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EXPERIMENTAL TRANSMISSION OF HUMAN LEPROSY INFECTION TO A SELECTED, LABORATORY-BRED HYBRID BLACK MOUSE

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INTRODUCTION

Since the discovery of *Mycobacterium leprae* in 1873, all attempts at animal transmission until very recently have failed. Failure in transmission to experimental animals might have been due to unsuitability of the animal used, or to faults in the technique employed. Many hold that untried new species of animals should be used for this experimentation, while others prefer to change the technique employed. Bergel⁽¹⁻³⁾ has recently claimed to have produced infection with the leprosy bacillus in white rats by keeping them on a "prooxidant" diet.

The present investigation, aimed at transmitting and establishing infection with *M. leprae*, was begun in 1956 and is still continuing. We have used a new type of animal and have also varied the technique. The observations reported here show that experimental transmission has been successful. The study also included 48 hamsters, in which a certain degree of success was obtained⁽⁵⁾, but that part of the work is not included in the present report. The substance of this report has been reported locally in a preliminary way⁽⁶⁾.

MATERIAL AND METHODS

Animal used.—A selected hybrid black mouse (Fig. 1), bred under controlled supervision in our institute by crossing male Indian house mice (*Mus musculus*) and female Swiss white mice, was chosen for most of the experiments. This crossing produced mice of various colors, the blacks in the smallest numbers. The black ones were selected to form a special colony, and they were the ones used in the experiment. Only very young animals, aged between 10 to 15 days, were used.

Inoculum.—The following method was used in the preparation of the inoculum. Bacilli from lesions of active, untreated cases of lepromatous leprosy were made practically tissue-free by differential centrifugalization and diluted in physiologic

saline to contain a known number of bacilli per cubic centimeter of suspension. Even during passing from animal to animal, in the preparation of inocula from organs of infected animals the bacilli were separated from the tissue before being inoculated.

Method of inoculation.—The subcutaneous or intraperitoneal route, or a combination of them, was usually chosen. However, in a number of animals the intracerebral and intrascrotal routes were employed, and a few were inoculated otherwise. The animals inoculated intracerebrally died within three months of inoculation without showing any gross manifestation of infection. Infection was produced by the other routes, but the animals were too few for special mention.

Dose of inoculum.—For inoculation of bacilli obtained from the human sources, the dose was 1,000 million bacilli. For passage from animal to animal this dose was much reduced, being between 1 and 20 million bacilli only. Each animal was inoculated only once during its lifetime.

Period of observation.—It was decided to allow the animals to live for a reasonably long period before they were sacrificed. However, some animals died during the period of observation. It was found that those which died or were sacrificed within five months of inoculation showed little if any evidence of infection. Subsequently no animal was sacrificed before the end of six months after inoculation. After this period animals were sacrificed at about monthly intervals.

Examination.—When an animal died or was sacrificed, it was examined for the presence of any macroscopic lesion in any part of the body. Tissues were then removed for sections. Smears were made from tissues for examination by the Ziehl-Neelsen technique for acid-fast bacilli. Sections were examined for histologic changes and the presence of acid-fast bacilli.

Inoculation of culture media.—From sacrificed animals which showed definite multiplication of the bacilli, tissues containing bacilli were aseptically triturated and suspended in physiologic saline. Petragani, Loewenstein, Petroff, and glycerine-agar media were then inoculated to rule out the possibility of contamination with the tubercle bacillus or any saprophytic acid-fast organism.

Skin tests of leprosy patients.—A Dharmendra-type antigen was prepared from affected tissues of infected animals rich in bacilli, and this preparation was tested on 9 patients with lepromatous leprosy, and 18 patients with nonlepromatous forms (all but 2 being tuberculoid), to compare the tissue reaction with that produced by Dharmendra's regular antigen (?). This was regarded as imperative to rule out possible contamination with the Stefansky bacillus or other acid-fast organisms. Only the 24-hour reactions were read.

OBSERVATIONS

To the time of writing a total 106 of the hybrid black mice have been inoculated with either original inocula of human lepromas or in serial passages, of which there have been three. Six of the total were lost, leaving 100 for consideration, as shown in Table 1. Of these 100, a total of 31 died during the experimental period, 51 were sacrificed at different intervals, and 18 are still living. Of the 31 deaths, 20 occurred within three months after inoculation (2 within 3 days), and those animals showed no evidence of infection. Furthermore, the 11 mice examined between 3 and 6 months after inoculation (1 of which had died, the other 10 sacrificed) showed very little evidence of infection when any. Of the 51 animals which died (10) or were sacrificed (41) during the period from 6 to 14

months, 8 dead animals were found decomposed and therefore could not be examined properly. The remaining 43 all showed mild to heavy generalized infections. In 23 of them the infection was graded as mild, in 11 as moderate, and in 17 as heavy.

TABLE 1.—*Findings in the mice examined of the four inoculation groups, original and three mouse-to-mouse passages.*

Source of inoculum	No. of mice inoculated ^a	Time of examination after inoculation						Living
		Within 3 months		3-6 months		6-14 months ^b		
		Examined	Infection	Examined	Infection	Examined	Infection	
Original human leproma	40	10	Neg.	3	Neg. 2 Few bacilli 1	24 (20)	Mild 14 Mod. 4 Heavy 2	3
First mouse passage	18	3	Neg.	3	Neg. 1 Few bacilli 2	9 (8)	Mild 1 Mod. 3 Heavy 4	3
Second mouse passage	17	4	Neg.	2	Few bacilli 2	6 (4)	Mod. 2 Heavy 2	5
Third mouse passage	25	3	Neg.	3	Neg. 1 Few bacilli 2	12 (11)	Mod. 2 Heavy 9	7
Totals	100	20		11		51 (43)		18

^a Not including the 6 animals lost, 1 or 2 from each group.

^b The figures in parentheses represent the numbers actually examined, after deducting the dead animals which decomposed.

Gross findings.—Heavily infected animals started showing signs of sluggishness in their movements from 6 months after inoculation onwards. Dryness around the mouth, dry and rough skin with discoloration and brittleness of hair were observed, and in advanced cases depilation with a fine nodular appearance of the skin. In advanced cases, too, the enlarged liver and spleen could be palpated, and on opening the abdomen the spleen and liver were found enlarged and with nodulations. Occasionally there were matting of the omentum, enlarged mesenteric lymph nodes, and enlarged testes.

Microscopic findings.—Smears from tissues of the animals inoculated either directly from the human source or from one animal to the other, showed the presence of intra- as well as extracellular acid-fast bacilli in the spleen, liver, omentum, glands, kidneys, testes or ovary, skin, nerves,

and occasionally in the spinal cord where granulomatous changes were also present. Nerve involvement was found in passage animals rather than those with the primary inoculation. Animals inoculated one year or more previously showed the heaviest infections. In them the tissue cells were crammed with innumerable bacilli, with globus formation. In the cases of mild infection, the affected tissues showed granulomatous changes with a few bacilli in them.

The animals inoculated directly with bacilli from the human lesions showed the presence of intra- and extracellular bacilli, but their numbers were seldom very large. However, there was evidence of multiplication and of the production of granulomas in the affected tissues. On passing, the animals showed progressively more generalized and heavier infections, so that of the third-passage lot 9 out of 11 examined were heavily infected, the other 2 moderately. The affected tissues and cells were found to be crowded with bacilli.

Histopathology.—This feature is yet under study. However, during the earlier period of infection, when the bacilli are very few in numbers and difficult to find, a type of fuchsinophilic cell of both reticuloendothelial and lymphoid origin (Fig. 2) can be found here and there in organs in which there are early granulomatous changes. When the disease has progressed further, occasional giant cells and macrophages, together with round-cell infiltration, appear in a somewhat focal type of distribution. But in heavy infection the tissue cells are found crammed with innumerable bacilli, the nuclei are generally pushed aside, and the cytoplasm in most cases cannot be differentiated and is often found to be vacuolated.

The degree to which bacterial invasion and multiplication, and granulomatous infiltration, can occur in heavily infected organs and tissues is shown in the photomicrographs which accompany this report, which (with one exception) are of paraffin sections stained for acid-fast bacilli by a modification of Fite's second (1947) technique. In most of them innumerable cells are so crammed with red bacilli that in these pictures they appear solid black and without detail. Unfortunately, circumstances prevent the reproduction of their color-film counterparts, which show the red masses and often—in the oil-immersion photomicrographs—individual bacilli in the masses.

The most solid massing is seen in the spleen (Figs. 3 and 4). In the lymph nodes the bacillus-laden cells are abundant but more focalized (Figs. 5 and 6). Lesions of the testes and vas deferens are shown in Figs. 8 and 9.

A significant feature of heavy infection is that it is not confined to the visceral organs but that the skin may be markedly involved, and also the peripheral nerves to some extent. In the skin there is, first, infiltration around the hair follicles, blood vessels and nerves. In advanced cases the dermis and subcutaneous tissues may be heavily infiltrated (Figs.

9 and 10), and the cells of the infiltrate are engorged with bacilli. The epidermis and subepidermal zones are relatively little affected.

Small foci of the granulomatous infiltration may be found inside peripheral nerves, the cells filled with bacilli (Figs. 11 and 12). However, such lesions are more common in the epi- and perineural sheaths of the peripheral nerve trunks.

In some advanced cases even the spinal cord was found somewhat infiltrated with bacilli. However, neither granulomatous changes nor bacilli have been detected in the central nervous system above the medulla oblongata.

Although histopathologic changes were found in almost all tissues, there was very little involvement of the lungs, and none has been found in the heart.

The histopathologic and histochemical studies of the tissues have not yet been completed. The detailed findings will be reported in a separate publication.

Results of skin tests of patients.—The tests with the Dharmendra-type antigen described resulted in negative 24-hour results in the 9 lepromatous patients tested, but positive results in all the 18 others except 4 which gave doubtful (\pm) reactions. The results with the Dharmendra antigen used for comparison were the same except that there were 3 negatives among the tuberculoid cases—2 of which were positive with the mouse-source antigen.

DISCUSSION

All previous animal transmission experiments suffered from what we concerned with this experiment have regarded as a serious handicap, namely, that the inoculum consisted not only of the specific organism but also of considerable amounts of human tissues and tissue juice. These components of the inoculum probably excite the defense mechanism of the animal to produce an antihuman-tissue factor unfavorable for the survival and multiplication of the leprosy bacilli of the inoculum. The more generalized and heavier infections observed on passaging tends to support this hypothesis, indicating that if the human tissue factor can be eliminated the possibility of successful transmission in a susceptible animal will be increased. There is no known method, serologic or other, which is sufficiently specific and delicate to demonstrate an antitissue factor after inoculations with as little human tissue as in the primary inoculations of this experiment. Furthermore, it is possible that there may have been in play a quite different factor, i.e., adaptation of the bacillus to the mouse in the primary inoculations, but there is no way of proving that. Be all that as it may, it is believed that the tissue-element factor, possibly nothing more than what would be adsorbed upon the surfaces of the bacilli, is the most likely deterrent one.

So far we are aware there is no record of using tissue-free leprosy bacilli for transmission experiments. With such inocula Chatterjee and Mukherjee (5) were successful in transmitting the leprosy-bacillus infection to Syrian hamsters, although the infection was not as heavy as in the black mouse, in which animal it has been possible to produce heavy infections. In the vast literature of animal transmission experiments no record has been found of the use of such a hybrid animal.

It has been observed in this experiment that, whether the inoculation was directly from the human source or from animal to animal, very little evidence of infection could be detected before the end of the sixth or seventh month after inoculation. Bergel also noticed this phenomenon in his transmission experiments.

As stated, various routes of inoculation were used in this work. The intracerebral route proved unfavorable, the mice dying within 3 months. Apart from that, no particular route was found significantly more favorable than the others.

The possibility of contamination with the tubercle bacillus or some saprophytic acid-fast organism was excluded, because no growth was obtained on any of the culture media used, on which these organisms grow well. The possibility that the Stefansky bacillus was involved is also excluded, (a) because the inocula used came from different lepromatous patients and were inoculated into different lots of animals with similar results; (b) because the Stefansky bacillus is not involved in human leprosy; and (c) because that bacillus is highly infectious for the hybrid black mouse, usually producing generalized infection in 6 to 8 weeks and death within three months (4).

The results of the skin tests in lepromatous and nonlepromatous cases of leprosy with an antigen made from infected mouse tissues as compared with Dharmendra's antigen indicate, because of the negativity in lepromatous cases, which is generally accepted as specific, that the bacilli from the mouse lesions were antigenically similar to *M. leprae*, which the Stefansky bacillus and other acid-fasts are not.

The nature of lesions produced in the tissues of the black mice suggests that this species of animal has greater susceptibility to infection by the human leprosy bacillus than any other animal so far used. This success in animal transmission has brought us nearer to the possibility of obtaining *M. leprae* in quantities to study its metabolism, pathogenicity, and natural or acquired resistance. It should be of great value in assessing chemotherapeutic agents for leprosy. There is a possibility of finding a clue for the *in vitro* cultivation of *M. leprae* from detailed studies of metabolism. Lastly, by passaging from animal to animal it may be possible to transmit the infection to other susceptible animals belonging to the same order.

SUMMARY

Success in transmitting and establishing infection with tissue-free *M. leprae* obtained from untreated cases of active lepromatous leprosy to a new type of selected, laboratory-bred, hybrid black mouse is reported. In all, 100 animals of tender age were inoculated and observed. The infection has so far been carried up to the third serial animal passage. With passaging the animals showed much heavier infection than with the original inoculum. Whether inoculated with bacilli from the human source or during passaging from infected mice, the animals usually began to show evidence of infection from the end of the 6th or 7th month onward. The heaviest infections were observed one year or more after inoculation.

The intra- and extracellular acid-fast bacilli were found in spleen, lymph node, liver, kidney, omentum, testis, ovary, skin, and peripheral nerves, where granulomatous changes were produced. The tissue cells were found crammed with acid-fast bacilli.

The possibility of contamination with the tubercle bacillus or with saprophytic acid-fast bacilli was excluded, because no growth was obtained on any of the culture media used, on which these organisms grow well. The Stefansky bacillus is also excluded, because of its high pathogenicity for the hybrid black mouse.

The results of skin tests in lepromatous and nonlepromatous cases of leprosy with an antigen made from infected mouse tissues as compared with Dharmendra's antigen indicate that the bacilli from the mouse lesions were antigenically similar to *M. leprae*, which the Stefansky bacillus and other acid-fast are not.

CONCLUSION

It may be concluded that, by using tissue-free bacilli from untreated cases of active lepromatous leprosy, and the selected hybrid black mouse developed in this laboratory, experimental transmission of infection by the human leprosy bacillus to this animal, and its establishment in them by passaging, has been successful.

RESUMEN

Descríbese el éxito obtenido al transmitir y establecer infección con *M. leprae* histoprivos procedentes de casos no tratados de lepra lepromatosa activa a una nueva especie de ratón negro híbrido, escogido y criado en el laboratorio. En conjunto, se inoculó y observó a 100 animales de edad tierna. La infección a esta fecha se ha llevado hasta el tercer pase seriado por animales. Con los pases los animales han mostrado una infección mucho más intensa que con el inóculo primitivo. Ya sean inoculados con los bacilos procedentes del foco humano o durante los pases derivados de ratones infectados, los animales suelen comenzar a manifestar signos de infección desde el final del 6o. o 7o. mes en adelante. Las infecciones más intensas fueron observadas al año o más de la inoculación.

Se encontraron bacilos ácidosresistentes intra- y extra-celulares en el bazo, los ganglios linfáticos, el hígado, el riñón, el epiplón, los testículos, los ovarios, la piel

y los nervios periféricos, en los que se produjeron alteraciones granulomatosas. Las células de los tejidos estaban atestadas de bacilos ácidosresistentes.

Quedó excluída la posibilidad de contaminación por el bacilo tuberculoso por no haberse obtenido colonias en ninguno de los medios de cultivos usados, en los que prolifera bien dicho microbio. También se excluye el bacilo de Stefansky, dada su elevada patogenicidad para el ratón negro híbrido.

Los resultados de la cutirreacciones ejecutadas en casos lepromatosos y no lepromatosos de lepra con un antígeno preparado de tejidos de ratones infectados, comparado con el antígeno de Dharmendra, indican que los bacilos procedentes de las lesiones murinas son antigénicamente semejantes al *M. leprae*, lo cual no sucede con el bacilo de Stefansky y otros bacilos ácidosresistentes.

ACKNOWLEDGMENTS

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DESCRIPTION OF PLATES

PLATE 15

(All but one of the pictures reproduced were black-and-white prints of color photographs, in which the bacilli, being stained red, were far better demonstrated than here. Unfortunately, for economic reasons, the color pictures cannot be reproduced.)

FIG. 1. The hybrid black mouse (inset), normal and heavily infected (the latter a somewhat unsatisfactory reproduction of the original color picture). The velvety appearance of the normal mouse is poorly represented. In heavily infected mice the hair becomes dry and brittle and the color tends to become lighter; in places there may be depilation revealing finely nodulated skin.

FIG. 2. An example of the fuchsinophilic cells found in the earlier period of infection of the mouse.

FIG. 3. Spleen of a heavily infected mouse, low power. The darkness of the tissue is not due primarily to the lack of contrast in the picture, but mainly to the massing of the red-stained bacilli throughout the entire section. (The color print shows little else than red in the massed cell areas.)

FIG. 4. Spleen, oil immersion. Showing the great numbers of bacilli. (The color print shows very clearly the clumps of bacilli, often distinguishable individually, and the peripheral displacement of the nuclei where the bacilli are intracellular.)

FIG. 5. Lymph node, low power. The bacillus-laden cells are not distinguishable at this magnification. (The color print shows large numbers of groups and strands of red-colored cells at all levels, giving the whole a reddish overcast although the majority of the cells are of normal blue color.)

FIG. 6. Lymph node, oil immersion. This represents one of the concentrations of cells crowded with bacilli. Many of the bacilli are distinguishable, and also a fine-grain vacuolation of the affected cells.

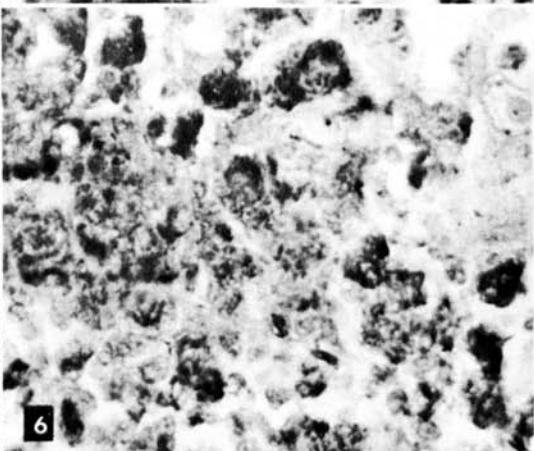
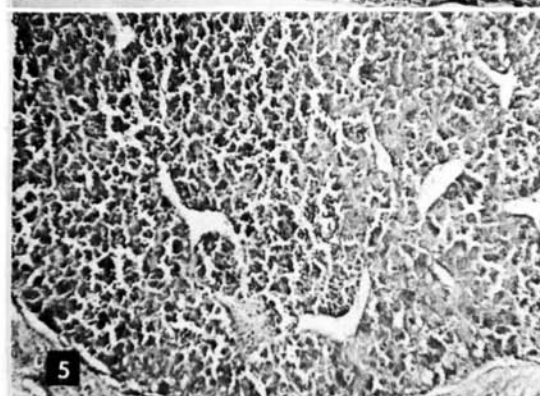
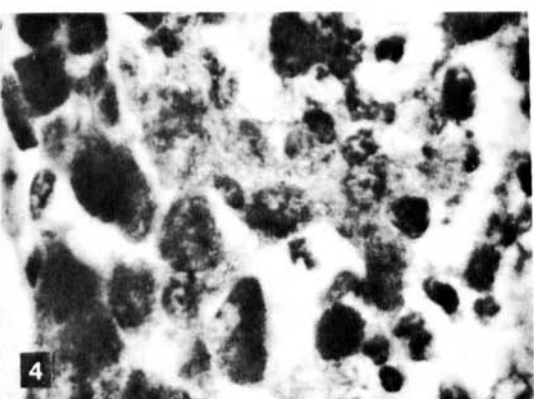
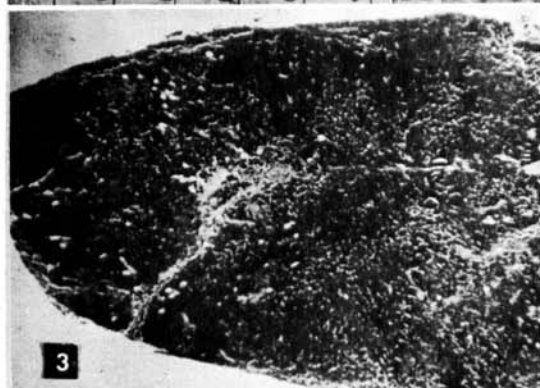
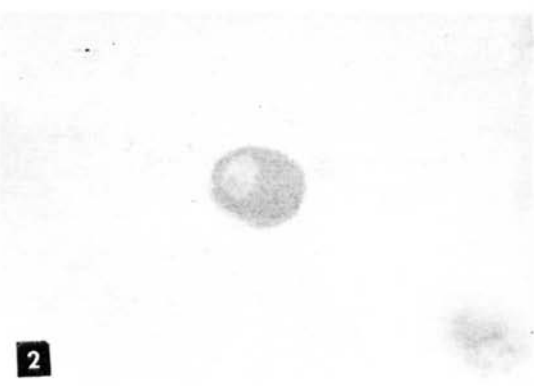
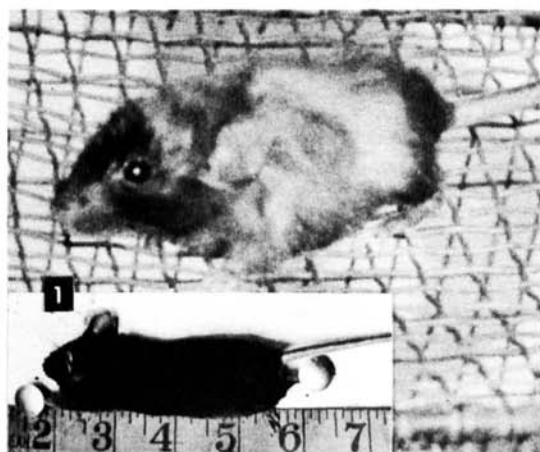


PLATE 15

PLATE 16

FIG. 7. Testis, oil immersion, frozen section. Showing cells full of bacilli.

FIG. 8. Vas deferens, low power. Showing two masses of cells loaded with bacilli. (The color print shows that most of the larger mass to the left of the central blood vessel is almost solid red, and also a large part of the cell cluster to the right of and below that vessel.)

FIG. 9. Skin, medium power. The cells clustered in groups and strands below the unaffected subepidermal zone are black because of the massing of red-stained bacilli. (This is well seen in the color print, in which the contrast is excellent.)

FIG. 10. Skin, medium power. Showing massing of bacillus-filled cells with better photographic contrast than in Fig. 9.

FIG. 11. Nerve, low power. The elongate dark streaks between nerve fibers are infiltrations of cells filled with bacilli (well demonstrated in the color print).

FIG. 12. Nerve, oil immersion. Showing more details of one of the infiltrates of bacillus-filled cells.

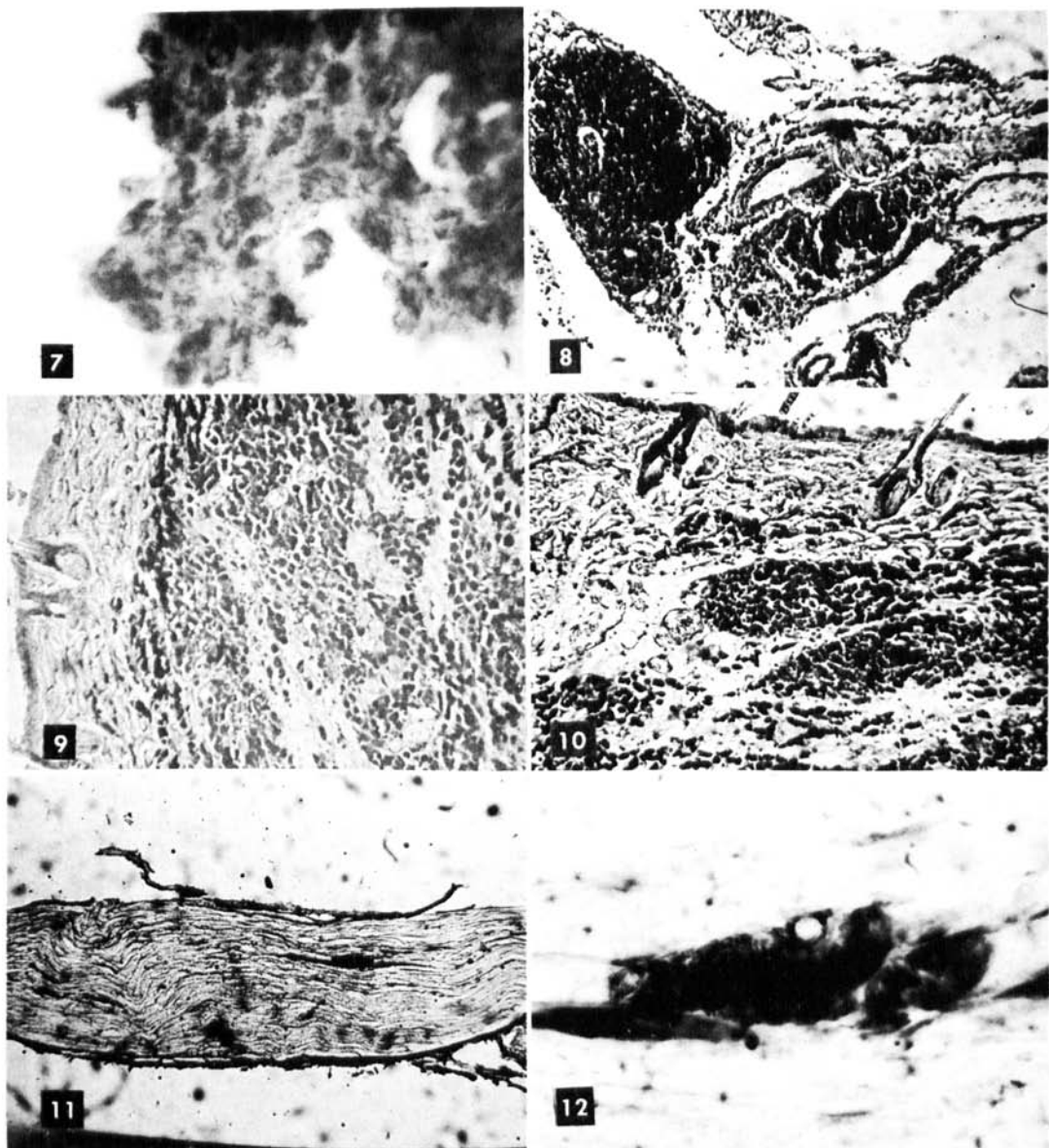


PLATE 16