STUDIES OF PATHOGENICITY OF THE ICRC BACILLUS ISOLATED FROM HUMAN LEPROMATOUS LEPROSY

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The *in vitro* cultivation of an acid-fast mycobacterium isolated from four different cases of lepromatous leprosy was first reported in July 1958 (1). These four strains of a microorganism, designated the "ICRC Bacillus," have been maintained in cultures for over three years, and their growth rate, growth pattern, and biologic behavior under culture conditions have been studied carefully (2). In the present article is reported a study of the pathogenicity of the organism in animals, and the possibility of producing disease in laboratory mice.

MATERIALS AND METHODS

Acid-fast bacillary material isolated from lepromatous leprosy and cultivated and maintained on the modified fluid of stock cell cultures for 1½ to 2 years was used for the animal inoculations. Most of the material used for inoculations came from stock cultures derived from Case I. Only four animals were inoculated with the strains of bacilli isolated from Cases II, III and IV (2).

Experiment 1.—Three strains of mice, C_3H(Jax), Little's dba, and a hybrid of a Paris strain XVII and C_{57}(Black), of ages varying between 7 and 13 weeks, were used for the first experiment. Twelve mice were inoculated intraperitoneally with a bacillus suspension prepared in Tyrode's balanced salt solution. A 7th generation stock subculture of Case I, and a 3rd generation stock of Cases II, III and IV, were used for this experiment. Eight mice received approximately 2×10^9 organisms each, and four mice of the dba(Bar) strain received 5×10^8 organisms each. Daily observations of the inoculated mice were recorded, and animals were sacrificed 6 to 17 weeks after inoculation at the age of 13 to 30 weeks, to study early reactions.

Experiment 2.—In the second experiment, 16 male mice, all of the dba strain, aged between 5 and 11 weeks, were inoculated intraperitoneally with smaller doses, ranging up to 3×10^8 organisms. The organisms belonged to the 12th generation of a subculture of Case I. All inoculations were made on the same day, and the animals were sacrificed 30 to 65 weeks after inoculation, at the late age of 35 to 75 weeks.

Experiment 3.—In the third experiment, 12 C_3H(Jax) males, 7-8 weeks old, were divided in 2 groups of 6 each. Of one group each animal was given an intraperitoneal inoculation of 4×10^8 organisms, while the other group was administered the same dose of bacillus suspension which had been autoclaved at 120°C at 15 lb. pressure for one-half hour. All these animals were sacrificed after 6 to 7 months, except three that were killed after 3-1/2 months.

Mice belonging to all the three experiments were killed by cervical dislocation and dissected for careful visceral examination. The skin, subcutis and the visceral organs were carefully examined with a magnifying lens for gross lesions. The pin-point lesions on the abdominal viscera, and pieces of liver, spleen, kidney, lymph nodes, testis, and skin of every animal were fixed as a routine. The tissues were fixed in Zenker-formol, and for locating acid-fast bacilli, sections were stained by Fite's modification of the Ziehl-Neelsen method (6). Fresh tissue smears of suspected gross lesions were also

made and stained by the regular Ziehl-Neelsen method. A detailed histopathologic study of these animals, specifically of changes in the organs of the reticuloendothelial system and other suspected foci, was undertaken to determine the pathogenicity of the bacillus maintained in cultivation, and the degree of progression of the infection produced.

EXPERIMENTAL FINDINGS

The *first* experiment was arranged to ascertain the response of different strains of mice to a particularly heavy inoculum (2×10^9 organisms per animal) and to determine the suitability of the mouse strains for the purpose of studying the pathogenicity of the microorganisms. Animals of these group were sacrificed 6 to 17 weeks after inoculation to study early lesions. Most of the animals showed pin-point nodules scattered over the surfaces of the liver, spleen, pancreas, diaphragm and intestinal coils. Some nodules were quite sizable. In some animals the lymph nodes were enlarged, and the skin at the site of inoculation was thickened and nodular. Mice of the dba strain showed an early response to inoculation and were found to be particularly susceptible to the organism.

In the *second* experiment 16 dba mice, 5 to 7 weeks old, were inoculated with a smaller dose of bacillus suspension (3×10^8 organisms per mouse) and were observed for 30 to 65 weeks. The animals started losing weight about 28 to 30 weeks after inoculation. Many of these mice (10 out of 16) developed palpable nodular thickenings below the testis at about 8 months after inoculation. Lymph nodes were enlarged and palpable in most animals. One mouse showed swelling of the hind limbs. The lesions in this group were similar to those in the first group, but they were more numerous and more progressive. There were many minute pin-point nodules on the visceral organs. The spleen was enlarged, with whitish patches on the surface. A peculiar thickening was noticed in the peritesticular tissues, and sometimes a large nodule pressed markedly on the testicular tubules (Fig. 1).

Tables 1 and 2 summarize the observations on individual animals in the first two experiments, and Table 3 gives a summary of the histopathologic types of these lesions.

HISTOPATHOLOGY

Liver. — Sections of the liver presented three types of lesions: (1) Small periportal and intralobular focal granulomas were commonly encountered (Fig. 2). (2) Occasionally multinucleate giant cells with intracellular organisms were seen (Fig. 3). These two lesions always remained microscopic. (3) The main macroscopic lesion was the third type, the "miliary leproma-like" nodules on the liver surface. The sharply circumscribed nodules were commonly located at the periphery of the liver lobe (Fig. 4). The nodules were composed mostly of elongated or irregularly spherical histiocytes, which were modified

Kupffer's cells. Scattered lymphocytes were also present. The histiocytes were laden with acid-fast bacilli, arranged in groups or forming "globus-like" bodies. Extracellular organisms were also plentiful in the central necrotic region of the nodule (Fig. 5). Many nodular liver lesions were found to develop progressively for as long as 10 to 12 months, and even longer.

Periportal and intralobular foci of small and large granulomas showed an increase of mesenchymal cells, mainly histiocytes.. Occasionally 2 to 3 giant cells were seen in these foci. The histiocytes as well as giant cells contained varying numbers of acid-fast bacilli, depending on the development of the lesion. Isolated large multinucleate giant cells with few intracytoplasmic acid-fast organisms were frequently observed (Figs. 2 and 3). These lesions always remained microscopic, but the "leproma-like" lesions were usually larger and were visible to the naked eye (Figs. 4 and 5).

Spleen.—The inflammatory reaction in the spleen differed to a certain extent from the reaction in the liver. Circumscribed nodular accumulations were present on the surface of the spleen in most of the animals, both young and old. Microscopically, these nodules were similar to the miliary leproma-like lesions in the liver, but such lesions were rarely encountered in the spleen. Much more frequently the general pattern of the splenic pulp was disturbed by diffuse bands and sheets of large, rather spherical, pale-staining cells (Fig. 6). Only a few mice in the older group showed microscopic granulomas with occasional acid-fast organisms (Fig. 7).

Lymph nodes.—Enlargement of regional lymph nodes was usually observed, with complete distortion of their structure by irregular accumulations of large, vacuolated foamy cells with bacilli. Sometimes the lymph nodes were seen to be full of histiocytes laden with organisms, an appearance which could be easily demonstrated in an impression smear (Fig. 8). Minute nodules on the peritoneal surface of the abdominal viscera and on the lower surface of the diaphragm showed a structure similar to that described above, with histiocytes

DESCRIPTION OF PLATE

FIG. 1. Abdominal viscera of a dba(Bar) male mouse, killed 54 weeks after intraperitoneal inoculation of 5 x 10^8 organisms. Besides minute pin-point nodules, large whitish leproma-like nodules can be seen in the intestinal loop (high, near the top) and in the peritesticular regions (lower, bilateral).

FIG. 2. A section of the liver of a dba mouse killed after 23 weeks of treatment with the dose of organisms 3 × 10^8. Periportal and intralobular focal granulomas are quite conspicuous. ×90.

FIG. 3. Higher magnification of one of the focal liver granulomas from Fig. 2. Typical histiocytic reaction, showing a good number of acid-fast bacilli. ×750.

FIG. 4. Miliary leproma-like lesion at the edge of the liver of dba mouse K/524. ×45.

FIG. 5. Higher magnification of a portion of FIG. 4, showing "leproma-like" nodule swarming with bacilli. ×150.

FIG. 6. Section of spleen of a dba mouse 23 weeks after treatment, showing disturbed pattern of splenic pulp. ×45.

of varying forms and containing foamy cytoplasm and abundant bacilli. Similar nodules were also common on the pancreas (Fig. 9). Occasionally such nodules were found on the capsule of the kidney; but pulmonary involvement was conspicuous by its absence.

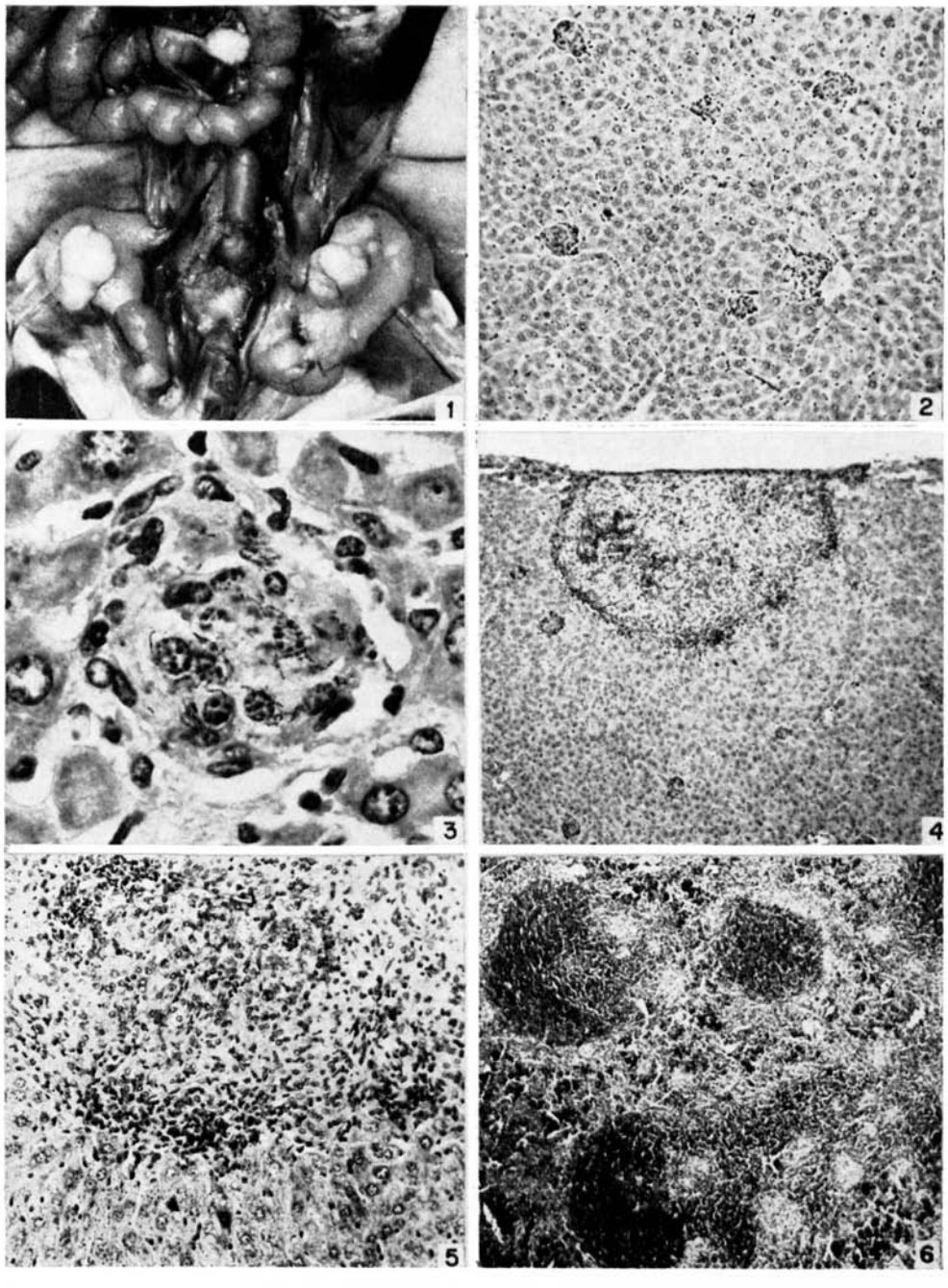


TABLE 1.—*Observations on animals inoculated intraperitoneally with ICRC bacilli in Experiment 1, the dose approximately* 2×10^9 *to* 5×10^8 *per mouse.*

Animal No.	Strain	Age when inoculated (weeks)	Age when killed (weeks)	Quantitative index of organisms in visceral lesions							
				Liver	Spleen	Testis	Lymph node	Kidney	Pin-point nodules on viscera	Skin	Lung
J/2769 ♂	dba(MTI)	7	13	3+	2+	1+	3+	1+	3+	—	—
J/2770 ♂ (PM)	dba(MTI)	7	13	3+	2+	1+	3+	1+	3+	—	—
J/2215 ♂	C_3H(Jax)	7	14	3+	2+	1+	3+	—	3+	—	—
J/1230 ♀	Hybrid-XVIIxC_{57}	13	20	3+	3+	—	2+	1+	1+	—	—
J/2212 ♂	C_3H(Jax)	7	15	3+	2+	1+	3+	1+	3+	—	—
J/1227 ♀	Hybrid-XVIIxC_{57}	13	25	2+	1+	—	2+	—	2+	—	—
J/1228 ♀	Hybrid-XVIIxC_{57}	13	30	1+	2+	—	1+	—	±	—	—
J/1229 ♀	Hybrid-XVIIxC_{57}	13	30	1+	2+	—	±	—	±	—	—
K/524 ♂	dba(Bar)	9	15	3+	3+	—	3+	—	2+	—	—
K/525 ♂	dba(Bar)	9	18	3+	1+	1+	—	—	2+	—	—
K/531 ♂ (PM)	dba(Bar)	7	16	—	—	1+	1+	—	1+	—	—
K/532 ♂	dba(Bar)	7	18	3+	2+	1+	2+	—			

TABLE 2.—*Observations on dba male mice inoculated intraperitoneally with ICRC bacilli in Experiment 2, the dose approximately* 2×10^9 *to* 5×10^8 *per mouse.*

Animal No.	Age when inoculated (weeks)	Age when killed (weeks)	Quantitative index of organisms in visceral lesions							
			Liver	Spleen	Testis (adven.)	Lymph node	Kidney	Pin-point nodules on viscera	Skin	Lung
L/1599	5	25	3+	2+	2+	3+	−	2+	3+	−
L/1594	6	34	3+	2+	3+	3+	1+	3+	2+	−
L/1598	5	33	2+	1+	3+	−	−	±	3+	−
L/1600	5	35	2+	2+	2+	2+	−	3+	2+	−
L/1593(PM)	6	40	3+	2+	3+	2+	−	3+	2+	−
L/359	11	45	1+	1+	−	−	−	2+	−	−
L/668	10	44	2+	1+	1+	1+	−	3+	−	−
L/1597(PM)	6	50	3+	1+	3+	2+	1+	3+	2+	−
L/358	11	57	3+	2+	−	−	−	2+	−	−
L/357	11	67	±	1+	−	2+	−	1+	−	−
M/2952	10	75	2+	2+	−	−	−	1+	−	−
N/127	10	67	3+	1+	2+	1+	−	±	−	−
O/5(PM)	7	57	3+	1+	3+	1+	−	3+	1+	−
O/6(PM)	7	52	3+	1+	2+	2+	−	2+	−	±
O/4	7	57	2+	2+	3+	2+	−	2+	−	−
O/8(PM)	7	51	2+	1+	1+	1+	−	2+	±	−

TABLE 3.—*Summary of histopathology of lesions*

	Liver			Spleen		Testis		Kidney (capsular region only)	Enlarged lymph nodes	Intraperitoneal pinpoint nodules	Histiocytic reaction in the abdominal muscle	Nodule in the lung	Reaction in the skin and subcutis
	Miliary lepromas	Granulomas periportal and intralobular	Giant cells with organisms	Disturbed splenic pattern: Nodules on the surface	Disturbed splenic pattern: Small granulomas	Nodules in: peritesticular tissue	Nodules in: testicular tubules						
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
Experiment 1. Early reaction; age group 3½ to 7½ months													
No. of mice with lesion/ Total No. of mice	8/12	9/12	9/12	10/12	3/12	6/12	—	3/12	7/12	9/12	3/12	—	2/12
Quantity of bacilli	3+	1+	1+	3+	±	2+	—	±	2+	3+	1+	—	1+
Experiment 2. Late reaction; age group 7 to 18 months													
No. of mice with lesion/ Total No. of mice	10/16	12/16	6/16	6/16	10/16	11/16	1/16	1/16	8/16	15/16	5/16	1/16	7/16
Quantity of bacilli	3+	1+	±	3+	1+	3+	±	±	2+	3+	—	±	3+

Skin and subcutis.—Thickening of the skin at the sites of the skin punctures for the intraperitoneal inoculations was observed in 6 experimental mice in Experiment 2. Secondary skin lesions in the older group of dba mice were much more advanced than those in the younger group of Experiment 1. Section of the skin showed typical lepromatous nodule formation consisting of histiocytes laden with acid-fast bacilli (Figs. 10 and 11). The nodule was either immediately below the epidermis or in the subcutis.

Testes.—In the younger group of mice of the first experiment, microscopic histiocytic lesions were seen in the epididymis and the capsule of the testis. It was possible to locate only few organisms in such lesions. However, in the older group of animals belonging to the second experiment, in 10 out of 16 mice such lesions were larger and became palpable, indicating progressive growth (Fig. 1). In some of these animals there were discovered hard palpable nodules fixed to the testicular mass, which were necrosed centrally and were swarming with acid-fast organisms. Occasionally the testicular tubes were atrophied and distorted as a result of the mechanical pressure exerted by the nodule (Fig. 12). Very rarely, isolated acid-fast organisms were located in the testicular parenchyma.

Experiment 3. Inoculation of autoclaved bacillary material.— The control group of 6 young C$_3$H(Jax) mice of this experiment, inoculated with 400 million living organisms per animal, presented results similar to those in the previous experiments except that the reaction was not so extensive. A few pin-point nodules were present on visceral organs. The spleen and lymph nodes were slightly enlarged, but there was no naked-eye evidence of progressive lesions. On microscopic examination the liver showed typical granulomas, and a small number of microscopic leproma-like lesions were found in internal organs such as the liver, spleen and pancreas. The skin and the peritesticular tissues showed comparatively small numbers of bacilli.

In the other group of mice inoculated with the same dose of bacillary material which had been autoclaved, response to the inoculation was definitely poor but not totally absent. Minute nodules with numerous acid-fast organisms were seen on the surface of the spleen and the pancreas in 3 out of 6 animals. One animal had a couple of nodules on the liver, but the organisms were granular and degenerated. In the remaining 3 animals there was no reaction whatever, and only an occasional isolated acid-fast organism could be detected on careful examination. There was no sign of a progressive lesion in any of these 6 animals inoculated with "inactivated" bacillary material.

DISCUSSION

Many attempts to transmit human leprosy to laboratory animals have been recorded in the literature since 1874 ([9]). Studies on different

aspects of the subject have been receiving considerable attention lately ([3, 4, 5, 10]). We attempted in 1958 to investigate the biologic factors responsible for transmission of the disease, and reported ([7,8]) some success in transmitting the infection to a small group of golden hamsters. Simultaneously with these animal experiments, *in vitro* studies on *Mycobacterium leprae* have been in progress. Observations on experiments arranged to test the affinity of that organism to fetal nerve elements were reported in 1958 ([10]). Further experiments in tissue culture with the use of human spinal ganglia fibroblasts as substrate yielded interesting results. An acid-fast organism isolated from human lepromatous leprosy was later successfully cultivated in tissue culture and designated as the "ICRC bacillus" ([1, 2]).

Growth-characteristic studies on the organism grown on the modified fluid of stock cultures and adapted to standard bacteriologic media have recently been completed ([2]). Animal experiments have now been carried out to test the pathogenicity of this cultivated organism. Experiments to compare its *in vivo* behavior with that of *M. leprae* are under way. It may perhaps be worthwhile to compare the histopathology of lesions induced by the ICRC bacillus with that of lesions reported by Binford in hamsters and by Chatterjee in hybrid black mice. It may also be interesting to compare the lesions of the internal organs, particularly of the reticuloendothelial system, with some of the clinical reports on human material described by Tilden ([11]).

A careful study of the tabulated data in Table 3 of the present report shows that typical histiocytic reaction was evoked in the organs of the reticuloendothelial system of almost every animal. These lesions have also been observed in the skin and subcutis and in the tissues surrounding the testes. A very heavy dose of the inoculum (approximately 2×10^9 organisms) killed two mice during 6 to 9 weeks of treatment. In this group, gross pin-point lesions were numerous and quite extensive, particularly in the liver, pancreas and all abdominal viscera. Lymph nodes were badly affected.

In the later age groups of 30 to 40 weeks and 50 to 75 weeks, many of the nodular liver and spleen lesions, as well as the skin and peritesticular lesions, presented an indication of progressive growth. Such

DESCRIPTION OF PLATE

FIG. 7. Section of spleen under higher magnification, showing occasional focal granulomata. ×150.

FIG. 8. Impression smear of an enlarged lymph node swarming with bacilli, from a dba mouse killed 6 weeks after inoculation of ICRC bacilli. ×850.

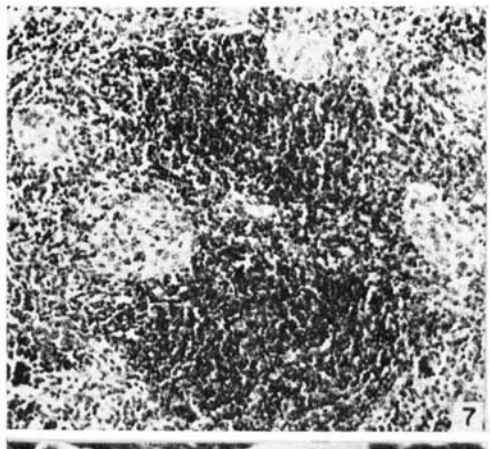
FIG. 9. A typical leproma-like nodule in the pancreatic tissue, showing bacilli. ×300.

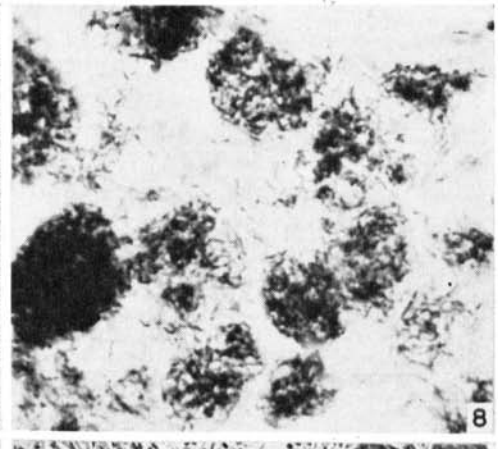
FIG. 10. A section of the skin of a dba mouse killed 43 weeks after treatment. Note nodule formation in the subcutis. ×150.

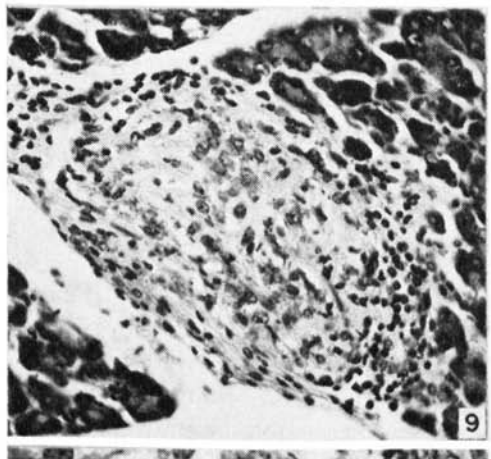
FIG. 11. Nodule in the subcutis of Fig. 10 under higher magnification, showing typical histiocytic reaction and cells loaded with acid-fast organisms. ×750.

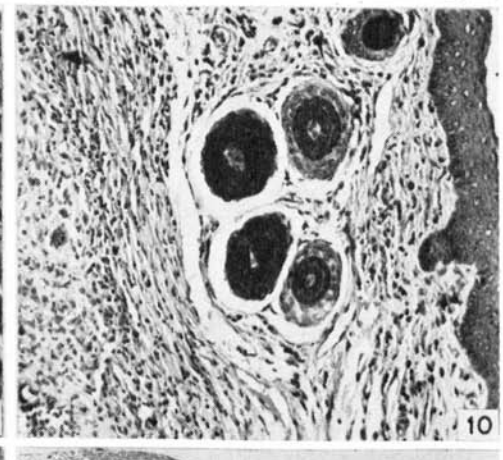
FIG. 12. A section of testis of a dba mouse killed 65 weeks after inoculation. A large leproma-like nodule full of bacilli and with central necrosis is seen in the peritesticular region. ×12.

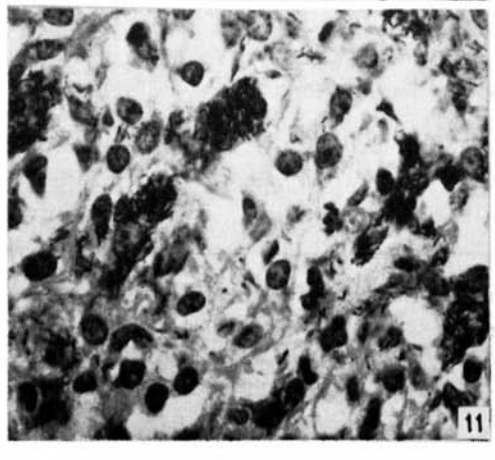
progressive testicular lesions were not observed in the earlier group. The bacillary material that was thus recovered after 6 to 8 months of inoculation and later, up to 12 to 18 months, was probably not just an accumulation and deposition of bacilli which had been inoculated but suggested a multiplication of the organisms and the formation

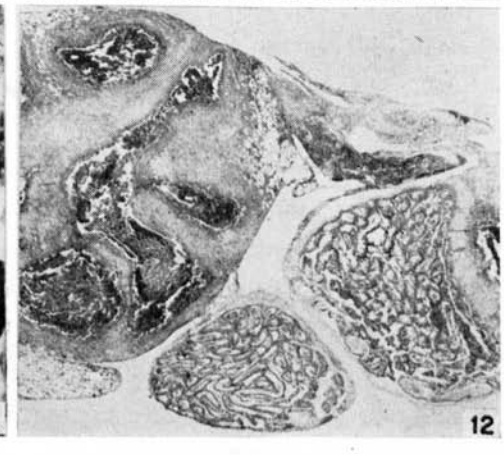












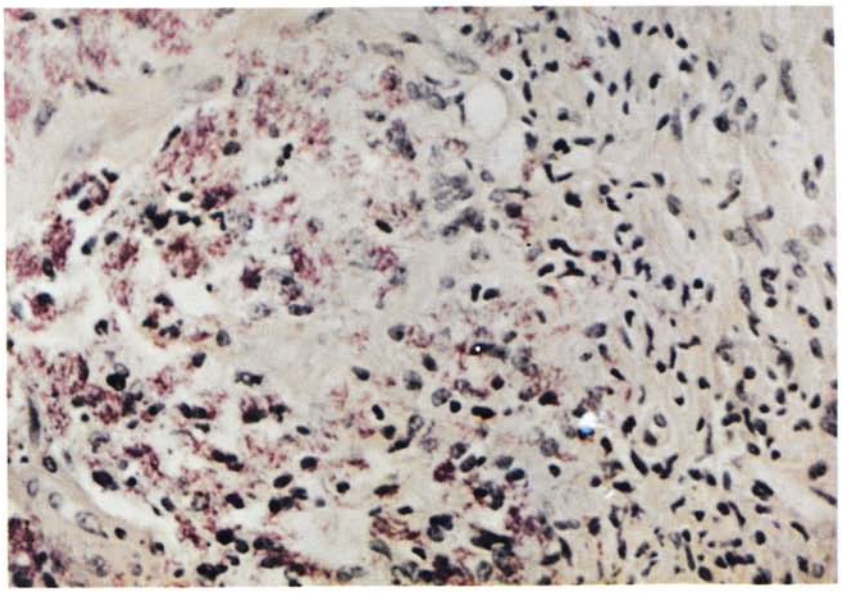
of progressive nodules. The intracellular organisms were frequently in the form of globus-like structures.

The histopathology of the nodular histiocytic lesions in all the organs of the reticuloendothelial system was closely comparable to the miliary lepromas described by Tilden from his clinical material. He described small granulomas of histiocytic origin around sinusoids with intracellular organisms, and large nodular lepromas at the edge of the liver lobe, closely comparable to the lesions observed by us in all the experimental mice. He observed a different reaction in the spleen with a disturbance of the splenic pulp pattern, and the formation of rare granulomas. A similar splenic condition has been seen in the experimental material described. Minute nodules on the surface of the spleen were abundant, although the formation of granulomas in the organ was rare. Tilden was of the opinion that leprosy was essentially a disease of the reticuloendothelial system, and the lesions encountered in our animals were comparable to those described by him in the reticuloendothelial system.

Skin lesions were observed frequently only in the older dba mice, as also were lesions in the peritesticular tissues. Such lesions without an involvement of the tubular parenchyma would only suggest a spread of the organisms through the lymph channels. The entire reticuloendothelial system and the skin at the site of inoculation were the organs particularly involved. Lungs were conspicuously free of any lesions, as reported before by others in both clinical material (Tilden) and experimental material (Chatterjee).

Binford's experiments produced similar histiocytic lesions comprised of cells laden with numerous bacilli in the form of varied size groups and "globi," but these are restricted to the sites of inoculation. He selected the testis and earlobe as the sites for inoculation, with special consideration of the temperature factor. Chatterjee demonstrated numerous organisms in the spleen, lymph nodes, testis, skin, etc., but did not describe the histopathology of the lesions in much detail. He succeded in passaging the nodular lesion, and so also has Binford. Our animals lately inoculated in the intraperitoneal cavity with nodular lesions are still under observation, but this line of investigation has been suspended for the time being because of lack of dba (Bar) animals.

The six mice in the third experiment that were inoculated with autoclaved material did not show progressive reaction, although an occasional nodule found in some mice on the surface of organs like the spleen perhaps represents a reaction to the inactivated material and mere accumulation of the intraperitoneally-inoculated acid-fast organisms. In general, the lesions of the reticuloendothelial system, skin and testis developed in the experimental mice—particularly in the inbred dba strain—with the ICRC bacillary inoculations were comparable to clinical and experimental lesions described previously.



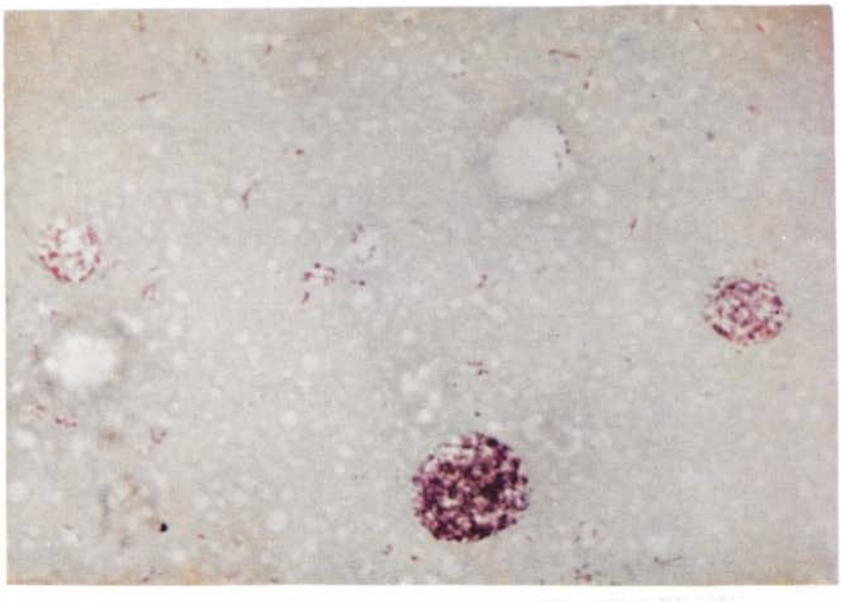


FIG. 13. A smear of the ICRC bacillus from a culture in a fluid medium, stained by Ziehl-Neelsen. Showing typical globus formation of the acid-fast organisms. 750×.

FIG. 14. A section of a histiocytic nodule from the pancreas. Stained by Ziehl-Neelsen, to show groups and clumps of acid-fast bacilli in the cells. 300×.

The cultivated organism maintained for several months under the condition of culture described ([2]) is thus capable of producing lesions in the mouse. More extensive studies on a larger group of dba mice, particularly to investigate the reaction of the nervous system to bacillus inoculation and to study the behavior of the organism on transplantation from animal to animal, are under way. It has already been possible to isolate the ICRC bacillus from the leproma-like lesion of the dba mouse, and the bacillus has been kept in continuous cultivation in the modified fluid culture medium for the last six months.

Experiments are under way to study the immunologic characters of the ICRC bacillus. Immunologic studies with a "lepromin" prepared from the organism are also in progress, the results of which will be reported separately.

SUMMARY AND CONCLUSION

An acid-fast microorganism isolated from lepromatous leprosy, designated the "ICRC bacillus" (Indian Cancer Research Centre bacillus) has been maintained in *in vitro* cultivation for over four years. To test the pathogenicity of the organism in laboratory mice, intraperitoneal inoculations of heavy doses, ranging from 5×10^8 to 2×10^9 organisms per mouse, were given to young mice of three strains C_3H(Jax), Little's dba, and a hybrid of strains XVII and C_{57} (Black).

In the first experiment, designed to study early visceral response, the animals were sacrificed 6 to 17 weeks after inoculation. Early histiocytic reactions of granuloma type, further developing into pinpoint nodules and "miliary leproma-like" lesions swarming with bacilli, were quite common in the liver, spleen, lymph nodes and other visceral organs. The dba mouse was found to be particularly susceptible to the bacillary infection.

The second experiment was carried out exclusively with dba males, with the same range of dosage. These animals, sacrificed between 20 to 65 weeks after inoculation, presented progressive histiocytic lesions in the liver, spleen, pancreas, lymph nodes, skin, subcutis and the peritesticular tissue.

The histopathology of the progressive lesions was closely comparable to some of the clinical material and experimental lesions described before. The histopathology of the lesions induced by the ICRC bacillus is illustrated by suitable photomicrographs, and the data are discussed in the light of the relevant literature.

RESUMEN Y CONCLUSION

Un microbio ácidorresistente, aislado de lepra lepromatosa y denominado "Bacilo ICRC" (bacilo del Centro Indio de Investigación del Cáncer), ha sido mantenido en cultivo *in vitro* por más de cuatro años. Para comprobar la patogenicidad del microbio en ratones de laboratorio, se hicieron inoculaciones intraperitonealee de dosis masivas,

variando de 5×10^8 a 2×10^9 microbios por ratón, en ratoncillos de tres cepas: C_3H (Jax), el dba de Little y un híbrido de cepas XVII y C_{57} (negro).

En el primer experimento, destinado a estudiar la reacción visceral temprana, se sacrificó a los animales de 6 a 17 semanas después de la inoculación. En el hígado, bazo, ganglios linfáticos y otras vísceras, fueron bastante frecuentes las reacciones histiocíticas tempranas de forma granulomatosa que se convirtieron más tarde en nódulos en punta de alfiler y lesiones "parecidas a leproma miliar," que rebosan de bacilos. El ratón dba resultó ser en particular susceptible a la infección bacilar.

El segundo experimento se llevó a cabo exclusivamente con dbas machos, con el mismo régimen posológico. Estos animales, sacrificados de 20 a 65 semanas después de la inoculación, presentaban lesiones histiocíticas evolutivas en el hígado, bazo, páncreas, ganglios linfáticos, piel, subcutis y el tejido peritesticular.

La histopatología de las lesiones evolutivas era íntimamente comparable con la de algún material clínico y la de las lesiones experimentales descritas anteriormente. La histopatología de las lesiones inducidas por el bacilo ICRC es ilustrada con microfotografías apropiadas discutiéndose los datos a la luz de la literatura pertinente.

RESUMÉ

Un micro-organisme acido-résistant isolé de lèpre lépromateuse et désigné sous le terme de "bacille ICRC" (Indian Cancer Research Centre bacillus) a été maintenu *in vitro*, en culture, pour plus de 4 ans. Afin de tester le pouvoir pathogène de cet organisme chez la souris de laboratoire, des injections intra-péritonéales de fortes doses ont été pratiquées chez de jeunes souris de trois souches, C_3H(Jax), dba de Little, et un hybride des souches XVII et C_{57} (noir). Les doses se sont élevées de 5×10^8 à 2×10^9 organismes par souris.

Dans la première experience, ménée pour étudier l'action précoce sur les viscères, les animaux ont été sacrifiés 6 à 17 semains après l'inoculation. Une réaction précoce histiocytique de type granulomateux fut trouvée communément dans le foie, la rate, les ganglions et d'autres organes internes. Cette réaction se developpait ensuite en nodules de la grosseur d'une tête d'épingle, avec lésions ressemblant à une "miliaire lépromateuse", bourrées de bacilles. Les souris dba furent trouvées particulièrement susceptibles à l'infection bacillaire.

La seconde experimentation fut menée exclusivement sur des mâles de la souche dba, avec des inoculationes du même ordre de grandeur. Ces animaux, sacrifiés 25 à 65 semaines après l'inoculation, ont présenté des lésions histiocytiques progressives dans le foie, la rate, le pancréas, les ganglions, la peau, l'hypoderme et le tissu péritesticulaire.

L'histopathologie des lésions progressives était fort comparable à celle du matériel clinique et des lésions expérimentales décrites ci-dessus. L'histopathologie des lésions provoquées par le bacille ICRC est illustrée de façon adéquate par des photomicrographies et les résultats sont discutés à la lumière des données pertinentes de la littérature.

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