On 5/20 the guinea-pig died with symptoms of dyspnea.

*Guinea-pig No. 3.*—Vaccination with 3.0 mgm. of BCG. First injection of lepromin: Negative results. Second lepromin test, 4/16: Also negative. Third test, 4/23: Erythematous, slightly infiltrated reaction in 24 hours, persisting for another day and then disappearing, with no late development. Fourth test, 5/3: Erythematous reaction in 24 hours, diminishing on the next day and then disappearing. This guinea-pig died on 5/14, also with symptoms of severe dyspnea.

*Guinea-pig No. 4.*—Vaccination with 3.0 mgm. of BCG mixed with 2 cc. of lepromin. First injection of lepromin: Negative until 5/3, when a nodule was seen which persisted until 5/11, when it was biopsied for histological examination. Second lepromin test, 4/16: Erythematous infiltration in 24 hours, persisting for two days to disappear on the 4th day.

On 5/5 there was a small nodule which was visible until 6/5, when it was biopsied. Third test, 4/23: Erythema and infiltration in 24 hours, biopsied on the next day. Fourth test, 5/3: Erythema and infiltration in 24 hours, the reaction remaining positive until 5/15. Fifth test, 6/4: Slightly infiltrated erythema in 24 hours, stronger at 48 hours, and assuming a nodular erythematous aspect on the 4th day; this persisted until 6/13 when there was observed a punched-out ulceration which healed by 7/4, leaving a scar.

*Guinea-pig No. 5.*—Vaccination with 4.5 mgm. of BCG. First injection of lepromin: Negative until 5/3, when a nodule was observed; this persisted until 5/11, when it was biopsied. Second lepromin test, 4/16: Erythematous infiltration in 24 hours, decreasing thereafter and gone after 72 hours. On 5/4 a mild reaction appeared which lasted for 4 days and then disappeared. Third test, 4/23: Erythema and infiltration in 24 hours, gone by the 3rd day. On 5/3 a small nodule was visible which remained until 5/11. Fourth test, 5/3: Erythema and infiltration in 24 hours, persisting until 5/11. Fifth test, 6/4: Slightly infiltrated erythema in 24 hours, becoming nodular on the 3rd day; on 6/13 this ulcerated, healing by 7/4 with a scar.

*Guinea-pig No. 6.*—Vaccination with 4.5 mgm. of BCG mixed with 3 cc. of lepromin. First injection of lepromin: Negative until 5/3, when a nodular reaction was observed which disappeared on 5/8. Second lepromin test, 4/16: Slightly infiltrated erythematous reaction in 24 hours which persisted for a day and then disappeared; no later development. Third test, 4/23: Erythematous infiltration in 24 hours which lasted until 5/11. Fourth test, 5/3: Erythema and infiltration in 24 hours, lasting until 5/11. Fifth test, 6/4: Slightly infiltrated erythema in 24 hours, becoming nodular by the 3rd day; this ulcerated on 6/13, healing by 7/4 leaving a scar.

#### DISCUSSION OF RESULTS

Of the six guinea-pigs used in this experiment, three (Nos. 1, 3 and 5) were inoculated exclusively with BCG in variable doses, while the other three (Nos. 2, 4 and 6) received mixtures of BCG and lepromin. The results obtained showed that the addition of lepromin did not appreciably influence the results, for which reason we regard them as referable exclusively to the effects provoked by the BCG.<sup>3</sup>

<sup>3</sup> It should be said that, in previous work, attempts have been made to induce sensitization in guinea-pigs by lepromin injections, with negative results.

Considering first the doses of BCG employed, guinea-pig No. 1, which received 1.5 mgm., reacted to lepromin after 56 days without ulceration. In the guinea-pigs which received double or triple that dose, the reactions 56 days after vaccination were more intense, terminating in frank ulceration.

Perhaps more important than the dose is the time interval between the vaccination and the lepromin testing. The results were very demonstrative.

(a) When the lepromin was given simultaneously with the vaccination, the results were negative in all instances. It is true that a local reactivation was observed in five out of the six animals from 24 to 28 days after vaccination, but this we attribute to the phenomenon called "remote positivization" by Rosemberg and associates (9).

(b) When the lepromin test was made 7 days after vaccination, all of the guinea-pigs gave a fleeting early reaction which disappeared within 72 hours. Reactivations were observed in Nos. 2, 4 and 5, these also interpreted as "remote positivization."

(c) When the lepromin test was made 14 days after vaccination, all of the guinea-pigs gave the brief early reaction, with the exception that in No. 6 it persisted for 18 days. Three of the animals, Nos. 1, 2 and 5, showed the late nodular reactivation.

(d) When the lepromin test was made 24 days after vaccination, the reactions were characterized by their persistence, the infiltrations lasting from 8 to 12 days. Guinea-pig No. 3 was an exception, there being only brief early reaction; this animal died with manifestations of dyspnea 8 days later.

(e) Observations on the lepromin test made 56 days after vaccination were confined to four guinea-pigs, since the other two had died before that time. The reactions were persistent, lasting for approximately 4 weeks. In No. 1 the reaction, which was a slightly infiltrated erythema after 24 hours, had become nodular by the end of the 4th day; it remained so for 30 days, when it became soft to the touch. In the other 3 animals (Nos. 4, 5 and 6) the development was similar up to the 11th day, when the nodules were replaced by punched-out ulcerations; 30 days later they had healed with cicatrization.

*Remote positivization:* In this experiment there was repeatedly observed the phenomenon called "remote positivization" by Rosemberg and associates. When the lepromin tests were made on the same day as the vaccination, although there were no early reactions, late ones occurred in 5 of the 6 animals after 24 to 28 days, manifested by the development of a small nodule which later subsided without trace. With the tests made 7 days after vaccination, after the passing early reactions 3 of the animals (Nos. 2, 4 and 5) showed similar late reactions after 21, 18 and 19 days, respectively. On testing after 14 days, after the passing early reactions, late ones ap-

peared between the 10th and the 15th day in 4 of the animals (Nos. 1, 2, 5 and 6).

In summary, the late reactions observed in the guinea-pigs which gave negative or brief early reactions appeared between the 24th and 28th days after the vaccination. The periods between the time of the intradermal injections and the appearance of the late reactions varied according to the time of testing after vaccination: between the 24th and 28th days when the lepromin was given simultaneously with the vaccination, between the 18th and 21st days when the test was made after 7 days, and between the 10th and 15th days when the test was made after 14 days.

#### CONCLUSIONS

The results obtained in this experiment have led us to draw the following conclusions:

1. Vaccination of healthy, lepromin-negative guinea-pigs with BCG is capable of creating in them a special biological state which makes them sensitive to the injection of lepromin.
2. This condition of acquired sensitization we interpret as a manifestation of the phenomenon of group cosensitization described by Fernandez.
3. This phenomenon of cosensitization to lepromin provoked by BCG may vary in its clinical manifestations according to the time elapsing between vaccination and lepromin testing.

The observed phenomena are of three categories: (1) The lepromin tests made at the time of vaccination evoked no early reaction, but a late small nodular one which subsequently disappeared—"remote positivization." (2) Lepromin tests made 7 and 14 days after vaccination caused (a) an early reaction, strongest at 48 hours, which diminished or disappeared after another day; and, after a period of negativity, (b) a late nodular one of variable duration—also regarded as "remote positivization." (3) When the lepromin test was made after the third week following vaccination, there occurred an early reaction which either persisted as an erythematous infiltration or transformed to a nodule which later ulcerated, this late reaction being the more intense and prolonged the later the test was made.

4. These phenomena are interpreted as follows: (1) The first period, comprising the first week after vaccination, we

designate the *preallergic period*, there being no manifestation of sensitivity to lepromin. (2) The second period, comprising the second and third weeks after vaccination, we designate the *period of hypersensitivity*; the organism reacts differently from normal because there has been established a state of allergy to lepromin, resulting in an early reaction of the tuberculin type. (3) The third period, beginning in the third week, we call the period of *hypersensitivity and resistance*, there being seen an early reaction with the characteristics of hypersensitivity which, however, without a period of negativity, is transformed into a persistent nodular reaction which may ulcerate. Thus is reproduced faithfully the so-called Koch phenomenon, presenting the clinical characteristics of a positive BCG re-vaccination.

5. The late reactions seen in the first and second of these three periods of immunoallergic phenomena are believed to be of extraordinary interest in the interpretation of the pathogenesis of the phenomenon of cosensitization to lepromin by the BCG, the "remote positivization."

The fact that, when lepromin causes no reactions or exclusively the early ones in the first and second periods, nodular reactions appear in the injection sites after approximately the same time (24 to 28 days after vaccination), demonstrates that at the end of the third week the BCG-vaccinated guinea-pig has developed an immunoallergic state which renders it capable of reacting to the presence of the dead Hansen bacillus which had been injected previously, as well as those injected after this condition has developed. This is in accord with the experience of Rosemberg and associates.

The occurrence of the late reactions, or of remote positivization, marks the limit between the period of pure hypersensitivity and that of the hypersensitivity-resistance phenomena.

6. It is believed that the present experiment confirms those performed by the investigators mentioned at the beginning of this report.

7. It is also believed that it would be of interest to continue the investigations on the phenomenon of group cosensitization between BCG and lepromin, for it may be of significance with respect to the control and eradication of leprosy.

#### SUMMARY

The state of acquired sensitization to lepromin resulting from BCG vaccination has been studied in a group of six

guinea-pigs. The phenomena observed are of three categories in relation to the time between vaccination and the lepromin testing, and these periods are designated: (1) the preallergic period, (2) the period of hypersensitivity, and (3) the period of hypersensitivity and resistance. The first period comprises the first week after the vaccination, when there is no early reaction to lepromin. The second period, which comprises the second and third weeks after the vaccination, is characterized by the appearance of brief early reactions. The third period, beginning after the third week, is characterized by the appearance of early reactions which persist and evolve to the formation of nodules which may ulcerate, reproducing the classical Koch phenomenon. The study of the phenomenon of "remote positivization" shows that it signifies a new biological condition with respect to lepromin which is established between the 24th and 28th days after the BCG vaccination. These findings confirm the investigations reported by various leprologists on this subject, and may be of significance with respect to the program of control and eradication of leprosy.

#### RESÚMEN

El autor estudia, en un grupo de 6 cobayos, el estado de sensibilización adquirido a la lepromina, por vacunación B.C.G. Los resultados obtenidos los agrupa en 3 categorías, relacionadas al tiempo de vacunación y que denomina: período prealérgico, período de hipersensibilidad y período de hipersensibilidad y resistencia. El primer período o período prealérgico, comprende la primer semana después de la vacunación; se caracteriza por la ausencia de reacciones precoces a la lepromina. El segundo período o período de hipersensibilidad, comprende la segunda y tercer semana después de la vacunación, se caracteriza por la aparición de reacciones precoces y fugaces a la lepromina. El tercer período o período de hipersensibilidad y resistencia, comprende desde la tercer semana en adelante, se caracteriza por la aparición de reacciones precoces, la que sin negativizarse evolucionan hacia la formación de un nódulo que puede ulcerarse y curar por cicatrización, reproduciendo el clásico fenómeno de Koch. Estudia el fenómeno de "positivización remota" determinando que dicho fenómeno significa una nueva condición biológica frente a la lepromina, y que se establece entre los 24 y 48 días de la vacunación. La experiencia realizada confirma las investigaciones comunicadas por diversos leprólogos sobre el tema y el autor insiste en su valor que puede tener en un plan de profilaxis para la erradicación de la lepra.

#### REFERENCES

1. AZULAY, R. D. A ação do B.C.G. sobre a reação lepromínica. Mem. V Congr. Internac. Lepra, Havana, 1948; Havana, 1949, pp. 1142-1145.

2. CHAUSSINAND, R. Para-allergies bactériennes dans la tuberculose. *Ann. Inst. Pasteur* **73** (1947) 814-815.
3. CHAUSSINAND, R. La réaction de Mitsuda, indice de l'immunité relative antilepreuse. *Mem. V Congr. Internac. Lepra, Havana, 1948; Havana, 1949, pp. 595-597.*
4. FERNANDEZ, J. M. M. Estudio comparativo de la reacción de Mitsuda con las reacciones tuberculínicas. *Rev. argentina Dermatosisif.* **23** (1939) 21-22.
5. FERNANDEZ, J. M. M. Influencia del factor tuberculosis sobre la reacción a la lepromina. *Rev. argentina-norteamericana Cien. Méd* **1** (1943) 592-597.
6. FLOCH, H. and DESTOMBES, P. Allergie et para-allergie dans la lèpre. Réaction de Mitsuda; allergie lépreuse et allergie tuberculoïde; vaccination par le B.C.G. *Internat. J. Leprosy* **18** (1950) 177-183.
7. GINÉS, A. R. and POLETTI, J. G. La reacción de Mitsuda en los vacunados con BCG. (Posibilidades de la vacunación BCG en la profilaxia de la lepra.) *Hoja Tisiol.* **5** (1945) 284-292; *also An. Fac. Cien. Med. Asunción* **5** (1945) 101-112.
8. OLMOS CASTRO, N. Reacción a la lepromina en cobayos calmetizados. Trabajo de tesis presentado al Instituto de Anatomía Patológica de la Universidad Nacional de Tucumán. (To be published.)
9. ROSEMBERG, J., SOUZA CAMPOS, N. and AUN, J. N. Da relação imunobiológica entre tuberculose e lepra. I. Ação positivante do BCG sobre a lepromino-reação. *Rev. brasileira Leprol.* **18** (1950) 3-23.