PATHOMORPHOLOGICAL CHANGES INDUCED IN THE INTERNAL ORGANS OF *Dasypus novemcinctus* LINN. BY INFECTION WITH *Mycobacterium leprae**

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The nine-banded armadillo (*Dasypus novemcinctus* Linn.) is nowadays used the most adequate object for obtaining a model of experimental leprosy. The first experiments on experimental infection of the armadillo with human strains of *Mycobacterium leprae* were carried out in 1971 [8]. The idea of using this "relict" for the reproduction of experimental leprosy was based on the fact that the armadillo has a body temperature which is relatively lower (from 30 to 35°C) than that of other mammals, and it was suggested that this corresponds more closely to the condition for reproduction of *M. leprae*.

Many facts on the general pathology of *D. novemcinctus* when infected experimentally with human *M. leprae* have been reported in the literature in recent years [3, 4, 9]. It is stated that the disease develops 1.5-2 years after inoculation with infected material and that it follows a very similar course to the lepromatous type of leprosy in man. At the same time, frequent involvement of the lungs by the specific process has been reported, which is not typical of human leprosy, as well as spreading of the granuloma in the skin as far as the stratum basale of the epidermis (in man the subepidermal zone is always unaffected in the lepromatous type of leprosy) and invasion of bacteria into the cytoplasm of hepatocytes, with their transformation into foam cells [5-7].

Research into experimental infection of aramdillos with *M. leprae* is being undertaken in the USSR for the first time. It is taking place by agreement with the World Health Organization (Project ID 780259, Project Director A. A. Yushchenko).

EXPERIMENTAL METHOD

The Leprosy Research Institute received 26 nine-banded aramdillos from the state of Florida (USA). During the first two months eight animals died from various causes (trauma during transportation, hypothermia, and possibly, adaptation). During the same period twelve pregnant females gave birth to 46 young armadillos. Of this number 2 survived 2 months, 5 lived 2-15 days, and the rest were stillborn, premature, or eaten by their mothers immediately after birth. After a three-month quarantine period 15 armadillos were infected with a suspension of *M. leprae* obtained from a leproma in a patient with an untreated lepromatous type of leprosy. Infection was carried out by intravenous and subcutaneous injection of 10^7-10^8 *M. leprae* cells per animal. Four armadillos died 3-4 months after infection. At autopsy on the animals which died pieces of organs were embedded in paraffin wax and sections cut on freezing microtome. Sections were stained with hematoxylin and eosin, with picrofuchsine by Van Gieson's method, with Sudan III, with carbol fuchsine by the Ziehl-Nielson method for acid-fast mycobacteria, the PAS reaction, and Perls' reaction for iron. In all cases microbiological studies were done but yielded negative results.

EXPERIMENTAL RESULTS

In the stillborn armadillos the pulmonary alveoli and renal glomeruli were underdeveloped, Signs of perivascular and pericellular edema were clearly visible in the brain. In armadillos which survived for 2 or 3 days and 2 months after birth, evidence of bilateral bronchopneumonia was present. Macroscopically there were tiny, dense collapsed areas, grayish-red on section. Histologically the alveoli and bronchioles were filled with polymorphonuclear leukocytes and the blood vessel walls were swollen. Trophic disturbances were found in other organs.

In the uninfected armadillos which died after giving birth hypertrophy of the myomedtrium and decidual transformation of the endometrium were present, with multiple foci of necrosis and of infiltration by leukocytes. All the females had

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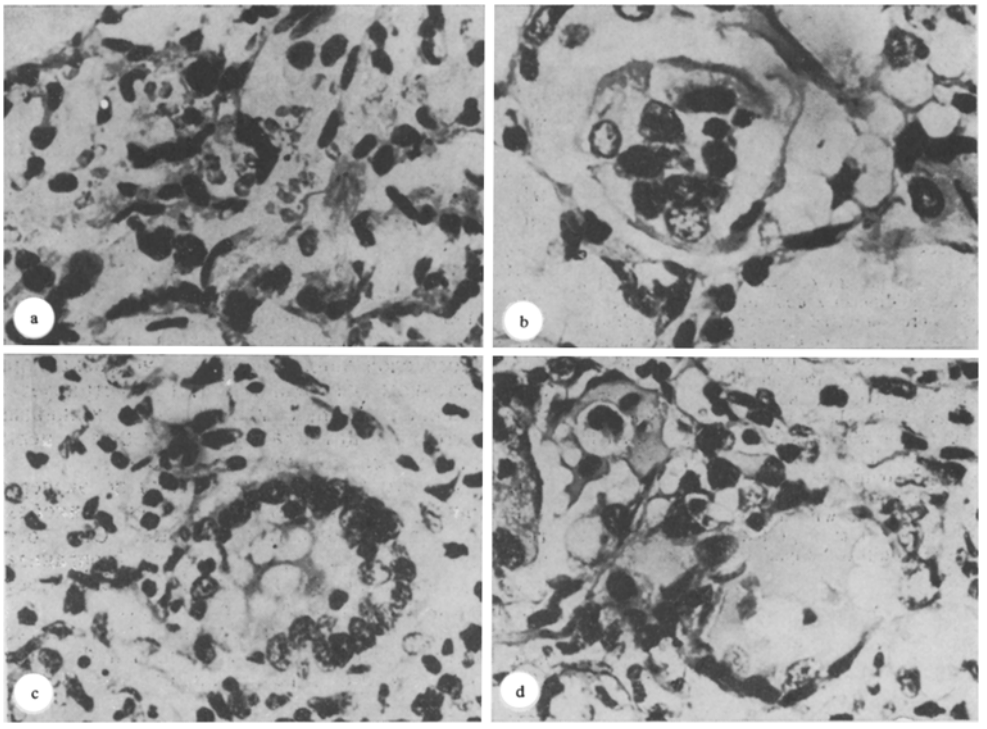

Fig. 1. Tuberculoid changes in lungs of armadillos 4 months after infection: a) foci of infiltration with histiocytes and epitheiloid cells, b-d) various types of polynuclear giant cells, 500 X. Here and in Figs. 2 and 3, sections stained with hematoxylin–eosin.

considerable traumatic wounds on various parts of the skin, more especially in the region of the tail. In one case a massive hemorrhage was found into the right pectoralis mucle. Histologically the wounds were characterized by considerable bleeding, infiltration with leukocytes, and granulation tissue development. Changes in the lungs in 2 animals of this group consisted of small foci of bronchopneumonia, but no changes were present in one female. Fatty degeneration of the liver was found in all animals, diffuse in one case. The liver of one animal contained multiple nodules of infiltration with polymorphonuclear leukocytes and lymphocytes. In most cases marked hemosiderosis of the liver could be observed. In the interstitial tissues of the kidneys and myocardium tiny foci of infiltration containing mainly lymphoid cells, mixed with a few polymorphonuclear leukocytes, were discovered.

In the remaining uninfected animals which died signs of bilateral bronchopneumonia were found, and in one case there were considerable changes in the liver in the form of multiple foci of necrosis with infiltration by leukocytes against a background of diffuse fatty degeneration. Examination of the skin in the region of the wounds revealed areas of necrosis, infiltration with leukocytes, and signs of regeneration. In the kidneys there were multiple foci of infiltration of the stroma by leukocytes and lymphocytes.

Investigation of the infected armadillos which died showed widespread granulomatous changes of tuberculoid type in two of the four cases. The lungs of these animals contained extensive areas consisting of nodular and diffuse tuberculoid structures with an abundance of giant cells. These cells were numerous, they occupied whole areas, and were distributed in groups or singly. The giant cells varied considerably in shape: typical cells of Langhans with the nuclei located peripherally, large cells with central nuclei, and cells with 3 to 5 large nuclei were encountered (Fig. 1). Besides giant cells, numerous lymphoid cells and histiocytes, together with solitary polymorphonuclear leukocytes were found in the zone of infiltration. In one of these cases there was distinct evidence of calcinosis of the lung tissues. Wedge-shaped areas of the lungs on macroscopic examination occupied part of a lobe, they were whitish in color, and dense and leathery in consistency. In two cases thin-walled cysts equal in size to 5-10 alveoli were found in the lungs, and the walls of some cysts contained lines (Fig. 2). On investigation of the skin of the abdomen and limbs, foci of infiltration with epithelioid cells, mixed with a few lymphoid cells, were found in the dermis. Nodules consisting of lymphoid and giant (of the Langhans' type) cells were found in the liver. In the case with calcinosis of the lungs multiple fibrous nodular structures were found in the liver, some of them with residual features of a granuloma and with single Langhans' cells (Fig. 3). In two other infected armadillos signs of bilateral bronchopneumonia and degenerative changes in the organs were present.

Tuberculoid structures containing giant cells were thus observed to have developed in the lungs and liver, and epithelioid-cell nodules in the dermis of two armadillos which died 4 months after experimental infection with human strains

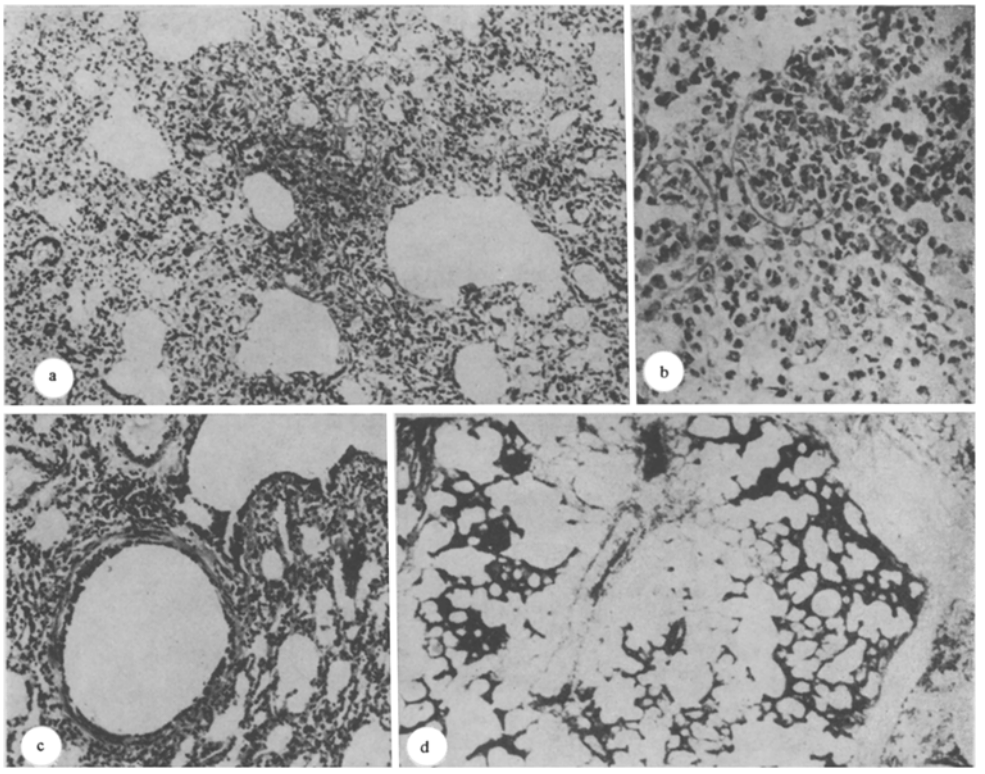

Fig. 2. Changes in lungs of armadillos 4 months after infection: a) focus of giant-cell pneumonia, with microcysts; b) formation of microcysts; c) deposition of line in walls of microcysts; d) diffuse calcinosis of lung tissue, 120 X.

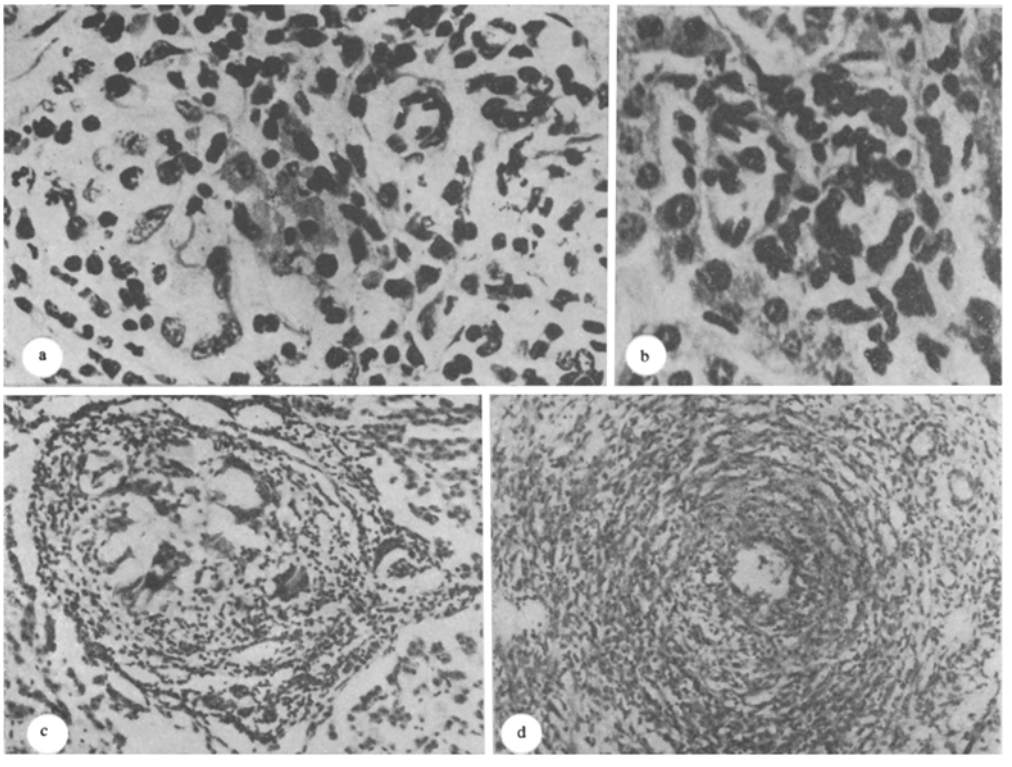

Fig. 3. Granulomatous changes in liver and skin of armadillos 4 months after infection: a, b) tuberculoid structures in liver, 500 X; c) cicatrizing granuloma in liver, 120 X; d) granuloma in skin, 120 X.

of *M. leprae*. In all cases studied staining for micobacteria gave negative results. Changes in the lungs similar to those described above have been described in sporadic diseases in laboratory animals [1] and during an epizootic in rats [2]. Despite a certain morphological similarity, the discovery of tuberculoid structure only in the armadillos which were infected with leprosy, and also the fact that they were found not only in the lungs, but also in other organs (liver, skin), gives grounds for attributing these changes to inoculation of the armadillo with *M. leprae*.

Information on the development of disseminated lesions of tuberculoid type in *D. novemcinctus* could not be found in the literature on experimental leprosy. There are reports [4] of the development of granulomas of tuberculoid type without mycobacteria in armadillos of another species – the seven-band armadillo (*Dasypus sabanicola*) when infected with human leprosy. On this basis the author cited concluded that *D. sabanicola* gives two types of response to infection: systemic lesions similar to those in *D. novemcinctus* and lesions with a tuberculoid structure without mycobacteria. In *D. novemcinctus* tuberculoid changes have been described extremely rarely and observed only at the sites of inoculation of the mycobacteria, and only in animals considered to be "resistant" to infection with leprosy. From this standpoint it is possible to interpret the changes discovered in the present experiments as a result of a reaction to massive inoculation of mycobacteria in armadillos resistant to infection with leprosy. The presence of changes not only in the skin, but also in the lungs and liver, must be explained by the fact that infective material was injected in large doses.

Tuberculoid structures thus develop in the lungs, liver, and skin of nine-banded armadillos infected with human strains of *M. leprae* in some cases. This indicates that the course of experimental leprosy in *D. novemcinctus* may follow either the lepromatous or the tuberculoid type.

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