INFECTIONS PRODUCED IN HAMSTERS WITH THE HUMAN LEPROSY BACILLUS

A CRITIQUE OF RECENT STUDIES [1]

JACINTO CONVIT, M.D.

Director of the Division of Sanitary Dermatology
Ministry of Health and Social Welfare
Caracas, Venezuela

Since the discovery of the leprosy bacillus by Gerhard Armauer Hansen nearly a century ago physicians and bacteriologists have felt challenged to solve the problems of cultivating it and determining if it would fulfill Koch's postulates. Attempts to inoculate animals with material from human leprosy have also been carried out for the greater part of this century. Both matters have caused a great deal of controversy, which has been kept alive to this day. The attitude of workers in the field of leprologic bacteriology has varied from clear acceptance to skepticism and flat denial regarding the identity of the bacillus cultivated outside the human skin with the true etiologic agent. The present attitude has been characterized by a keen but naturally cautious interest in the experiments that various workers in different countries have carried on with the two problems in the last decade.

It is not necessary to go into detail about the work of the early workers. Their perseverance in the face of adverse criticism and their faith in the validity of their results kept the issue alive and prepared the way for later experiments on the basis of newer knowledge and new methods.

The issue as regards both animal inoculations and cultures has been plagued by rigid concepts concerning bacterial species inherited from the time when a species, even at the lowest level, was considered to be an unchangeable taxonomic entity that should always respond to the same test of identification. Though it might "adapt" its metabolism to the use of new substances in a new environment, it was expected to remain intrinsically the same. If it did not conform to the standards set for its ancestors, it was considered as another species fortuitously introduced. Leprologists have been notably slow to interpret the facts of their experiments in the light of the vast literature that has accumulated in the last few decades following observations on spontaneous or induced bacterial mutation.

Genetic aspects of *M. leprae* have been ignored by most workers and great emphasis has been laid on susceptibility attributable to particular strains of mice, as in the work of Chatterjee and Rees ([11]), or to susceptibility induced by a prooxidant diet. To this latter device Bergel ([1–5]) attributes his success in producing lesions in rats.

Although Chatterjee ([10]) obtained extensive lesions in a selected strain of hybrid black mice, it is very doubtful that hybridism had anything to do with the evident susceptibility. This may more logically be attributed to susceptibility in the wild gray ancestors, if the albino strains of mice were considered as resistant. If he had bred a pure

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strain of gray mice he might have developed a susceptible strain from it by selection as easily as from the black hybrid. Chatterjee's claim for the identity of his strain of *M. leprae* from the mouse lesions with the human precursors from lepromatous patients, is based on similar patterns of diffusion in agar of antigens prepared from the two strains. As both his antigens were prepared by the Dharmendra method, by which specific lipids were removed, the two antigens might well give similar diffusion patterns and still be different in essential immunologic tests. The mere fact that the mouse antigen gave a diffusion pattern different from that of an antigen from *M. lepraemurium* does not necessarily prove that the strain is identical with that from the precursors of the bacilli in human lepromatous lesions. When we consider that Chatterjee used inocula in his mice containing as many as a billion bacilli, it seems highly improbable that there should not be one genetic variant capable of responding to natural mutagens in the intracellular environment of the new host.

In his immunologic tests with the mouse antigen in comparison with human lepromin, Chatterjee used only the early reading and failed to mention the late reaction. On this basis there is room for a good deal of doubt as to the identity of the strain developed in his mice with precursors in human lesions.

The supposed identity of the two strains was not supported by electron microscopic studies, and the observations made on the behavior of the bacillary antigen from the mouse lesions when administered to lepromatous patients are insufficient. During the conferences in Rio de Janeiro I had an opportunity to examine slides brought by Dr. Chatterjee. They showed a great number of bacilli within histiocytes and apparently in a very active phase that reminded me strongly of the situation we have observed repeatedly in our hamsters in Venezuela. Our strain of the bacillus developed in hamsters is certainly not identical with its precursors in human lesions when its immunologic aspect is used as the criterion.

The work of Bergel ([4]) is more difficult to appraise justly. His idea that a prooxidant diet makes the rat susceptible to human leprosy bears no relevance to what is known about the endemicity of leprosy in relation to diet. Yet it must be granted that there is room for both a yes and a no in the question of defense as a function of nutrition. If we suppose that certain highly unsaturated fatty acids are essential parts of a specific lecithin serving as a lipid haptene or cohaptene in the formation of a specific antibody against *M. leprae*, the oxidation of their double bonds would certainly alter their chemical properties and invalidate them as participants in the specific defense. The crucial point in Bergel's work is the genetic identity of the successors of *M. leprae* in his rats with their precursors in human skin. He claims that his antigens from the rat lesions give negative reactions in lepromatous patients, as is the case with human lepromin. His attempts to cultivate the bacilli from the rat lesions failed.

During the Rio de Janeiro Congress I saw the slides that Bergel had brought. My impression was that the lesions were regressive. They reminded me of old lepromas in treated patients in times past. They were certainly different from the active lesions seen in my own hamsters and in Chatterjee's mice. Bergel, perhaps, implanted fragments of a leproma in the process of elimination.

In the work of Binford ([6]) with hamsters no emphasis is laid on the selection of the animals for supposed special susceptibility, but sites of lowest body temperature are chosen for intradermic inoculation. This is a perfectly logical procedure and would at least make the intracellular environment similar in temperature to that of human skin.

There is a progressive development in the work of Binford, that may be divided into three stages, viz., one prior to the Tokyo Congress in 1958, one prior to the Baltimore Symposium in 1961, and subsequent work presented to the Congress in Rio de Janeiro in 1963.

In the first stage of his work, Binford's fundamental ideas, in his comparison of his strain developed in hamsters with its precursors in human lesions, are clinical and histopathologic. The same is the case with the second stage. The observations presented in Baltimore are similar to those reported at the Tokyo Congress ([8]), but the scope of his work had been amplified by a successful attempt to cultivate on Löwenstein-Jensen medium the strain developed in his hamsters. Shepard ([18]) at the Baltimore Symposium reported that he and Kirsh had classified Binford's cultivated strain as belonging to Group III in the Runyon classification.

In the third stage of his work reported at the Congress in Rio de Janeiro, Binford and Madison ([9]) reported lesions in the ears of hamsters in which involvement of the small dermal nerves was a prominent feature. The strain of the bacillus involved in those lesions was uncultivable in contrast with that producing the lesions in hamsters reported in the second stage of his work.

The work of Binford was not amplified by electron-microscopic studies of the animal lesions and the strains of the bacillus that caused them. The immunologic tests of his antigen from lesions in hamster testes and cultures were carried out by Guinto in the Philippines with lepromatous patients ([7]), and the reactions were positive, in contrast with the current reaction to human lepromin. It was evident that the causative agent of the hamster lesions was not intrinsically identical with its precursor in human lesions. Antigens of the same lots tested by Guinto were tested immunologically by Lapenta of the Cabo Blanco Sanatorium, as was also Binford's antigen from testicular lesions in the hamster. Both the testicular antigen and the antigen from cultures gave positive reactions in lepromatous patients. Lapenta applied the test with human lepromin on the forearm of the patient opposite to the one on which the Binford antigens were administered, and contrary to expectations the reactions to that test were also positive. The same had been the case with human lepromin administered several months before

in a similar way jointly with our antigen from the uncultivable strain of the bacilli of borderline origin developed in our hamsters at Cabo Blanco. However, an antigen made from our cultivable strain obtained directly from lepromatous human lesions failed to cause the regular Mitsuda test site to become positive, though it gave a positive reaction at the site where it was administered.

The uncultivable strain of the bacillus developed by Shepard and Guinto ([19]) in the footpads of mice kept at a temperature of 18°C seems to come closest to being identical with the strain from human lepromatous lesions, in that the antigen prepared from it gives a negative reaction when injected in lepromatous patients and a positive one in tuberculoid cases, as shown by Guinto and Fajardo of the Eversley Childs Sanitarium of Cebu, Philippines. As in the work of Binford, no study was made with the aid of the electron microscope.

While at Rio de Janeiro I had the opportunity to see the preparations from the biopsies of the footpad lesions of Shepard's mice. They differed greatly from the lesions in Chatterjee's mice and in our hamsters in Venezuela in that they were formed by a small number of cells, some of which were filled with apparently active bacilli.

Shepard has made successful mouse to mouse passages and in some he obtained an infiltration of the small nerves, although not with the frequency that occurred in Binford's passages from hamster to hamster.

Shepard emphasized that the multiplication of the bacilli could be observed better with the use of an inoculum with preferably 5×10^3 but not more than 5×10^5 bacilli. His criterion for determining the identity of a strain in animal lesions with its human precursors is the immunologic skin test in leprosy patients and not the known serologic tests, which are complicated by cross reactions.

The work carried out in Venezuela by myself and coworkers ([13–15]), was based on the concept that success or failure in producing leprous lesions in animals would depend on the intrinsic nature of the bacilli inoculated. We considered the lepromatous and tuberculoid forms of leprosy as polar types of the disease, and their bacilli as terminal forms in two distinct trends of genetic change from common precursors in the borderline form. In the latter we supposed that the not very numerous bacilli present were in various transitional forms, of which some would proceed in their evolution to represent either the lepromatous or the tuberculoid form. It seemed logical to suppose that some of them would be capable of changing into a stable form adapted to the intracellular environment of an animal host. This idea was strengthened when it was revealed by electron-microscopic studies that the vast majority of bacilli in human lesions of the lepromatous form were in a state of degeneration, semidisintegrated and probably dead, while, in contrast, those from borderline lesions were intact and showed no signs of a degenerative process. The latter seemed by far the most promising material for experimental transfer to another host. Our choice was

amply rewarded. The bacilli from borderline lesions produced a strain viable in the hamster, but the initial incubation period was eight months. This was shortened with each successive passage until it became less than four months. As the period of incubation shortened, and the animals lived longer with active lesions, infiltrations were observed in their livers and spleens, but we have had no conclusive evidence of nerve involvement.

In our inoculations we disregarded the concepts of previous workers concerning the preparation of animals or the site of inoculation, except for ordinary sterilization. We selected no strain of hamsters or mice for any supposed special susceptibility. However, we followed the logical principle of Binford in inoculating the animals in body parts of lowest temperature.

Our repeated attempts to cultivate the bacilli of borderline origin from hamster lesions failed completely.

The attempts that we made concurrently to produce lesions in hamsters, mice, and rats with bacilli from lepromatous lesions, failed continually from September 1959 until late in 1962, when, rather surprisingly, lesions appeared in a group of hamsters so inoculated. A strain from those lesions proved to be cultivable in the Löwenstein-Jensen medium with retention of their pathogenicity for the hamster.

While we have attributed great importance to transitional forms of the bacilli in paucibacillary borderline lesions, as compared with those from lepromas, the final validity of the idea will depend on continuation of the work.. By the end of February 1964 we had obtained positive results in 11 out of 30 groups inoculated with bacilli from borderline lesions, but the proportion may ultimately be higher, as several of the groups are of comparatively recent inoculation. The result is significant nevertheless, when we consider that we inoculated 50 groups with bacilli from lepromas to obtain a positive result in one group only.

Our criterion for determining the identity of the strains of *M. leprae* developed in the hamster with their precursors in human lesions was that the immunologic reaction in skin tests should be identical for the two strains. If identical, both should give negative reactions in lepromatous patients. This was the crucial test that failed, but it revealed unexpected properties in the strain of borderline origin from hamster lesions. When the antigen from that strain was applied on one forearm of the lepromatous patient and the regular lepromin from human lesions on the other, there was a strong positive late reaction to the hamster antigen accompanied by a positive late Mitsuda reaction. This positivization was the same, if the human lepromin was applied 30 days after the hamster antigen, and positivization could still be demonstrated one year later.

The antigens for these tests were prepared by the trypsinization method. The concentration of bacilli obtained was 480 million per ml. As the doses used were of 0.2 ml. the number of bacilli present in each injection was 96 million.

As it is, the work presented by the various investigators who have successfully inoculated animals with *M. leprae* from human lesions presents a confusing array of contradictory hypotheses and experimental data. The cause of this confusion must be sought in a too rigid adherence to the current hypothesis that *M. leprae* is a taxonomic entity, that may or may not be contaminated by adventitious mycobacteriaceae from outside sources. The situation is aggravated by an almost general disregard of the facts of modern bacterial genetics.

In a paper published as late as 1963, Nishimura ([17]) touches only casually on bacillary mutation as the cause. He dismisses such a postulate as being experimentally unverifiable, to take refuge in the assertion that in reality all small rodents used in the experimental transmissions of leprosy in Japan are carriers of *M. lepraemurium,* the Stefansky bacillus, and that the injection of *M. leprae* only serves to arouse it from latency into active proliferation.

We have, of course, used *M. lepraemurium* in the preparation of antigens for comparison, but the material was always obtained from a colony kept at El Algodonal some 12 kilometers from our Caracas laboratory. No rats or mice inoculated with that bacillus are kept anywhere near our premises. If our hamsters were carriers of rat leprosy, it would seem exceedingly strange that in all our experience we have never observed one spontaneous lesion in them. We could not accept the Nishimura postulate without supposing that they were all equally resistant and only awaited an inoculation with human leprosy to become susceptible to their latent *M. lepraemurium* and develop a nodule at the site of the injection.

It would seem equally strange, if the concepts of Nishimura were valid as regards our hamsters, that not one animal of the control groups injected with heat-killed bacilli from lepromatous patients developed any lesions. Even if the hypothetic factor in *M. leprae* necessary for the proliferation of *M. lepraemurium* were very labile to heat, it should have been present in the inocula from lepromatous lesions that we used for three years in 50 groups of hamsters before getting a positive result.

We have prepared antigens from *M. lepraemurium* isolated from rat lesions. They give positive reactions in lepromatous patients, as do so many other forms of acid-fast bacilli, but *they do not positivize the reaction to human lepromin.* As the antigen from hamster lesions did positivize that reaction, and Binford's antigen from his cultivated strain did the same, it is evident that they differ intrinsically from both *M. leprae* and *M. lepraemurium.* They synthesize something different in enzymatic capacity, which suggests a difference in the nature of their proteins. This difference must be sought in the nature and sequence of their constituent amino acids. This in turn depends on the code given by the sequence of the purine and pyrimidine side chains in the nucleic acid functions. Thus we have plainly a case of mutation. The mutagenic factors may be purines or pyrimidines present in the host cell. They are of a molecular weight sufficiently low to pass the mycobacterial

cell wall in company with other nutrients and to pass intracellular cytoplasmic membranes so as to come into contact with the nucleic acid chains. The actual exchange of radicals and the consequent change in the nucleic acid code would depend on the same situations as in all phenomena of radical exchange.

It might be expected that mutants occurring within hamster skin cells, through mutagens that are genetic determinants of protein synthesis in the hamster, would acquire some features of internal morphology possessed also by the acid-fast bacilli of rodent leprosy. However, morphologic similarity, whether external or internal, would hardly be a guide to the intrinsic enzymatic capacities of a microorganism. As Nishimura himself says, the immunologic test should determine the question of identity or difference. The reactions to that test with our antigen, and with those of Binford, differ from reactions to both human and rat lepromin.

The intrinsic difference may be connected with a capacity possessed by the mycobacterial mutant to synthesize a cytolipin intimately connected with the positive reaction to human lepromin or a factor needed by the host to convert such a cytolipin into the specific haptene. This concept is strengthened by the fact that our antigen from the strain of the bacillus developed in the hamster from borderline precursors failed utterly to positivize the reaction to human lepromin when it was prepared by the Dharmendra method, but brought about a good positive reaction when prepared by trypsinization. Apparently the chloroform used in the Dharmendra method to float the bacilli had removed one or more factors of immunologic importance.

We may be sure that the immunologic process is accomplished through a train of enzymes. When a healthy person reacts positively in the Mitsuda test, while a lepromatous patient does not, it is a logical conclusion that in the former the train of enzymes involved in the process is complete and that in the latter some link in the train is missing or inhibited. When positivization is induced in a negative reactor by an antigen from a mutant of the bacillus, it may further be concluded that the new strain has produced a factor either capable of mobilizing the inhibited link or of enabling the host to bypass it through another enzymatic pathway. The capacity becomes general, but we cannot from present day knowledge form a precise idea of the mechanism by which it is relaid throughout the skin.

As we see in retrospect the work of the various investigators of the problem of transmitting human leprosy to animals, as well as that of cultivating the bacillus, we find a strange array of contradictory hypotheses and seemingly irreconcilable data. There is not only a great need for coordinating methods through cooperation among the various groups, but an even greater need for clarifying the whole situation in the light of modern biochemical and genetic knowledge.

The Congress in Rio de Janeiro ([12]) adopted certain resolutions in

the interest of reconciling different results of investigation. The principal recommendations were:

1. Interchange of material between the various investigators, and
2. Determination of the importance of the neural lesions and their exact localization.

As regards the second point, it is unfortunate that all the investigating groups, except ours in Venezuela, have used only the light microscope for their studies. They have not shown whether the bacilli of the neural lesions in their animals are found within the axon or within the Schwann cells. Within the latter, Imaeda *et al.* ([16]) failed to find bacilli in their electron microscopic study, but we do not know if that is also the case in the materials of other workers.

As much as we may coordinate and cooperate, we must realize that the element of chance that has to do with the creation of new bacterial genotypes will be with us always to perturb our ambitions of finding a stable, cultivable form of the bacillus with the identical antigenic properties of *M. leprae* in lepromatous leprosy.

When we consider that the cultivable strain produced by Binford has acquired the property of bringing about a positive response to the Mitsuda test in lepromatous patients in a manner similar to the antigen from our uncultivable strain in the hamster, it would seem that, whereas one chance mutation has confounded the primary object of the investigation, another more distant leprologic goal has been reached in obtaining a stable variant of *M. leprae* with immunologic properties of therapeutic application.

The origin of the proteins that are active enzymatically in the synthesis of the specific immunologic factors remains a secret in the genetic code of the mutants. An investigation of that situation lies beyond the applicable knowledge of present day leprology. However, I see no reason why the leprogenic mycobacteriaceae should not be investigated as regards their innermost biochemistry, as has been the case with *Escherichia coli*, which for many years has been the exemplary microbe for research in bacterial genetics. There are by now perhaps more than a thousand publications dealing with that subject.

The occurrence of mycobacterial mutations should no longer be ignored as unverifiable by leprologists, nor should it be brushed aside lightly as of little comparative interest. On the contrary, leprologists should enlist in their investigation the collaboration of men eminent in the field of biochemistry as applied to bacterial genetics. We should stop clamoring before the door to which we have no key and can make none. Let us hope that biochemists and geneticists will eventually give us the open sesame that will unlock the door from within.

SUMMARY

There is need for a thorough revision of the theories that have served as criteria for the many attempts to transmit human leprosy to laboratory animals. Previous workers have made no attempts to see

M. leprae in the light of bacterial genetics as applied to the facts of bacterial mutation.

The theories to which success in transmission has been attributed deal with the selection of supposedly susceptible animals (Chatterjee), or nutritionally induced susceptibility (Bergel). Each of these investigators has claimed that the bacilli in the animal lesions of his experiments are identical with those of human lesions, but their proofs are not very convincing.

The experiments of the author and his coworkers are based on the transitional phase of the relatively few bacilli in borderline lesions. These bacilli are considered to be genetically unstable and capable of forming a strain adapted to the intracellular environment of a host of another species. Material from borderline cases gave positive results in 11 groups of hamsters out of 30, while the abundant bacilli from lepromatous patients gave lesions in only one group out of 50. Differences in the electron-microscopic appearance of the two inocula were strongly in favor of the bacilli of borderline origin, most of which appeared intact and alive, whereas in lepromatous lesions the vast majority of the bacilli were degenerated and perhaps dead.

The central point is the intrinsic difference between the bacillus developed in the hamster and its precursor in human lesions, as proved by the reactions to their antigens in lepromatous patients. The hamster antigen induced positive late reactions. Moreover, its effect had the faculty of bringing about a positive late reaction to human lepromin (in the originally lepromin-negative cases) when that antigen was given simultaneously on the opposite forearm of the patients, and also when given a month or even a year after. This induction of the capacity to react positively to the Mitsuda test is a significant phenomenon, in view of the persistent negativity of the patients in all previous tests.

The induced positive reactivity appears closely connected with a chloroform-soluble factor in the hamster antigen. When an antigen was prepared by the Dharmendra method instead of being purified by trypsinization, it failed to bring about a positive reaction to the human antigen.

Two antigens prepared from the cultivable bacillus of Binford, one from infected testicular tissue of hamsters and one from a culture, were tested at the Cabo Blanco Sanatorium and found to possess the same property of bringing about positive reactions to human lepromin in lepromatous patients. However, an antigen prepared from *M. lepraemurium*, while causing positive reactions, had no effect on the reactions to human lepromin.

The author is convinced that a new strain of *M. leprae* has been developed in the hamster by the mutation of a transient phase of the bacillus in borderline lesions. He makes a strong plea for a biochemical investigation of the immunogenic lipid synthesized by the mutant, and of the possibility of using that factor therapeutically, at least in early lepromatous leprosy. He also pleads for a biochemical investigation of

the genetic mechanisms that have to do with the appearance of new strains of *M. leprae*.

RESUMEN

El autor aboga por una completa revisión de las teorías que han servido de criterios para los muchos ensayos que se han hecho hasta el presente, de transmitir la lepra humana a animales de laboratorio. Los investigadores anteriores no han considerado el bacilo a la luz de la genética bacterial relacionada íntimamente con la mutación.

Los éxitos obtenidos en la transmissión de la enfermedad se han atribuído a la selección de animales de supuesta alta susceptibilidad como en los trabajos de Chatterjee, o a una susceptibilidad inducida por la nutrición según Bergel.

Cada uno de estos investigadores ha afirmado que los bacilos de las lesiones de sus animales son idénticos con los de las lesiones humanas, pero las pruebas aducidas no son muy convincentes.

Las experiencias del autor y sus colaboradores se basan en la fase transitoria de los bacilos comparativamente escasos de las lesiones borderline. Se considera que estos bacilos son genéticamente inestables y capaces de formar cepas adaptadas al ambiente intracelular de otra especie.

El material obtenido de lesiones borderline dió resultados positivos en 11 de 30 grupos de animales inoculados mientras que los bacilos de origen lepromatoso, siempre muy abundantes, no dieron lesiones sino en un solo grupo de los 50 inoculados.

Las diferencias observadas en el microscopio electrónico entre los dos materiales usados demostraron que los bacilos de origen borderline parecían intactos y vivos, mientras que los de lepromas eran en su vasta mayoría degenerados y tal vez muertos.

El punto céntrico del trabajo se encuentra en la diferencia intrínseca entre el bacilo desarrollado en el hamster y su precursor en lesiones humanas. Es una diferencia inmunológica comprobada por las reacciones a sus antígenos en pacientes lepromatosos. El antígeno del hamster provoca una reacción retardada, pero al mismo tiempo tiene la facultad de evocar una reacción positiva, tardía, al test de Mitsuda en pacientes anteriormente negativos cuando se les inyecta la lepromina humana en la piel del otro antebrazo. Esta reacción positiva es la misma, cuando la lepromina humana se administra simultáneamente con la de hamster, sea cuando se administra un mes y hasta un año después. Esta inducción de la capacidad de reaccionar positivamente en pacientes negativos en todos los tests anteriores es significativa.

La reacción positiva inducida parece que está estrechamente relacionada con un factor presente en el antígeno bacilar proveniente del hamster y removible con cloroformo. Cuando el antígeno se prepara por el método de Dharmendra, en lugar de ser purificado por la tripsinización, deja de evocar una reacción positiva al antígeno humano.

Dos antígenos preparados por Binford fueron sometidos a prueba por Lapenta en el Sanatorio de Cabo Blanco en Venezuela. Uno de ellos era de lesiones testiculares del hamster y el otro de un cultivo. Ambos tenían la misma propiedad que el antígeno de los hamster del autor de evocar una reacción positiva a la lepromina humana. Un antígeno preparado en Venezuela con *M. lepraemurium* no tenía dicha propiedad, aunque sí dió una reacción positiva en el sitio de la inyección.

El autor está convencido de que una cepa nueva de *M. leprae* ha sido desarrollada en el hamster por la mutación de una fase transitoria del bacilo en lesiones borderline y subraya la necesidad de una investigación bioquímica del lípido inmunógeno sintetizado por el mutante. Señala además la posibilidad de usar dicho lípido en la terapia de la lepra—por lo menos en los casos lepromatosos incipientes. Arguye en favor de una investigación bioquímica del mecanismo genético que origina posiblemente nuevas cepas de *M. leprae*.—

RÉSUMÉ

L'auter est convaincu de la nécessité de reviser à fond les théories qui ont servi de base pour les expériences faites avec la transmission de la lèpre humaine aux animaux

de laboratoire. Il déplore le fait que les investigateurs antérieurs n'aient pas consideré *M. leprae* au point de vue génétique.

Jusqu'à présent les résultats positifs obtenus par l'inoculation de la maladie aux animaux ont été attribués soit au choix d'animaux susceptibles comme dans les essais de Chatterjee, soit à la susceptibilité induite par la nutrition, comme dans les essais de Bergel. Chatterjee, comme Bergel, affirmait que les bacilles des lésions produites dans leurs animaux étaient identiques à ceux des lésions humaines. Selon l'opinion de l'auteur leurs preuves ne sont pas suffisamment convaincantes.

Les expériences de l'auteur et de ses collaborateurs sont basées sur la phase transitoire de *M. leprae* des lésions borderline. Les bacilles de cette phase sont probablement d'un type génétique instable et peuvent former dans un hôte d'une autre espèce de nouveaux types qui deviennent génétiquement stables. Les résultats des inoculations faites avec des bacilles choisis des lésions borderline oú ceux-ci ne sont pas nombreuse, ont été positifs en 11 de 30 groupes d'animaux, tandis que sur 50 groupes inoculés avec les bacilles provenent des lésions lépromateuse oú ils abondent toujours, un seul groupe positif a pu être trouvé. La différence observée dans le microscope électronique entre ces deux types de bacilles employés dans les inoculations étaient en faveur des bacilles d'origine borderline en les comparant avec ceux des lésions lépromateuse, la plupart d'entre eux étant dégénérés et peut-être morts, les premiers, au contraire, vifs et intacts.

Ce qu'il y a de plus intéressant dans ces expériences est la différence observée entre les bacilles développés dans le hamster et leurs précurseurs des lésions humaines. Cette différence est immunologique comme il a été prouvé par le réactions del malades lépromateux aux antigènes préparés avec les deux.

L'antigène préparé avec les bacilles des lésions du hamster a produit une réaction tardive positive chez malades lépromateux, tandis que la réaction à la lépromine humaine chez les malades de ce type était toujours négative. Néanmoins, en injectant l'antigène humain à l'autre avant-bras du malade avec l'antigène de hamster simultanément ou un mois après, la réaction tardive devint positive. Cette positivisation se manifestait encore une année après. La capacité induite chez le malade de réagir positivement à la preuve de Mitsuda est un phénomène de grande signification en considerant que tous les cas lépromateux étaient auparavant négatifs.

La réaction positive dépend d'un facteur présent dans l'antigène de hamster et soluble en chloroforme. Cet antigène préparé par la méthode de Dharmendra, au lieu de le purifier par trypsination, perd sa capacité d'évoquer la réaction positive a l'antigène humain.

Les antigènes preparés par Binford, l'un d'une culture et l'autre des bacilles obtenus del lésions testiculaires du hamster, provèrent dans le Sanatorium de Cabo Blanco posséder la même propriété de positiviser la réaction à la lépromine humaine.

L'antigène préparé avec *M. lepraemurium* a produit une réaction positive à l'endroit de l'injection mais ne possède pas la propriété d'évoquer une réaction positive à la lépromine humaine.

Selon l'opinion de l'auteur un précurseur génétiquement transitoir d'origine borderline a produit par mutation dans le hamster un nouvel type de *M. leprae*. L'auteur recommande de faire des investigations biochimiques sur les lipoïdes synthétisées par les mutants et qui possedent l'activité immunisante. Il suggère la possibilité de les employer dans la thérapie de la lèpre lépromateuse—au moins dans les cas pas trop avancés. Il souligne la nécessité de faire des investigations sur le mécanisme génétique qui puissent produire un nouvel type de *M. leprae*.

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[Author's note] It is difficult to correlate the general facts of bacterial mutation with any one publication out of the many hundreds available. Leprologists will be interested in the work on bacterial genetics by Edward A. Adelberg, Little, Brown & Co., Boston, 1960, and papers on bacterial viruses by Gunther S. Stent, Little, Brown & Co., Boston, 1960, and also in the general literature contained in *Genetics,* published for many years by Genetics, Inc. at the University of Texas.