EXPERIMENTAL TRANSMISSION OF MURINE LEPROSY TO THE GUINEA-PIG BY MEANS OF INDUCED MACROPHAGE EXUDATE AND SUPPRESSION OF THE NATURAL DEFENCE MECHANISM

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The problem of transmission of human leprosy to laboratory animals remains unsolved, and presents a serious barrier to the study of the disease. Very many attempts have been made without success to produce the infection in animals, usually without but sometimes with attempts to suppress their defence forces.

In approaching this problem it seemed to be logical to undertake to lower the native resistance of the animal to be used. The experiments were unavoidably conducted on a rather empirical basis, since the factors which govern resistance and susceptibiltiy are still unknown. Until more basic research is done to obtain fundamental information concerning the pathogenesis of the disease, and concerning factors which influence the host-parasite relationship and determine the outcome of the infection, the probability of success in the experimental transmission of human leprosy is rather small.

Studying the behavior of macrophages from susceptible and resistant animals toward the murine leprosy bacillus (24, 25), some observations were made which suggested the possibility of transmission of rat leprosy to the resistant guinea-pig. The theoretical basis, the experimental set-up, and the negative and the positive results obtained will be reported here, since the experiment may serve as a model for similar studies with the human leprosy bacillus.

The problem can be formulated as follows: Would it be possible to transfer experimentally into a resistant host a chronic infectious disease caused by an obligate intracellular parasite, characterized by an intense tendency to invade specific host cells and by extreme susceptibility to extracellular inhibitors? The following factors must be taken into consideration.

THE THEORETICAL BASIS OF THE APPROACH

The antihistamines.—Our earlier investigations with the tubercle bacillus showed that the cellular defence can be markedly stimulated by appropriate treatment (8, 9, 10, 21), and that it can be inhibited by synthetic antihistaminic substances (8, 22). These observations suggested similar investigations on experimental murine leprosy, the expectation being that antihistaminics would have an aggravating effect as they had in experimental tuberculosis. As a first attempt, 100 albino rats were inoculated with murine leprosy bacilli and 50 were treated with an antihistaminic (mepyramine

[1] With the technical assistance of Miss Estelle Giasson and Miss Monique Sabourin.

maleate), the other 50 serving as controls. Treatment was given daily for two months. Unexpectedly, large granulomas developed in both groups of animals, with no macroscopic or histologic differences.

It was then decided to investigate more closely this peculiar difference between the two experimental infections, tuberculosis and murine leprosy. Using the technique of surviving macrophages, we found that the cells of both species, the rat and the guinea-pig, are stimulated to an increased phagocytic activity against tubercle bacilli by histamine, whereas small amounts of antihistaminic substances inhibit phagocytosis of those bacilli (22, 24). On the contrary, with the murine leprosy bacilli the presence of synthetic antihistaminic substances failed to inhibit the rapid invasion of the macrophages of both species. In these experiments, again, striking differences were found in the macrophage-parasite relationship in tuberculosis and murine leprosy.

A noteworthy observation was that, normally, the murine bacilli are rapidly reduced to acid-fast debris or nonacid-fast particles in the macrophages of the guinea-pig, but that in the presence of the antihistamine they remain unchanged for a relatively long period of time. It seemed that the lytic activity of the nonsusceptible macrophage was paralyzed by the antihistamine, and that an undisturbed biosphere was created between the nonsusceptible host cell and the parasite. This observation suggested the possibility of experimental transmission of rat leprosy to the guinea-pig under the protection of the antihistamine.

Furthermore, this investigation seemed promising because synthetic antihistaminics inhibit the following defence processes closely related to the functions of the reticuloendothelial system: (*a*) the increased capillary permeability in the inflammatory process (4, 7, 20, 28); (*b*) the acquired phagocytic activity of the capillary endothelium (18, 4, 26, 7, 20); (*c*) the activation of histamine in the tissue (18, 28); (*d*) in larger doses, the chemotactic action of leucotaxin (4); (*e*) the action of the leucocytosis-promoting factor in the bone marrow (27, 28); (*f*) the phagocytic activity of Kupffer cells of the liver (18, 19); and (*g*) the phagocytic activity of the reticuloendothelial system (18, 19, 20, 23).

Halpern and his co-workers (13) treated guinea-pigs with antihistamines and found that a nonpathogenic flora of the intestinal tract became pathogenic; and they also reported (11, 12) that a local inflammatory process in guinea-pigs induced by intradermal injection of *Salmonella typhi murium* developed fatally. Petri *et al.* (28) observed an aggravation of the inflammatory process in rabbits infected intradermally with *Micrococcus aureus* and treated with antihistamine. The harmful effects of antihistamines on experimental tuberculosis of mice and guinea-pigs (8) has been mentioned.

An anticomplementary compound, sodium polyanethol sulphonate.—This compound, called Liquoid (Roche), is known as a powerful anticoagulant (5). Battistini (3) found that it inactivates complement and destroys the bactericidal power of blood plasma. Auxilia (2) showed that it interferes with the phagocytic activity of leucocytes. According to Allgöwer (1), traces of Liquoid not only prevent phagocytosis completely, but exert an inhibitory effect on the migration of leucocytes. I found (25) that it inhibited phagocytic activity of monocytes of the guinea-pig and the rat versus tubercle bacilli, and, among several substances tested, was the only one which prevented the phagocytosis of murine leprosy bacilli by the macrophages of the rat and the guinea-pig.

The following observation was made, among others. If 10 to 100 μmg/ cc of Liquoid is added to a suspension of guinea-pig monocytes containing 5 per cent serum, phagocytosis of murine leprosy bacilli is considerably inhibited. In control tubes without Liquoid most of the intracellular bacilli, and those which are extracellular and therefore exposed to the environment, are reduced to acid-fast debris within a few hours or a day, whereas in the presence of Liquoid the bacilli remain unchanged, acid-fast rods, for the same period of time. This finding can easily

be explained by the complement-inactivating action of Liquoid, in consequence of which all the cellular and humoral defence processes mediated by complement are inhibited. According to Hanks and Gray (17), the hydrogen transfer capacity of *M. leprae murium* is considerably reduced in the extracellular environment. However, the possible role of complement in this mechanism is not excluded.

Because of these observations, Liquoid was used in experiments made for the experimental transmission of rat leprosy into the guinea-pig.

Other anticoagulants.—Since Liquoid is anticoagulant as well as anticomplementary, an experiment was devised to ascertain whether its action in animals is due to its inhibition of fibrin formation or to its inhibition of complement. The pathways of blood clotting—in which 29 substances participate—can be inhibited at different levels. Therefore, the influence of two types of anticoagulants on the transmission of rat leprosy to the guinea-pig was studied. These were heparin, which inhibit the action of thromboplastin, thus increasing the clotting time, and the coumarin derivatives, which lengthen prothrombin time by decreasing prothrombin concentration of the blood, presumably by retarding its production in the liver.

Administration of both anticoagulants may lead to an excess of fibrinogen instead of profibrin or fibrin in the inflammatory process induced by the injection of the murine leprosy bacilli. Therefore the action of fibrinogen and fibrin on the outcome of experimental transmission was similarly investigated.

Choice of animal.—So far as has yet been shown both human leprosy and rat leprosy are very host-specific. All other species except the susceptible host can be considered resistant, and experimentation with any animal species not yet used would be but a shot in the dark. However, I share the opinion of Feldman (6) that "failure of transmission is not due to the type of animal used but rather to the wrong methodology." It seems most promising to turn to investigations of factors which govern native resistance, although it has to be recognized that very little is known of the matter. We have proposed (24, 25) to create models *in vitro*, like surviving macrophages of the resistant host, and to study systematically the fate of the murine bacilli in the macrophages of the susceptible and of resistant hosts under the influence of mediators and substances which are known to inhibit or stimulate the ingestive and digestive activities of the reticuloendothelial cells. The methods used until now to lower the native resistance, like cortisone, alloxan, propyl-thiouracyl, avitaminosis, protein deficiency, silicon dioxide, etc., are but adventuring in the solution of this enigma, and a systematic search for a specific antagonist of intracellular and extracellular inhibitors of the infectiousness of murine leprosy bacilli is a basic necessity for any further advance in this field.

Once it was observed that in the presence of mepyramine maleate the macrophages of the guinea-pig lose their capacity to digest rapidly the murine leprosy bacilli, and that in the presence of Liquoid the bacilli remain unchanged in the extracellular environment, the guinea-pig was the animal of choice for the experimentation in transmission. There are also the advantages of known uniformity of response to infections, and the fact that a uniform peritoneal macrophage exudate can be induced.

Route and method of transmission.—The experiments of Hanks and coworkers (14-17) have brought us nearer to an understanding of the role of factors which influence the infectiousness of the transferred inoculum and the transmissibility from one animal to the other. We learn from their findings that during the preparation of the rat leproma homogenates for inoculation the bacilli are invariably exposed to unfavorable environmental factors. After the inoculation the bacilli lie in the tissues of the new animal, again exposed to the damaging action of extracellular inhibitors. Hanks and Gray (17) also postulated that natural extracellular inhibitors may be responsible for the failure of experimental transmission of human leprosy.

One may speculate how it would be technically possible to transfer murine leprosy from one animal to another without exposing the parasite

to injury by the natural extracellular inhibitors. It is necessary to create favorable environmental conditions in the resistant host, and for that the following conditions must be fulfilled: (1) an infecting material must be obtained which is as pure a suspension as possible of bacilli with adequate hydrogen transfer capacity; and (2) a favorable biosphere must be induced in the animal to be infected at the site of inoculation, containing a huge number of healthy macrophages ready to be parasitized at the time of inoculation.

To fulfill the first requirement, we took advantage of Hanks' experiments in preparing the bacillary suspension. To obtain a favorable environment for the transmission, the following technique was devised. A cellular exudate is induced in the peritoneal cavity of the guinea-pig as by Suter (29). On the fifth day after administration of glycogen, when the peritoneal exudate contains a great number of monocytes, the infective material is injected intraperitoneally. Without being exposed for a long time to the damaging body fluids, the bacilli are ingested within minutes by the macrophages. The method will be referred hereafter as the "macrophage-exudate technique" (MET).

MATERIALS AND METHODS

The macrophage-exudate technique (MET).—The Hawaiian strain of *M. leprae murium* was maintained in Wiersing rats, as previously described (24). The infective material for the inoculations was prepared from 12-weeks-old lepromas. The nonnecrotic granulomatous tissue, cleaned of connective tissue and fat and tested with tetrazolium violet, was ground under aseptic conditions with sea sand in a mortar and gradually diluted with saline to obtain a 1:10 suspension. The bacilli were not washed, but the complete tissue homogenizate was used as the infective material to avoid the separation of metabolites, or factors necessary for infectiousness. Heat-killed bacilli used in certain control experiments were obtained by heating the diluted leproma homogenizate at 100°C for 20 minutes.

Guinea-pigs of both sexes weighing 450±20 gm. were used. The animals were fed with pellet diet and cabbage and carrots *ad libitum*. They were of mixed colors, to avoid the use of more resistant individuals within the species, as it is known that white ones are somewhat more resistant to tuberculosis than others. Five days before the inoculation, the animals were injected intraperitoneally with 10 cc. of physiologic saline containing 0.1 mgm. of glycogen. This is the most "physiological" way to obtain a peritoneal exudate containing more than 90 per cent of macrophages among the cellular elements. On the fifth day, at which time an immense number of macrophages are present in the peritoneal cavity, 1 cc. of the freshly-prepared leproma suspension was injected intraperitoneally.

Transmission from one guinea-pig to another was done with the MET as from the rat to the guinea-pig, with the difference that a 1:5 leproma suspension was used for the inoculation. Transmission from the guinea-pig back to the rat was performed by subcutaneous injection of 0.5 cc. of a 1:5 suspension of the peritoneal granuloma of the guinea-pig.

Experimental substances used.—The synthetic antihistamine used was mepyramine maleate (Neoantergan)[2] dissolved in saline to contain 8 mgm. in 0.5 cc. and sterilized at 60°C for one hour. Guinea-pigs received 0.5 cc. subcutaneously daily (except Sundays) during the whole period of the experiment. The first injection was given

[2] Pyranisamine, or N-p-methoxybenzyl-N-N'-dimethyl-α-pyridylethylene diamine.

30 minutes before the inoculation. As in all other experiments, the control animals received daily injections of the same amount of physiological saline.

The complement inhibitor used was sodium polyanethol sulphonate (Liquoid, Roche), 0.5 per cent in saline. Guinea-pigs received intraperitoneally, daily except Sundays, 2.5 mgm. in 0.5 cc. of the solution throughout the experiment. The first dose was given 30 minutes before inoculation.

Three anticoagulants were used. A 0.5 per cent saline solution of commercial heparin, sterilized at 60°C, was injected daily intraperitoneally in 0.5 cc. doses, containing 2.5 mgm. of the substance. The two anticoagulants which retard prothrombin production were Marcumar (Roche)[3] and Syntron (Geigy).[4] Both compounds were given orally, mixed in the ground pellet food. The daily dose of Marcumar was 1 mgm. and of Syntron 0.5 mgm. In a few cases, if bleeding from the ears or mouth or vagina occurred, administration of the drug was interrupted for 24 hours. This interruption did not increase the prothrombin time.

Commercial fibrinogen was injected intraperitoneally, 3 mgm. in 0.5 cc. saline, daily during the first 20 days of the experiment when it was used. Fibrin powder was ground fine in a mortar and suspended in saline, approximately 3 mgm. being given intraperitoneally during the first 5 days. These two substances were not sterilized, but prepared under aseptic conditions.

Evaluation of lesions.—This was done by visual readings at autopsy. Smears were made from the peritoneal granulomas, and from the liver, spleen, suprarenal gland, testicle, and the peritoneal and mediastinal lymph nodes. Histologic sections were made of the granulomas, the liver, and the spleen.

RESULTS

Effects of heat-killed bacilli.—Eight guinea-pigs were inoculated by the MET with the heat-killed bacillus suspension. Four of them were treated with the antihistamine, while the 4 controls received only saline. All were killed after 9 weeks, and their peritoneal cavities were washed with 10 cc. of saline. No acid-fast bacilli were found in the centrifugates. In 2 animals in the control group a few small pinpoint granulomas were found on the omentum, and smears contained a very little extracellular acid-fast debris and a few histiocytes and fibrocytes. The spleens of all animals were of normal size, with no acid-fast elements in smears.

Effects without the macrophage exudate technique.—Another 8 guinea-pigs were inoculated intraperitoneally without using the MET, 4 receiving the antihistamine and 4 serving as controls. All remained of healthy appearance and gained weight normally. In the animals of both groups, killed after 9 weeks, the omentum lay retracted high under the stomach and showed a fibrous inflammatory reaction and dilated capillaries. In both groups a few small compact masses were found in the retracted omentum, some of them as hard as cartilage. These masses were encapsulated, not larger than 1-5 mm. in the control group and 1-8 mm. in the antihistamine group. Smears of these masses revealed, in both groups, very few acid-fast bacilli, large amounts of acid-fast debris, and nonacid-fast particles. No bacilli were found in the spleen, liver, suprarenal glands or testicles, or

[3] Marcumar (Roche), phenylpropylhydroxycoumarin = 3-(1′-phenyl-propyl) -4-hydroxycoumarin.

[4] Syntron (Geigy), nitrophenylacetylethyloxycoumarin = 3- [α- 4′-nitrophenyl-acetylethy *1*)]-4-oxycoumarin.

the peritoneal or pelvic lymph nodes. The omental lesions of all 8 animals, weighing 1.6 gm. all together, were made into a 1:5 suspension. This homogenizate was injected subcutaneously into 8 Wiersing rats, which were killed in groups of 2 after 4, 8, 12 and 18 months. No sign of the disease was found in the gross, in smears, or in tissue sections. The inoculated material had disappeared without trace.

Transmission to the guinea-pig by the macrophage exudate technique. —Twelve guinea-pigs were inoculated with rat leproma homogenizate intraperitoneally with the MET, and 6 were treated with mepyramine maleate while 6 received only saline injections. One animal of each group was killed one hour after the inoculation. With each, great numbers of healthy macrophages were found in the peritoneal exudate. The peritoneal cavity was washed out with 25 cc. of Hanks' solution, and the wash recovered was found to contain about 160,000 mononuclear macrophages per cubic centimeter. The macrophages of both animals were heavily parasitized with acid-fast bacilli, as shown in Figure 1. Only relatively few acid-fast rods lay extracellularly, having escaped ingestion by the macrophages. There was no difference between the control and the treated group concerning the aspect of the parasitization and the shape, size and staining properties of the bacilli.

Twenty-four hours after inoculation a similar pair of animals was killed, one from each group. The findings this time were different. Both exudates contained immense numbers of healthy, mobile macrophages. In the control guinea-pig most of the bacilli were reduced to acid-fast debris and nonacid-fast particles, as shown in Figure 2, only a relatively small number of extra- and intracellular bacilli having retained their usual shape and acid-fastness. The exudate of the antihistamine-treated animal showed the same picture as the one-hour material, with a tendency of the macrophages to agglomerate (Figs. 3 and 4). The cells showed a sponge-like structure of cytoplasm, huge pseudopodia, and heavy parasitization with strongely acid-fast rods.

The remaining 8 guinea-pigs were killed and autopsied 8 weeks after inoculation. No pathologic changes were found in the organs of the 4 control animals, and no acid-fast rods or debris in smears of the organs. The omenta were thickened, and near the stomach 1 to 2 small pinpoint nodules were present. In smears of these there was found a very little acid-fast debris, and also some lymphoctyes, fibrocytes and destroyed macrophages inbedded in a fibrous network and fatty tissue.

Among the 4 mepyramine maleate-treated animals, one white female showed no lesions at all. In the three others there were huge granulomas of the omentum, loaded with bacilli. The lobulated growths were surrounded by a capsular tissue, seemingly the layers of the omentum, densely vascularized at the surface, and some of them were necrotic centrally. The weights of these tissue growths in the three animals, freed aseptically from loose connective tissue and fat, were 5.8, 8.9, and 9.6 gm., respectively.

Further bacillus-rich granulomas were found on the peritoneum and in the abdominal wall of two animals of the mepyramine group, at the site where the needle penetrated at the time of inoculation. Lesions were also found on the mesentery. Figure 6 shows the peritoneal granulomas gathered from the 3 antihistamine-treated guinea-pigs in comparison with those found in the untreated controls.

Spleens of all the antihistamine-treated animals were enlarged up to twice their normal size, and smears revealed large numbers of acid-fast rods, and debris. Similarly, acid-fast rods were found in the suprarenal glands, and in the enlarged peritoneal and pelvic lymph nodes. On the surface of the liver of one guinea-pig there was a necrotic layer 5 x 10 mm. in size and about 1 mm. deep; smears showed innumerable acid-fast bacilli. No acid-fast elements were found in the testicles, kidneys or liver, or in the inguinal, axillary or mediastinal lymph nodes.

A sample of omental granuloma was ground up in saline (1:5), and 8 Wiersing rats were inoculated (0.5 cc. subcutaneously), and 6 guinea-pigs (1 cc. intraperitoneally, by the MET). Various culture media (peptone bouillon, agar, blood agar, deep agar, VF medium, Dubos-liquid, Kirshner and Loewenstein) were inoculated from all the lesions in which acid-fast bacilli were found; all remained sterile. Specimens were saved for histologic examination.

The results of the histologic examinations were as follows:

Liver: Architecture well preserved. Scattered infiltrations throughout the area. Some regions show enormously dilated vessels, and around them dilated sinusoids. The small foci of infiltrations consist of large macrophages and a moderate number of small round cells. In many large areas, generalized necrosis of liver epithelial cells.

Spleen: Enlarged, showing hypermia and necrotic white pulps. The total depletion with necrosis of the white pulp cells is combined with the appearance of round cells and Langhans-type cells.

Abdnominal granuloma: The section is of a large granuloma from the omentum. This is encapsulated, and within its borders are shown different foci of smaller granulomas. The inter-granulomatous spaces consist of diffusely infiltrated omentum itself. Each of the granulomas consists of macrophages and large numbers of round cells and Langhans-type cells.

Transmission from guinea-pig to guinea-pig.—Of the 6 guinea-pigs that were subinoculated with the MET, 4 were treated with the antihistamine and 2 were left untreated as controls. All were killed 6 weeks thereafter. At autopsy, the same observations were made as in the original experiment: complete, or nearly complete, disappearance of the inoculum in the control animals, and the same macroscopic and microscopic lesions in the treated group. Again there was 1 animal, a white female, which developed practically no lesions despite the antihistamine treatment. The weights of the omenta of the 3 with lesions were 4.7, 6.2, and 8.2 gm.

Every 6 or 12 weeks thereafter the same transfer to new guinea-pigs was performed, and continual treatment with mepyramine maleate. Similar lesions deevloped in the course of seven subsequent transmissions, in the treated animals, during 18 months.

Reverse transfer, from the guinea-pig to the rat.—The 8 rats that were inoculated subcutaneously with the leproma suspension of the first experiment were all found to have small palpable nodules five weeks later. All were killed after 5 months, and all were found to have lesions macroscopically and histologically typical of rat leprosy, loaded with acid-fast bacilli typical of *M. leprae murium*. The 8 granulomas, which weighed 5 to 14 gm. each, were tested with tetrazolium violet and 7 gave positive reactions. The lesions found in the peritoneal cavities of the treated guinea-pigs will therefore be called guinea-pig lepromas, and the acid-fast rods found in them can be considered definitively as living *M. leprae murium* of high infectiousness.

Failure of transmission into white guinea-pigs.—After having seen, in two experiments, that white guinea-pigs inoculated with the MET and treated with the antihistamine developed no lesions, a rat leproma homogenizate was injected intraperitoneally into 8 white guinea-pigs and 2 of mixed colors with the MET. All animals were treated with mepyramine maleate and killed after 9 weeks. Both guinea-pigs of mixed colors developed the usual large lesions, whereas small granulomas (0.5-1 gm.) or none at all were found in the white ones, whether male or female.

Results with the anticomplementary substance, sodium polyanethol sulphonate.—Twelve guinea-pigs were injected as usual with rat leproma homogenizate, and 6 were treated with sodium polyanethol sulphonate (Liquoid, Roche). One hour later, 1 animal of each group was killed. In the control animal were found great numbers of healthy peritoneal macrophages, heavily laden with bacilli. In the Liquoid-treated animal, however, many of the macrophages were destroyed, and most of the remaining mononuclear cells were shrunken, rounded, and presented but small pseudopodia. The few healthy macrophages were parasitized with bacilli, but most of the acid-fast rods were extracellular, of normal acid-fast appearance.

After 24 hours another pair of guinea-pigs was killed. In the macrophages of the control animal the bacilli were reduced to acid-fast debris and nonacid-fast particles. Smears from the Liquoid-treated animal showed the same condition as before: mostly-destroyed macrophages and healthy acid-fast rods outside of the cells (Fig. 5).

The remaining 8 animals were killed 8 weeks after inoculation. The 4 controls showed practically no lesions. The 4 in Liquoid group all showed macroscopic lesions similar to those in the antihistamine-treated animals described above, except that there were more necrotic lesions on the surface of the liver which penetrated more deeply into the liver substance. Histologically these liver lesions showed periportal round-cell infiltration.

The nodular granulomas of the omentum were considerably larger than those of the antihistamine-treated group, weighing 5.5, 7.5, 9.8 and 12.2 gm., respectively. Acid-fast rods in extremely large numbers were found in these lesions, and in those of the mesentery, but few were present in

the smears of the spleen, suprarenal gland, or mesenteric lymph nodes. It was surprising to note that in smears from any of the lesions it was exceptional to find any cellular elements in large numbers. The acid-fast rods were mostly extracellular, although they were strongly acid-fast and of the usual shape; sometimes the smears had an appearance of a pure culture of acid-fast bacilli.

Without going into details, it may be said that the granuloma of the Liquoid-treated guinea-pig was transmissible to other guinea-pigs subjected to the Liquoid treatment. Similarly, the infection was also retransmissible to the rat.

Effects of other anticoagulants, and fibrinogen and fibrin.—Experiments comparable to those related were carried out with heparin, Marcumar, Syntron, and with fibrinogen and fibrin. These will not be discussed in detail because the results were entirely negative. No noteworthy lesions developed in any of the animals in observation periods of 8 weeks.

Tetrazolium violet tests with the peritoneal granulomas from the guinea-pig.—Small pieces of the granulomas of all the guinea-pigs were put into tubes containing 0.5 cc. of a freshly-prepared 0.2 per cent solution of tetrazolium violet and incubated for 15 minutes. The lesions from the mepyramine maleate-and Liquoid-treated groups infected with the MET became deep red, while those from the animals of all other groups were negative or showed only weakly positive reddening.

DISCUSSION AND CONCLUSIONS

1. A previous observation that, whereas ordinarily *M. leprae murium* is strongly damaged in the macrophages of the resistant host, the guinea-pig, that microorganism retains its acid-fastness and usual shape in the presence of an antihistamine (25), reflects an undisturbed intracellular biosphere for the bacillus in the cells of the resistant host when treated with such substances.

2. As postulated from that observation, it has been found that murine leprosy can be transmitted experimentally to the guinea-pig under the protection of an antihistamine, which suppresses important processes of the natural defence mechanism. Transmission, however, is successful only if a macrophage exudate is previously induced in the peritoneal cavity of the guinea-pig, this representing a favorable biosphere at the site of inoculation. With this technique the bacilli are ingested by the macrophages within a matter of minutes, quickly escaping the unfavorable extracellular environment.

3. These experiments constitute a further demonstration of the evidence presented by Hanks and Gray (17) regarding the harmful effects of the extracellular environment on the rat leprosy bacillus, since transmission to the guinea-pig is impossible if bacilli are exposed to the action of the body fluids, whereas it is successful if there are created experimental conditions which permit the rapid escape of inoculated bacilli from the unfavorable extracellular environment.

4. The fact that the administration of antihistamine suppresses successfully the natural resistance of the nonsusceptible host does not mean that histamine plays any role in the pathogenesis of murine leprosy, since antihistamines act directly on cells of the reticuloendothelial system (9, 22), and it was previously demonstrated (24) that neither histamine nor antihistamine interfers with the parasite-macrophage relationship in murine leprosy.

5. Another previous observation (25), that rat leprosy bacilli retain their acid-fastness and usual morphological shape in the presence of a complement-inhibiting compound, sodium polyanethol sulphonate (Liquoid, Roche), also reflects an undisturbed extracellular biosphere for the bacilli.

6. Again as postulated in advance, it was found that rat leprosy can be transmitted experimentally to the guinea-pig under the protection of that complement-inhibiting compound, which suppresses all the defence processes which are mediated by complement. In this case, also, transmission of the infection to the guinea-pig is successful only if a peritoneal macrophage exudate is established before the inoculation.

7. The observed effect of the complement-inhibiting compound suggests that it interferes with the action of the extracellular inhibitors described by Hanks. The present investigations have brought forth evidence that the unfavorable extracellular conditions which present a barrier to the experimental transmission of this obligate intracellular parasite can be surmounted by appropriate methodology.

8. It appears that the observed effect of sodium polyanethol sulphonate is connected with its anticomplementary action and not with the anticoagulant properties, since experiments with other anticoagulants (heparin and two dicoumarol derivatives), as well as an excess of fibrinogen or fibrin, had no influence in establishing infection.

9. Murine leprosy bacilli in the diseased guinea-pigs retained their infectiousness as shown by successful serial inoculations in that animal, and by transfer back to the original host, the rat. Evidence on this point was also supplied by the test with tetrazolium violet, according to Hanks. However, no direct evidence was obtained from the present experiments about the multiplication of *M. leprae murium* in the guinea-pig, because of lack of appropriate methods.

10. These experiments suggest the possibility of experimental transmission of human leprosy into a laboratory animal with the help of the macrophage exudate technique and simultaneous suppression of the natural resistance with an antihistamine or an anticomplementary compound.

SUMMARY

A method is reported for the experimental transmission of rat leprosy to a normally resistant host, the guinea-pig, by (1) inducing a macrophage exudate in the peritoneal cavity before intraperitoneal injection of the infectious tissue homogenizate, and (2) simultaneous suppression of the

natural resistance with an antihistamine (mepyramine maleate) or a complement-inhibiting compound (sodium polyanethol sulphonate). Large granulomas developed in 8 weeks in the peritoneal cavities of the guinea-pigs so treated. Using these granulomas, the infection was transmissable to subgeneration of the guinea-pig, and could be transmitted back again into the rat. In other words, *M. leprae murium* retained its infectiousness in the resistant host. Histologic examinations have shown that the granuloma of the guinea-pig is structurally similar to that of the rat. The tissue gives a strongly positive reaction to the tetrazolium violet test. The method described permits the parasite to escape the damaging action of the extracellular inhibitors which Hanks considers to be the barriers to experimental transmission.

RESUMEN

Descríbese un método para las transmisión experimental de la lepra murina a un huésped normalmente resistente, el cobayo: (1) induciendo un exudado macrófago en la cavidad peritoneal antes de la inyección intraperitoneal dek homogeneizato de tejido infeccioso (2) suprimiendo simultáneamente la resistencia natural con un antihistamínico (maleato de mepiramina) o un compuesto inhibidor del complemento (sulfonato de polianteol sódico). En o semanas se formaron granulomas grandes en las cavidades peritoneales de los cobayos tratados en esa forma. Usando estos granulomas, la infección fué transmisible a las subgeneraciones del cobayo y podía retransmitirse de nuevo a la rata. En otras palabras, el *M. leprae murium* retenía su infecciosidad en el huésped resistente. Los examenes histológicos han demostrado que el granuloma del cobayo es estructuralmente semejante al de la rata. El tejido acusa una reacción intensamente positiva al violeta de trazolio. El método descrito permite que el parásito evada el efecto nocivo de los inhibidores extracelulares que Hanks considera como las vallas de la transmisión experimental.

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Description of Plates

Plate (9)

(Figs. 1-4 after acid-fast staining; Fig. 5 taken by phase-contrast microscopy).

Fig. 1. Macrophages in the peritoneal cavity of an antihistamine-(mepyramin maleate) treated guinea-pig, one hour after intraperitoneal inoculation with a suspension of *M. leprae murium*. Cytoplasm and vacuoles thickly settled with acid-fast rods.

Fig. 2. Showing bacilli reduced to acid-fast debris in the macrophages of an untreated guinea-pig, 24 hours after intraperitoneal inoculation.

Fig. 3. The bacilli retain strong acid-fastness in the cytoplasm of peritoneal macrophages of guinea-pigs treated with mepyramine maleate, 24 hours after inoculation by the macrophage exudate technique.

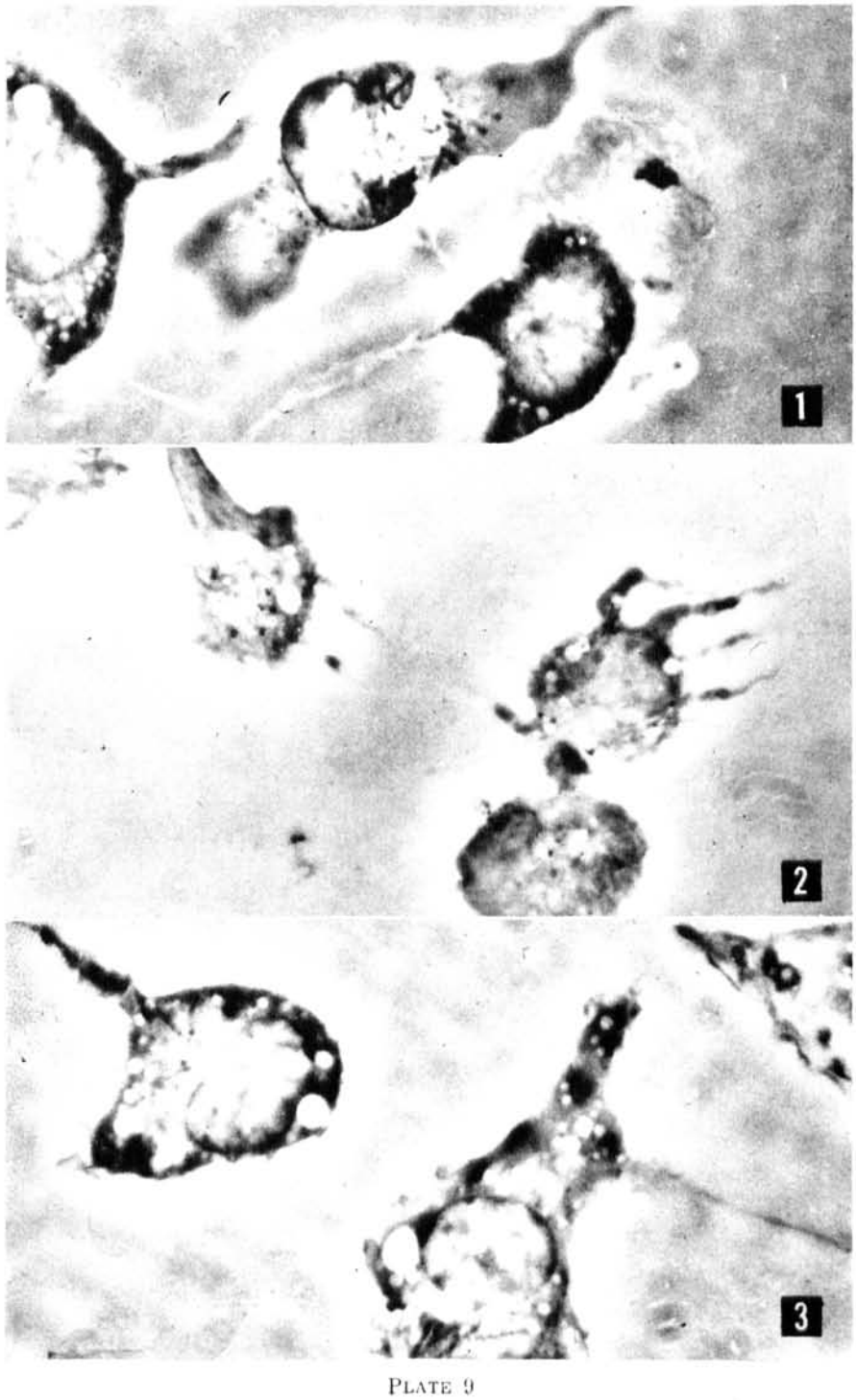

PLATE 9

PLATE (10)

FIG. 4. Same as Fig. 3. Note the tendency of the macrophages to agglomerate, the sponge-like vacuolization of their cytoplasm, and their heavy parasitization with acid-fast rat-leprosy bacilli.

FIG. 5. Shrunken and disrupted macrophages in the peritoneal cavity of a Liquoid-treated guinea-pig, as seen by phase contrast microscopy, 24 hours after intraperitoneal inoculation by the macrophage exudate technique. Bacilli lie extracellularly (see arrow).

FIG. 6. Lepromata of the 3 mepyramine maleate-treated guinea-pigs (left), as compared with the tissue growths of the 3 control animals (right). Autopsies done 8 weeks after intraperitoneal inoculation by the macrophage exudate technique in both groups.

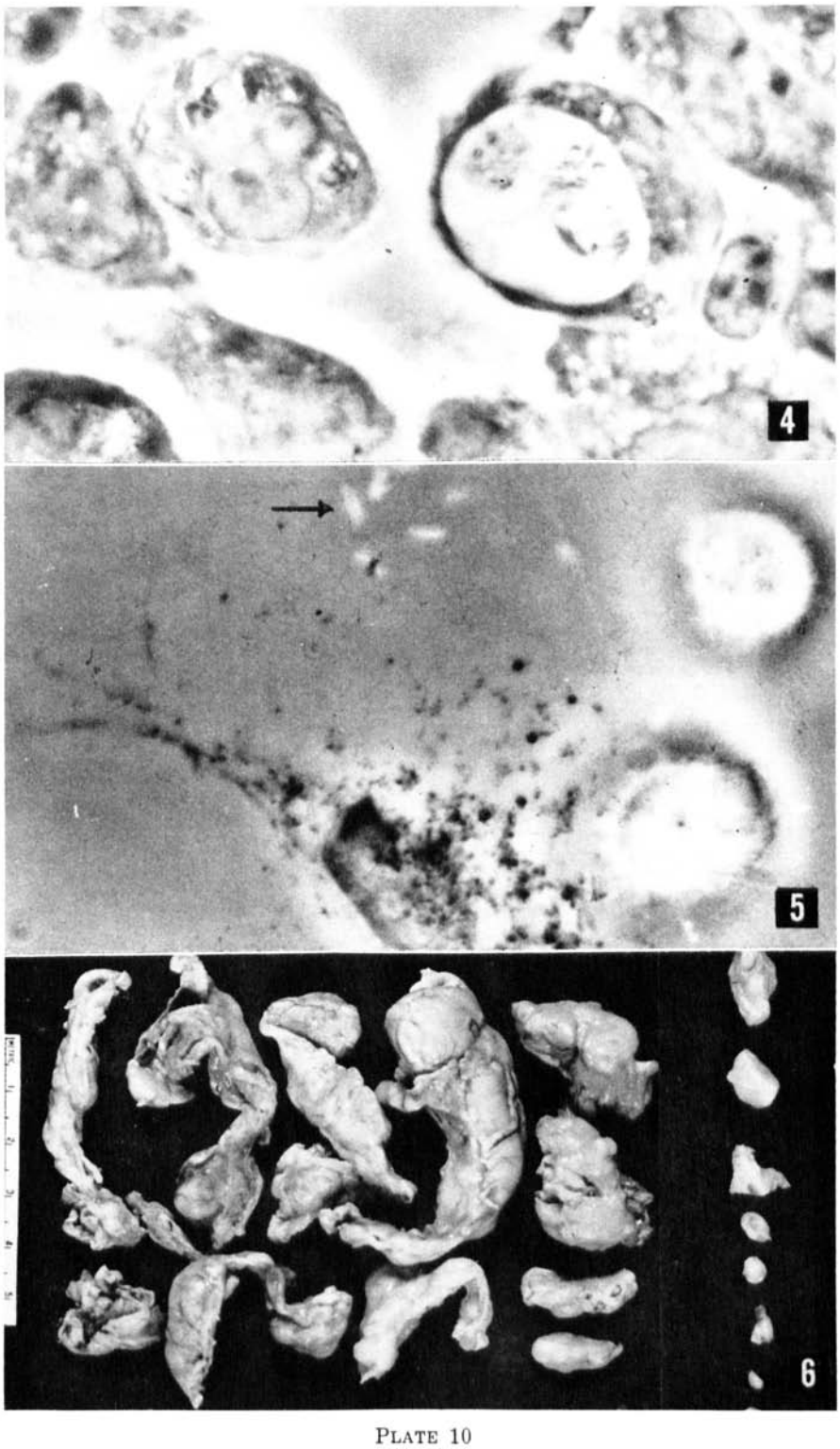

PLATE 10