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EXPERIMENTAL INOCULATION OF HUMAN LEPROSY IN LABORATORY ANIMALS[1, 2] I. CLINICAL, BACTERIOLOGIC, AND HISTOPATHOLOGIC STUDY

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During nearly a century many attempts have been made to transmit human leprosy to laboratory animals, but there is no convincing evidence that the objective has been achieved. Recent work, nevertheless, has served to stimulate and guide renewed efforts in this difficult branch of leprosy investigation. We shall refer only briefly to the more recent works.

Adler (1) reported having obtained visceral lesions in splenectomized hamsters (*Cricetus auratus*) in which he had implanted fragments of a leproma rich in bacilli. Other investigators using the same technique have not confirmed his findings.

Binford (6) reported positive results in hamsters with the inoculation of refrigerated lepromatous material, which produced nodular lesions at the sites of inoculation. Material from such lesions produced similar lesions in a series of hamster-to-hamster inoculations.

Shepard and Kirsh (13) have studied the acid- and alcohol-resistant bacilli from the lesions of Binford's hamsters, as well as those cultivated by Binford himself on Loewenstein-Jensen medium. They placed them taxonomically in the nonphotochromogen subgroup of Group III of Runyon's classification of "atypical" or "anonymous" mycobacteria.

Chatterjee (7) used a hybrid strain of black mice produced by crossbreeding males of the common mouse of India (*Mus musculus*) with

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[2]A preliminary version of this report was read at the Leonard Wood Memorial-Johns Hopkins University Symposium on Research in Leprosy, Baltimore, Md., May 8-10, 1961.

white, female Swiss mice. Generalized visceral lesions developed in that hybrid strain six months or more after inoculation, but attempts to cultivate the bacilli from the lesions in various media were without results.

Bergel ([2-4]) has published the results of work with rats kept on a prooxidant diet, and affirms that the bacilli have multiplied in their viscera. With an antigen prepared from the testis of one of his rats, he obtained skin-test reactions comparable with those produced by the standard Mitsuda-Hayashi antigen, positive in 3 tuberculoid cases and 4 normals, but negative in 2 lepromatous cases ([5]).

We, on our part, have made a series of experiments in the last two years, inoculating different animal species with material from human leprosy lesions containing *M. leprae*. In these experiments we have used approximately 1,000 animals, notably, about 600 hamsters, and the remainder hybrid black mice produced by crossbreeding the grey and white varieties, besides ordinary white mice, white rats, guinea-pigs and rabbits. The material used in the three latter species was obtained from lesions produced in hamsters. At the same time, 12 pigs were inoculated.

The working hypothesis on which this study was based takes into consideration not only the animal and the site of inoculation, but also —and especially—the nature of the inoculum.

As regards the selection of animals, the hamster was given preference, as it has been proved to be Mitsuda negative with the reaction unmodified by repeated injections of the antigen ([8]). In other words, the hamster does not react against an inoculum with the Mitsuda phenomenon, which constitutes a hostile milieu.

As for the site, places of lowest temperature such as the ears, the testicles and the plantar parts of the paws, were chosen for inoculation as suggested by Binford. No special preparation of the site of inoculation was done, and neither cortisone, nor x-rays, nor any other means of lowering the resistance of the animals was used.

Our second working hypothesis had to do with the inoculum. We have not only used the usual sort of material from lepromatous patients, but also material from the nonlepromatous forms and especially from borderline cases in the early stages, or, let us say, cases in which the lepromatous element is incipient. Great importance has been attributed to the stage of development of the disease ([10]). Borderline cases were selected for the following reasons:

The bacilli from lepromatous lesions have become completely adapted to the metabolism of an intracellular, human environment, and it would presumably be difficult for them to survive and multiply in the tissue of another species.

The bacilli obtained from borderline cases, in which the resistance of the host seems to oscillate, have not become completely adapted to

the intracellular environment, nor are they attacked by intracellular lysozymes. It seemed reasonable to suppose that they could give origin to a strain capable of surviving and multiplying when inoculated into animal skin tissue.

Studies made with the electron microscope tended to strengthen this hypothesis, for in the lepromatous granuloma, and particularly in the well-developed leproma, a great many of the bacilli show marked signs of degeneration, while those from the initial stage of borderline cases are of a rather wholesome aspect without degenerative phenomena and with their nuclear and cytoplasmic structures intact ([10, 12]).

MATERIALS AND METHODS

The material used in the inoculations was fresh, that is, recently taken from the patient and prepared and injected immediately without being submitted to refrigeration except for very short periods not exceeding a few hours. A few passages, however, were made with material kept under refrigeration for as long as a month. The tissue was ground in a mortar with normal saline until a more or less homogeneous suspension was obtained. The concentration of bacilli was always high in the lepromatous material, while it was much lower in the tissue from borderline cases, especially in that from one of the patients (A.M.) who was characterized as relapsing tuberculoid in transition to borderline and was rather poor in bacilli. The inoculations were made intradermally in the ears, the paws, the testicles or in the cheek pouches. The quantity injected was 0.1 cc.

A total of 26 groups were inoculated with material from different patients, 11 with material from two very similar cases of lepromatous leprosy, 7 with material from as many different borderline cases, 2 with material from indeterminate cases in transition to lepromatous, and 6 with material from tuberculoid cases, especially reactional forms. A control group consisted of animals inoculated with material sterilized at 121°C.

The inocula employed in subsequent passages from animal to animal were prepared in the same manner as the original inocula.

The animals were autopsied shortly after death. The autopsy included a careful examination of the inoculation site and any suspicious lesions of the skin, as well as examination of the viscera. All smears for bacteriologic examination were stained by the Ziehl-Neelsen method. Whenever a smear with an abundance of bacilli was found, material from the site was prepared for histopathologic study with the electron microscope and for inoculation and cultures.

Of the 1,000 animals used in the series of experiments, 427 had died at the time this paper was prepared. These include 330 hamsters, 72 mice and 25 white rats. The cause of death could be ascertained in only 43 per cent of the total number of dead, and included traumatism, pneumonia, gastroenteritis, hepatitis and septicemia. In the rest the cause of death was unknown.

RESULTS

In the first two passages, lesions were found only in the hamsters, and only in the ear, the site of inoculation. However, in the third passage we found small nodules also in the ear of the white mouse. In subsequent passages, lesions in the hamsters were obtained also at the sites of inoculation in the testicles, the paws and the cheek pouches.

The lesions produced by the inoculations from man to hamster were found after 8 to 10 months had elapsed. They were small nodules only

a few millimeters in diameter located at the site of inoculation in the ear (Fig. 1), but later they became surrounded by smaller nodules. They were found only in animals inoculated with material from borderline leprosy. In no instance were they found after inoculation with lepromatous material. It must be said, however, that in the 7 experimental inoculations made with borderline material from 7 different patients, we have obtained to date positive results from only two. It is an interesting fact that one of those patients (A.M. mentioned above), was a case of the form which Wade designates "relapsed tuberculoid in incipient transition towards borderline." The other patient (T.R.) was a more advanced borderline case with extensive ulcerating lesions of the trunk and limbs.

As regards the subsequent animal-to-animal passages with material from the initial lesions in the hamsters, the period of incubation was only 4 months compared with 8-10 months in the initial man-to-hamster groups. In later passages the incubation period was still less—in most cases only 2 months. These phenomena were observed in all sites of inoculation.

All initial lesions and all lesions appearing in subsequent passages in the hamster, as well as those developed in the mouse with material of the third passage, were studied, as we have already mentioned, bacteriologically, histopathologically, and by means of the electron microscope. At the same time, cultures of the bacilli were attempted.

Enormous numbers of acid-fast bacilli were observed in the smears (Fig. 2), a remarkable contrast to the scarcity of bacilli in the original inoculate. The histopathologic examination of various nodules from initial as well as subsequent passages revealed a monomorphous, histiocytic granuloma with extensive cellular vacuolization and large quantities of bacilli (Figs. 3 and 4). The granuloma was shown to occupy the entire dermis at the site of the lesion, and to be separated from the basal layer of the epithelium by a very narrow band of normal collagen in the vicinity of which there were numerous melanocytes. In many of the latter, acid-fast bacilli were found.

The testicular lesions were characterized as a histiocytic granuloma (Fig. 5) very rich in bacilli (Fig. 6), which was seen to be gradually replacing the parenchyma of the testicle.

All attempts to cultivate acid-fast bacilli in the Loewenstein-Jensen medium from the initial lesions and those of the subsequent series of passages have so far been without results.

DISCUSSION

The working hypotheses that have guided various investigators in their attempts to transmit human leprosy to laboratory animals have all been very different, but they all used lesion material rich in bacilli and consequently lepromatous.

We need to refer only to the works of Adler, Binford, Chatterjee and Bergel to get an idea of how diverse have been the thoughts underlying the experiments of these investigators. Adler thought to destroy the defenses of the animals by splenectomy before inoculation. Binford based his work on the lower temperature of such body parts as the ears and testicles. Chatterjee found, in preliminary experiments, that his hybrid black mouse was particularly favorable for the purpose. He also thought it important to use an inoculum as thoroughly purified of tissue elements as possible, on the supposition that their presence might cause a reaction in the animal that would prevent the inoculated bacilli from surviving long enough to multiply. Bergel based his work with rats on an aberrant metabolism induced by keeping them on a prooxidant diet.

Our own working hypothesis takes into consideration both the animal and the inoculum. We gave preference to the hamster, as it had been shown to be always Mitsuda negative in spite of repeated injections of BCG vaccine by various routes and repeated administration of lepromin ([8]). We also utilized the idea of Binford regarding the choice of site for inoculation. We were convinced, however, that the bacilli present in the inoculum should be of a form in the best possible condition for survival and multiplication in the tissues of the inoculated animal. In other words, it was the parasite-host relation that was uppermost in our minds as the criterion for selecting the inoculum.

If we consider the singular characteristics of lepromatous leprosy with its granuloma, in which bacillus and host cell form a kind of functional entity, it becomes evident that here the bacillus has adapted itself to an intracellular environment in a manner that permits it to multiply greatly. When there is also, as in the lepromatous granuloma, a localized proliferation of histiocytes, we may logically suppose that there is a mutual stimulation involving an exchange of growth factors. There seems to be a metabolic symbiosis in which the mycobacterial parasite proliferates as a stable mutant requiring that particular environment. This would explain its failure to survive when removed from the human host cell and inoculated into animal tissue. In our work we inoculated bacilli from various lepromatous sources into 11 groups of animals, and in no case was a positive result observed.

Another important observation must be mentioned here. In studying the lepromatous granuloma with the electron microscope, it was seen that a high proportion of the bacilli present bore evidence of degeneration, notably in the form of a condensation of the bacillary cytoplasm, and only a small proportion had the appearance of healthy, active germs. The ratio between the degenerate and intact forms varied with the age of the leproma. There was a greater proportion of intact bacilli in new lepromas, while the degenerate forms predomi-

FIG. 1. Induced nodular lesion of left ear of the hamster, the site of inoculation of material from the borderline case A.M. For orientation, one eye (left) is seen at the right end of the picture, and the right ear is at its lower edge. The lesion, as seen, has the appearance of a well-contained, bluish (or faintly greenish) bulb of some size.

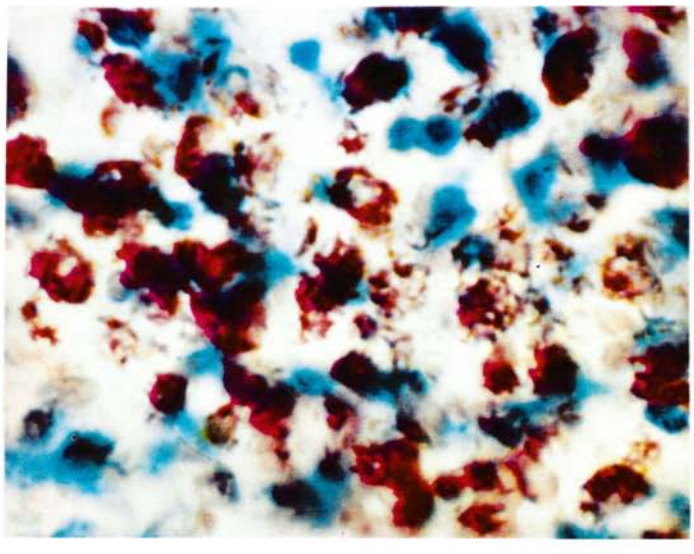

FIG. 2. Acid-fast bacilli in smear from the primary skin lesion shown in Fig. 1. They are mostly in clumps and tangled groups, but there is no evidence of globus formation.

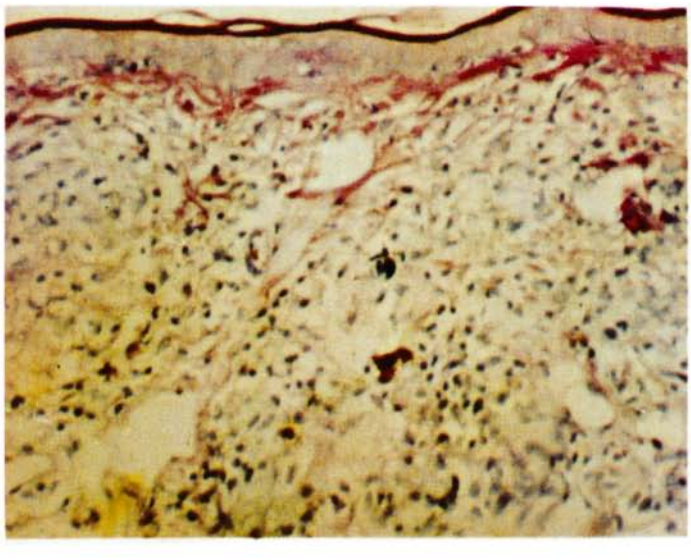

FIG. 3. Low-power photomicrograph of a nodular lesion of the hamster's ear, showing histiocytic granuloma. Hematoxylin and eosin, magnification 16×.

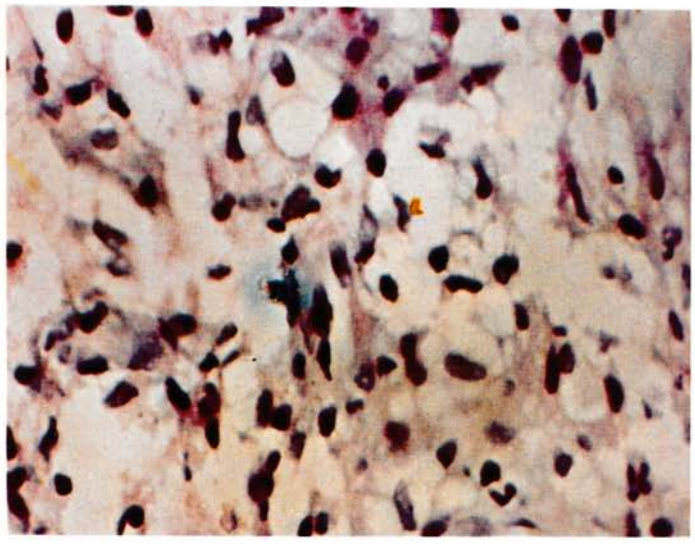

FIG. 4. High-power photomicrograph of the lesion shown in Fig. 3. Magnification 100×.

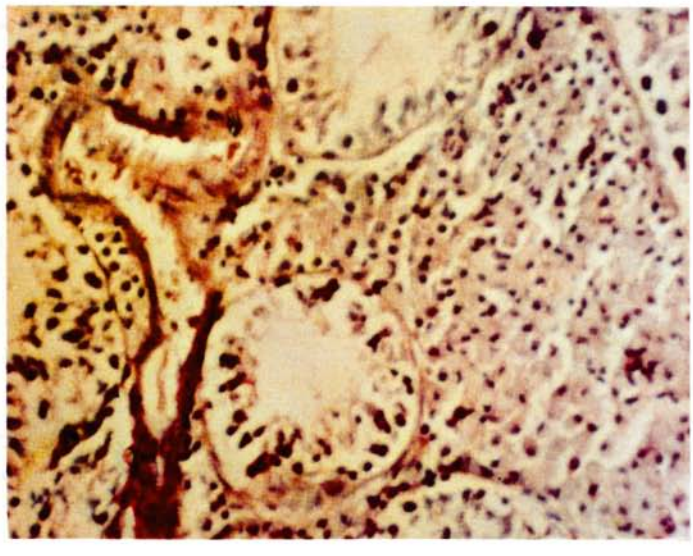

FIG. 5. Photomicrograph of a section of testis, second passage. There is a conspicuous area of histiocytic granuloma in the right end of the picture. Hematoxylin and eosin, magnification 16×.

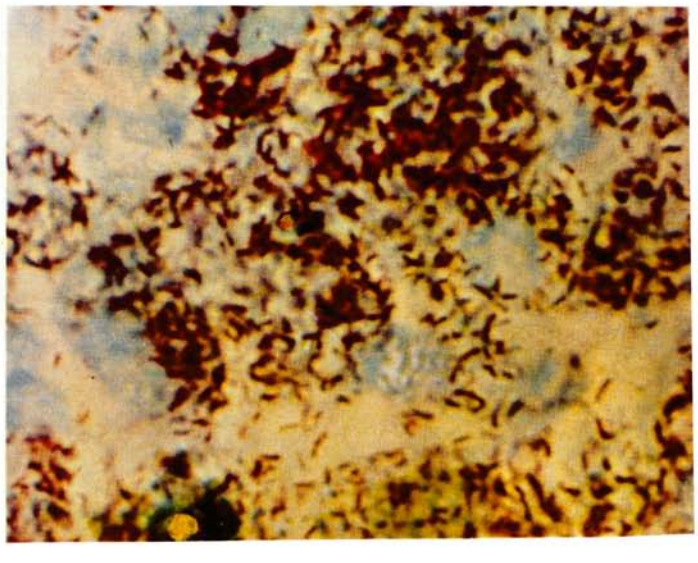

FIG. 6. Bacilli in section of second-passage testicular lesion. Fite-Cambre-Turner stain.

nated greatly in old ones. The materials used in our inoculations were obtained from lepromas in various stages of evolution, but as said the results have so far been negative.

This indicates, from our point of view, that all the bacilli in real lepromatous human tissue, regardless of whether they show up in the electron microscope as degenerate or intact, have adapted themselves completely to the environment. Their genetic type exists in obligatory dependence on the human host cell. In the borderline form of leprosy, on the other hand, we have an exceptional opportunity to secure a variety of *M. leprae* that is still susceptible to adaptation to a new living environment.

It is our impression that the probability of the bacillus becoming adapted to life as a parasite within the tissue of another species diminishes as the lepromatization of the human lesion advances. We thus have to think in terms, not only of borderline lesions as sources of the bacilli, but also of the phase of the bacilli in what is probably a genetic progression, in which the bacillus of early borderline leprosy is the temporarily unstable form capable of mutating to a genetically stable form within the same or another living environment. We have come to think of the bacillus of lepromatous leprosy as the stable terminal phase of the genetic progression, incapable of new adaptive mutations. This does not exclude the possibility of its being susceptible to lethal mutations.

A further consideration of ours has had to do with the peculiar immunologic conditions in borderline leprosy, in which the tissue resistance is of a variable character. Different phenomena of resistance are frequently observed in different sites on the body, as the lepromatization is often advanced or complete in some parts, while it is still in an initial stage in others. It should therefore be quite possible to obtain a positive result with the inoculation of material from one part of a lesion and a negative result with material from a different part of the same lesion. Even when lesions have been carefully selected for anticipated viability in animal tissues, it must be admitted that there is a considerable element of chance with respect to obtaining positive results.

As regards the two instances in our experiments in which the bacilli became adapted to the medium of the animal cell, it must be pointed out that in both of the borderline patients concerned the methylene blue test ([9]) was only weakly positive in certain areas. We conclude from this that the process of lepromatization was still incipient. At the same time, the electron microscope study showed that the bacilli were intact, without any signs of degeneration. They were apparently in a stage of development similar to the hibernation phase in the Schwann cells of the cord of Büngner described by Nishiura ([12]).

We suppose that material from positive reactional tuberculoid cases

could give positive results, while the cases are still in their early stage of evolution, i.e., before the bacilli have suffered the consequences of that tissue reaction that is seen in the later stages of this form of leprosy.

We were able, as said, to produce lesions in all of the hamsters inoculated in successive passages with bacilli of the type adapted in the first passage from man to hamster, and the incubation period finally shortened from 8-10 months to 2 months. Evidently, the bacillus strains have been undergoing gradually a more complete adaptation to the animal environment. At the time of writing the bacillus has proved viable in six successive animal passages.

A singular phenomenon has been observed which should be mentioned here. On two occasions we inoculated two groups of animals with the adapted strain. One of these groups was composed of well-developed, nearly adult hamsters, and the other of a female with her litter of newborn young. All of the animals of the first group developed nodules at the site of inoculation, but in the second group only the mother hamster did so, while nothing could be found in the offspring. At six months of age, no lesions have as yet appeared in that group of hamster siblings. We are inclined to believe that the newborn hamster, being quite imperfect, has not yet developed a capacity for tissue reaction with regard to the inoculated germ. The local phagocytosis, proper for the formation of a granuloma and a basic condition for the survival and multiplication of the bacillus, is probably absent.

The results of our experiments lead us to believe that the most favorable condition for a positive inoculation in animal tissue is one of very slow intoleration to the germ with commencement *in situ* of a chronic inflammatory, granulomatous process by macrophagé cells capable of phagocytizing the bacillus without destroying it, so that it has a chance through adaptation to survive and multiply. This is a somewhat gross, but to our minds logical, interpretation of the delay of 8-10 months before nodules appeared after the primary inoculations. It is to be hoped that the intrinsic mechanism of the adaptation will be explained in a not too distant future in terms of bacterial genetics.

There is a possibility that the formation of a phagocytic granuloma could be induced by injecting, together with *M. leprae*, certain inert substances or other mycobacteria killed by heat. We suppose that *M. leprae* would be phagocytized together with the material introduced for the purpose of local provocation.

In considering the slow growth rate of the bacillus after the primary inoculations, it must be remembered that the numbers of bacilli in the inocula were very small, and yet positive results were obtained. And 60 nodules have been obtained in the six successive hamster-to-hamster passages made. There is no question but that the few bacilli of the original inocula have multiplied greatly.

We must repeat that no local lesions were produced in experimental animals other than the hamsters by material from human lesions. Only with material from the third passage in hamsters did we get a nodule in a mouse seven months after inoculation. This contrasts sharply with the progressive infiltration and large generalized lesions produced in mice by *M. leprae murium*, to which the hamster is said to be particularly susceptible ([7]).

We have had no success in the attempts to cultivate the bacillus, either with material from the initial lesions, or with material from any of the six passages in hamsters. The strain of *M. leprae* that has become viable in the hamster is noncultivable, like its precursor from human leprous lesions.

If it should turn out that it is not true *M. leprae* which produced the lesions obtained, we have at least obtained a mutant strain of that bacillus which is as dependent on the intracellular environment of hamster skin tissue as its precursor would have been on living human skin tissue, if it had reached its final genetic phase.

The difficulties of evaluating the results of these experiments in the light of current knowledge of leprosy are great, the more so as we have no information in terms of bacterial genetics as to what incapacity or aberrance on the part of the bacillus makes it an obligatory intracellular parasite. We believe that further investigation of this phenomenon should be made in the light of the already vast store of facts that have been made available by geneticists and biochemists in the fields of bacterial genetics and bacterial physiology.

One inconvenience in our current investigation has been the comparatively short life-span of the hamster under the conditions of the experiments. It probably does not live long enough for the disease to manifest itself by generalized lesions. It would be a great advantage in future research if another animal, nonreactive to lepromin, with a longer life span could be found.

Our program for future animal experiments includes submitting the animals to the action of substances known to accelerate the process of generalization. We refer to cortisone, x-rays, ultra-violet rays, local traumatization, and local application of substances favoring the extension of lesions. We shall also try to prepare an antigen from the animal lesions in order to compare the reaction it provokes with that of regular lepromin in patients and contacts.

SUMMARY

During the last two years we have conducted a number of experiments, inoculating small laboratory animals with material from human lesions containing *M. leprae*. About 1,000 animals have been used, including close to 600 hamsters, which are nonreactive to lepromin, the rest being mice, both black and white, rats, guinea-pigs and rabbits.

No preparation of any kind was made of the site of inoculation, neither was cortisone or X-rays used to lower the resistance of the animals.

The sites of inoculation selected were the body parts with lowest surface temperature, as recommended by Binford. However, we used another working hypothesis based on the selection of material from different types and forms of leprosy, that is, borderline, lepromatous, reactional tuberculoid, and indeterminate in the course of transformation. At the same time, we considered the stage of development of the disease.

The following considerations were used for the selection of the nonlepromatous cases, especially those of the borderline type in the early stage: The bacilli in lepromatous cases are completely adapted to the intracellular environment, and their metabolism has become genetically fixed to depend on that of the human host cell. It would be difficult for them to survive and multiply in another medium. On the other hand, the bacilli from nonlepromatous cases, in which the resistance of the host fluctuates, have not developed into a genetically stable mutant in the human intracellular environment, although they may in time reach a stage in the same or in another host.

Furthermore, the electron microscope study of bacilli from lepromatous cases usually show important degenerative changes, while those from the initial stages of the borderline form are of uniform aspect and structurally intact. They therefore seemed to us to have greater possibilities of surviving and multiplying when transferred to a host of another species.

The animals were inoculated with lesion suspensions intradermally in the ears, the footpads, the testicles, and in the case of the hamster also in the cheek pouches, in which the temperature is different from that of the other sites mentioned. The material was inoculated in the fresh state immediately after being taken from the patients, and in no case was it submitted to refrigeration. For controls, sterilized material was used.

The bacilli present in the material from the borderline cases were rather scarce, while they were abundant in the lepromatous material—yet the results were positive in the former and negative in the latter. The material from one of the borderline patients (A.M.), a case of relapsing tuberculoid in transformation to borderline, was the poorest in bacilli, and yet it produced the most marked results in the hamsters.

As yet, we have obtained positive results only with the hamster. However, using material from the third passage in hamsters, we also obtained positive results in the ear of a white mouse.

The first lesions obtained in the hamsters were in the ears, but in subsequent passages lesions were also obtained in the testicles, the paws and the cheek pouches.

The period of incubation or adaptation was 8-10 months in the first

passage, with the production of a small nodule at the site of inoculation in the ear and the subsequent appearance of small nodules in the immediate vicinity. Such nodules have developed only with the inoculation of borderline material, and in no case have we observed them in animals inoculated with lepromatous material.

We have made to date six successive passages from hamster to hamster, all with positive results. In the first passage from hamster to hamster the period of incubation or adaptation was 4 months, but in later passages it has been only 2 months and even less.

The hamster nodules were studied histologically and bacteriologically, as well as with the electron microscope. Attempts were made to cultivate their bacilli in various media.

Acid-fast bacilli were found in great numbers in the smears, their abundance contrasting remarkably with the scarcity of bacilli in the first inoculum from a human source, indicating undoubted multiplication.

Histologically, the lesion is a granuloma resembling the human leprous granuloma, containing numerous bacilli that could be demonstrated by the Fite-Cambre-Turner method.

The electron microscope study showed intracellular features similar to those of lepromatous leprosy.

All attempts to cultivate the bacilli in different media have so far given only negative results.

RESUMEN

Durante los últimos dos años hemos hecho un número de experimentos con la inoculación de pequeños animales de laboratorio con material de lesiones humanas que contenían *M. leprae*.

Hemos usado aproximadamente 1,000 animales en estos experimentos, inclusive más o menos 600 hamsters, siendo el resto ratones, tanto negros como blancos, ratas, cobayos y conejos. Un total de 12 cerdos también fueron inoculados.

Non se hizo preparación alguna del sitio de la inoculación, in se usó cortisona, ni rayos x para bajar la resistencia de los animales.

Escogimos como sitios de inoculación las partes de temperatura superficial mas baja como lo recomenda, Binford. Sin embargo, nuestra hipótesis de trabajo fué otra, basándose en el uso de material de diferentes tipos y grupos de lepra, es decir, borderline, lepromatosa, tuberculoide reaccional e indeterminada en transformación lepromatosa. Al mismo tiempo hemos tomado en consideración la etapa evolutiva de la enfermedad.

Nuestro criterio para la selección de los casos no lepromatosos, especialmente los del tipo borderline en sus etapas tempranas, ha sido el siguiente: Los bacilos de los casos lepromatosos se han adaptado completamente al ambiente intracelular y su metabolismo ha sido fijado geneticamente para depender obligatoriamente de la célula huesped humana, de modo que les sería dificil sobrevivir y multiplicarse en otro medio. Por otra parte, los bacilos de casos no lepromatosos, en los cuales la resistencia del huesped oscila, no han llegado a formar un mutante estable en el ambiente intracelular humano, aunque puede con el tiempo llegar a tal estado en el mismo o en otro huesped. Al mismo tiempo, el estudio con el microscopio electrónico reveló que los bacilos de casos lepromatosos habian sufrido importantes cambios degenerativos, mientras que los de casos borderline en sus etapas iniciales eran de aspecto uniforme y de estructura intacta.

Esta forma de bacilo nos pareció que tenía más probabilidad de sobrevivir y multiplicarse al ser traspasado a un huesped de otra especia.

Los animales fueron inoculados intradermicamente en las orejas, las plantas, los testículos y algunos de los hamsters también en las bolsas faríngeas. La temperatura en estas es diferente de la de los otros sitios mencionados.

El material fué inoculado en estado fresco, es decir, inmediatamente después de haberse removido del paciente. El material humano no fué sometido a refrigeración en ningún caso.

Para los grupos de control se usó material esterilizado. Los bacilos presentes en el material de los casos borderline eran escasos, mientras que abundaban en el material de los lepromatosos, pero con este los resultados fueron negativos, mientras que con aquel fueron positivos. El material más pobre en bacilos fué el del paciente A.M., un caso tuberculoide de recaida en transformación a borderline y, sin embargo, produjo los resultados más notables cuando fué inoculado en el hamster.

Hasta el presente solamente hemos obtenido lesiones en el hamster dorado (*Cricetus auratus*) cuando inoculamos material humano, pero con material del tercer pase en hamsters obtuvimos también nódulos en la oreja de un ratón blanco.

Las primeras lesiones observadas en el hamster fueron en la oreja, el sitio de la inoculación, pero en los pases subsiguientes se observaron lesiones también en testículos, patas y bolsas faríngeas.

El período de incubación o adaptación fué de 8 a 10 meses en los hamsters del primer pase en que se produjo un pequeño nódulo en el sitio de inoculación en la oreja, seguido más tarde por nódulos más pequeños en el mismo sitio. Estos nódulos se produjeron solamente con la inoculación de material borderline y en ningún caso los hemos observado en animales inoculados con material de casos lepromatosos.

Hasta la fecha hemos realizado seis pases de hamster a hamster y todos han sido positivos. En el primer pase de hamster a hamster el período de incubación fué de 4 meses, es decir, la mitad del tiempo observado en el pase de hombre a hamster y en los pases subsiguientes se redujo a 2 meses y hasta menos.

Los nódulos fueron estudiados desde el punto de vista histopatológico y bacteriológico, como tambien en el microscópio electrónico. Se hicieron ensayos de cultivo en diferentes medios.

Bacilos acidorresistentes se encontraron en gran cantidad en los frotis. Su multiplicación extraordinaria contrastaba grandemente con su escasez en el inóculum de lesiones humanas.

El examen histopatológico puso en evidencia un granuloma parecido al granuloma leproso humano y que contenía numerosos bacilos coloreables por el método Fite.

El examen en el microscopio electrónico mostró que las células tenían ciertos componentes semejantes a la lepra lepromatosa.

Todos nuestros ensayos de cultivo en diferentes medios no han dado sino resultados negativos hasta ahora.

RESUMÉ

Au cours des deux derniéres années, nous avons mené un certain nombre d' experiences en inoculant de petits animaux de laboratoire avec du matériel provenant de lésions humaines et contenant *M. laprae,* Environ 1,000 animaux ont été inocules, parmi lesquels prés de 600 hamsters, qui ne réagissent pas à la lépromine, le reste consistant en souris, blanches et noires, rats, cobayes et lapins. Le site d'inoculation n'a été préparé en aucune manière; de même ni cortisone ni rayons X n'ont été utilisés pour dimineur la résistance des animaux.

Les endroits choisis pour inoculation étaient situés sur les parties du corps à température la plus basse, comme il a été recommandé par Binford. Notre hypothése de travail, toutefois, était autre, et basée sur le choix du matériel d'inoculation, qui

provenait de différents types et de différentes formes de lèpre : border-line, lépromateux, tuberculoide réactionnel et indeterminé en voie de transformation. En même temps, nous avons tenu compte du stade de la maladie.

Les données suivantes ont été prises en considération pour la sélection des cas non-lépromateux, et particulièrement pour celle des border-line débutants. Chez les lépromateux, les bacilles sont complétement adaptés a leur environnement intra-cellulaire, et leur génotype a subi une transformation qui rend leur métabolisme étroitement dépendant de celui de la cellule humaine qui leur sert d'hôte. Il leur serait difficile de survivre et de se multiplier dans un autre milieu. D'autre part, chez les cas non-lepromateux, ou la resistance de l'hôte oscille, les bacilles n'ont pas donne naissance à un mutant génetiquement stable dans le milieu humain intra-cellulaire, quoiqu'ils puissent à la longue atteindre un tel état chez le même hôte ou chez un hôte différent.

De plus, chez les bacilles provenant de lépromateux, le microscope électronique montre géneralement des signes importants de dégenerescence, alors que ceux provenant des border-line débutants sont d'un aspect uniforme et témoignent d'une structure intacte.. Il nous a dè lors sembléque ces derniers bacilles avaient plus de chances de survivre et de se multiplier lors du transfert à un hôte d'une espèce differente.

Les animaux ont été inoculés avec des suspensions provenant des lésions et introduites par voie intra-dermique dans les oreilles, la plante des pattes, les testicules, et aussi chez le hamster dans les bajoues, oú la température est differente de celle des autres sites mentionnés. Le matériel a été inoculé à l'état frais, immédiatement après avoir été prélevé chez le malade. En aucun cas il n'a été refrigéré. Comme contrôlen on a utilisé du matériel stérile.

Dans le matérial provenant des malades border-line, les bacilles étaient plutôt rares, alors qu'ils étaient abondants dans le matériel lépromateux—et pourtant, les résultats ont été positifs dans le premier cas et négatifs dans le second. Le matériel obtenu chez un des sujets border-line (A.M.), un malade atteint de lèpre tuberculoíde récidivante en transformation border-line, était le plus pauvre en bacilles, et cependant il a donné les résultats les plus prononcés chez les hamsters.

Jusqu'à présent, nous n'avons obtenu des résultats positifs que chez le hamster. Toutefois, avec du materiel provenant du troisième passage chez le hamster, nous avons également obtenu des résultats positifs dans l'oreille de la souris blanche.

Les premières lésions relevées chez le hamster étaient limitées aux oreilles, mais lors des passages ultérieurs nous en avons obtenu aussi dans les testicules, les pattes et les bajoues.

La période d'incubation, ou d'adaptation, a été de 8 à 10 mois lors du premier passage, avant qu'un petit nodule apparaisse à l'endroit d'inoculation au niveau de l'oreille, suivi de l'apparition ultérieure de plus petits nodules dans le voisinage immédiat. Les nodules décrits n'ont été constatés qu'àprès inoculation de matériel border-line, et nous ne les avons jamais observés chez des animaux inoculés avec du materiel lépromateux.

Jusqu'à present, nous avons procédé à six passages de hamster à hamster, tous avec des résultats positifs. Lors du premier passage de hamster à hamster, la période d'incubation ou d'adaptation était de 4 mois, mais lors des passages ultérieurs elle n'a plus été que de 2 mois ou même moins.

Les nodules du hamster ont fait l'objet d'études histologiques et bacteriologiques, aussi bien que d'examens au microscope électronique. Des essais ont été menés pour cultiver les bacilles de ces lésions sur différents milieux.

Des bacilles acido-résistants ont été trouvés en grand nombre dans les frottis, leur abondance contrastant de façon remarquable avec la rareté des bacilles dans le premier inoculat provenant de source humaine. Ceci indique une multiplication indubitable.

Au point de vue histologique, la lésion consiste en un granulome qui ressemble au granulome de lèpre humaine, et contient de nombreux bacilles acido-résistants qui peuvent être colorés par la méthode de Fite-Cambre-Turner.

Les études par le microscope électronique montrent des structures intra-cellulaires semblables à celles que l'on trouve dans la lèpre lépromateuse.

Tous les essais poursuivis pour cultiver les bacilles sur différents milieux sont, jusqu'à présent, demeurés négatifs.

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