HISTOCHEMICAL STUDY OF REVERSIVE REACTIONS IN EXPERIMENTAL LEPROSY IN ARMIDILLOS*

A. A. Yushchenko and F. E. Vishnevetskii

UDC 616-002.73-039.38-02:
615.281.873.21]-092.9

KEY WORDS: experimental leprosy; nine-banded armadillo (*Dasypus novemcinctus*); reversive reaction; morphology; histochemistry

During the treatment of leprosy patients transformation of the leprosy process, clinically and morphologically, toward forms of the disease with a more favorable course and prognosis is observed in some cases — from the lepromatous part of the spectrum toward the tuberculoid [6, 9]. The name "reversive reactions" has been given to this phenomenon. In 1984 Yushchenko [4] first described clinical observations of reversive reactions in armadillos with experimental leprosy. Armadillos experimentally infected with leprosy usually develop a morphological picture typical of the lepromatous type of the disease, with large numbers of mycobacteria in macrophages in the zone of infiltration [4, 5, 7, 12]. More recently the possibility that armadillos may develop leprosy of localized [10] and also of tuberculoid type has been reported [2, 8, 11].

This paper describes the results of morphological, enzyme-histochemical, and electron-microscopic investigations of armadillos in which the course of experimental leprosy can be assessed, on clinical and morphological grounds, as reversive transformation of the leprosy process.

EXPERIMENTAL METHOD

Nine-banded armadillos (*Dasypus novemcinctus* Linn.) were infected intravenously and subcutaneously with a suspension of a leproma from an untreated patient with the lepromatous type of leprosy in a dose of $6 \cdot 10^7$ bacterial cells per animal. The first lepromas (Fig. 1b) appeared in the armadillos 20-24 months after infection. Four animals developed confluent erythema of the abdominal surface of the body 10-14 months later, accompanied by quickening of respiration and a rise of temperature, together with the simultaneous development of multiple erosions of the epidermis and ulceration of large cutaneous lepromas (Fig. 2a). These features of the reactive state were observed for 15-20 days, after which regression of the lepromas began against the background of normalization of the animal's general state (flattening of the lepromas, a decrease in number and destruction of mycobateria). Biopsy specimens were taken from the primary lepromas (before the development of the reactive state) and from lepromas with evidence of regression.

Two armadillos were killed soon after the beginning of the reaction. In two other animals two reactions were observed in each case with an interval of about 2 months between them, after which they also were killed. Paraffin sections from the biopsy specimens and post mortem material were stained with hematoxylin and eosin and by the Ziehl—Nielsen method. Activity of the enzymes succinate (SDH), lactate (LDH), and glucose-6-phosphate (G6PDH) dehydrogenases, β-glucuronidase (BGL), acid phosphatase (AP), and α-naphthyl acetate esterase (NE) was studied. The enzyme reactions were conducted by the usual methods. Their intensity was estimated on an Opton SMP-01 scanning microscope-photometer. Measurements were made in monochromatic light at 540 nm for oxidative enzymes and 560 nm for AP and NE. The thickness of the probe was constant at 1 μ. Calculations were based on mean

*The investigation was partially subsidized by UNDP, the International Bank, and WHO, "Immunology of Leprosy," special program for the study of tropical diseases, project 780259, responsible executive A. A. Yushchenko.

Research Institute for the Study of Leprosy, Ministry of Health of the USSR, Astrakhan'. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 3, pp. 376-380, March, 1987. Original article submitted May 5, 1986.

0007-4888/87/0003-0427$12.50

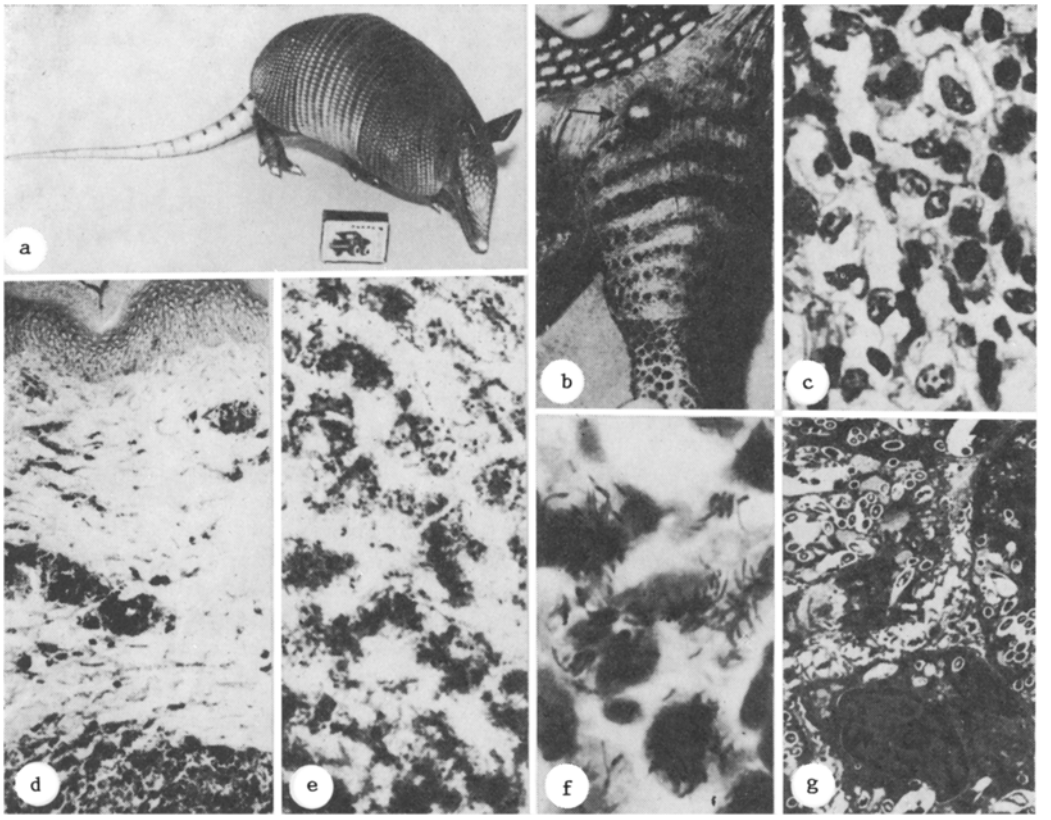

Fig. 1. Lepromatous leprosy in an armadillo before development of the reversive reaction. a) Nine-banded armadillo; b) leproma on outer surface of right hind limb (arrow); c) lepromatous infiltration of macrophages with foamy cytoplasm. Hematoxylin-eoxin, 400 ×; d) high esterase activity of macrophages in zone of infiltration and low in epidermis. Stained by method of Nachlas and Seligman in Gomori's modification. 630 ×; e) high G6PDH activity in macrophages in zone of infiltration. Method of Hess et al. 400 ×; f) mycobacteria in macrophages. Ziehl-Nielsen stain. 1000 ×; g) mycobacteria in cytoplasm of macrophages. 6500 ×

extinction (ME). Ultrathin sections through the lepromas were examined in the JEM-100S electron microscope.

EXPERIMENTAL RESULTS

Examination of biopsy material from primary lepromas showed the typical picture of the lepromatous type of leprosy. The zones of infiltration consisted mainly of large macrophages with a few vacuoles in their cytoplasm (Fig. 1c), which contained many acidfast mycobateria (Fig. 1f, g). Few solitary lymphoid cells were seen. High activity of oxidoreductases, especially G6PDH, was recorded in the cytoplasm of the macrophages (Fig. 1e). High activity of AP and NE was found in these same cells. Activity of the latter was considerably reduced in the epidermis above the leproma (Fig. 1d). The ratio between NE activity in the epidermis and macrophages of the leproma was 0.36 ± 0.01. The results of photometric and electron-microscopic investigations of all the enzymes were virtually identical with those in untreated patients with the lepromatous type of leprosy [1, 3].

A sharp decrease in the number of mycobacteria in macrophages of the granulomas down to single figures was discovered in lepromas with clinical manifestations of regression, by contrast with the primary lepromas (Fig. 2f). Morphologically three versions of reversive transformation of the zones of infiltration could be seen in armadillos with experimental leprosy: 1) the appearance of marked lymphoid infiltration (Fig. 2c) with solitary giant cells and a peripheral arrangement of the nuclei; 2) the presence of numerous giant cells (Fig. 2e) and of a very small number of lymphocytes; 3) a combination of marked lymphoid infiltration with considerable giant-cell transformation of the macrophages. In all three versions considerable concentrations of epithelioid cells could be seen, forming

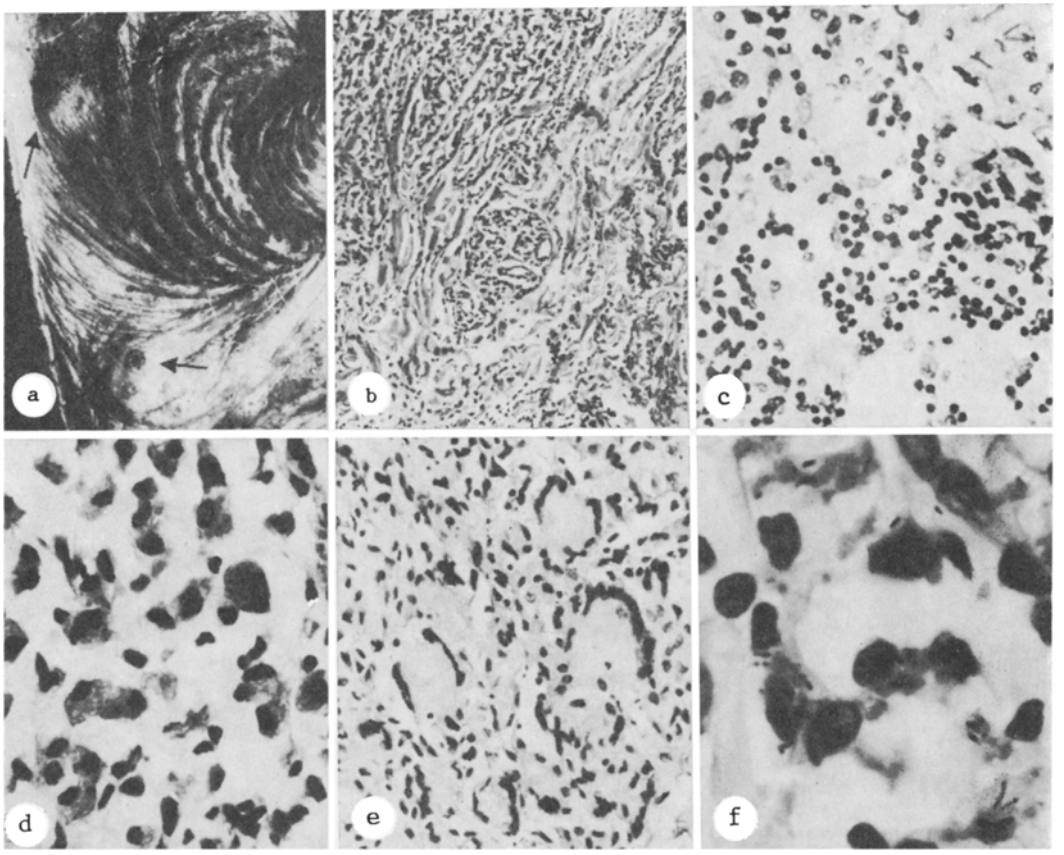

Fig. 2. Leproma in armadillo during period of reversive reaction. a) Flattening and ulceration of leproma; b) fibrous bands in zone of lepromatous infiltration. Hematoxylin-eosin (here and in Fig. 2b-e). 63 ×; c) infiltrating lymphoid cells in leproma. 160 × (here and in Fig. 2d, e); d) syncytium of epithelioid cells; e) group of multinuclear giant cells with peripheral location of nuclei; f) solitary and fragmented mycobacteria in macrophages. Ziehl-Nielsen's stain. 1000 ×.

TABLE 1. Dynamics of Enzyme Activity of Mononuclear Phagocytes of Leproma during Reversion of Leprosy in Armadillos (M ± m)

Enzyme	Biopsy material								Post mortem material			
	before reversion				in period of reversion							
	macrophage with many mycobacteria	macrophage with few mycobacterium	macrophage with no mycobacteria	giant cell with foamy cytoplasm	macrophage with few mycobacterium	macrophage with no mycobacteria	epithelioid cell	giant cell of Langhans	macrophage with few mycobacterium	macrophage with no mycobacteria	epithelioid cell	giant cell of Langhans
SDH	0,54 ± 0,02	0,26 ± 0,034	0,22 ± 0,006	0,34 ± 0,022	0,32 ± 0,008	0,30 ± 0,013	0,18 ± 0,015	0,48 ± 0,045	0,30 ± 0,011	0,28 ± 0,024	0,22 ± 0,016	0,52 ± 0,036
LDH	0,48 ± 0,072	0,34 ± 0,024	0,36 ± 0,011	0,52 ± 0,014	0,18 ± 0,016	0,28 ± 0,044	0,54 ± 0,012	0,76 ± 0,050	0,16 ± 0,010	0,20 ± 0,012	0,44 ± 0,036	0,54 ± 0,064
G6PDH	0,82 ± 0,057	0,52 ± 0,025	0,54 ± 0,017	0,64 ± 0,038	0,32 ± 0,022	0,46 ± 0,017	0,62 ± 0,025	0,58 ± 0,042	0,28 ± 0,011	0,38 ± 0,014	0,56 ± 0,021	0,48 ± 0,033
NE	0,96 ± 0,013	0,84 ± 0,034	0,92 ± 0,032	0,82 ± 0,022	0,62 ± 0,030	0,34 ± 0,010	0,46 ± 0,007	0,42 ± 0,012	0,60 ± 0,040	0,42 ± 0,014	0,44 ± 0,026	0,44 ± 0,018
BGL	0,42 ± 0,023	0,48 ± 0,017	0,48 ± 0,024	0,34 ± 0,011	0,62 ± 0,021	0,74 ± 0,044	0,76 ± 0,026	0,92 ± 0,033	0,64 ± 0,018	0,76 ± 0,057	0,78 ± 0,054	0,86 ± 0,072
AP	0,92 ± 0,064	0,88 ± 0,057	0,64 ± 0,024	0,96 ± 0,034	0,92 ± 0,018	0,78 ± 0,022	0,70 ± 0,062	0,96 ± 0,046	0,86 ± 0,072	0,76 ± 0,033	0,82 ± 0,052	0,92 ± 0,036

syncytia (Fig. 2d). Occasionally single mycobacteria could be seen in the cytoplasm of the giant cells but no mycobacteria were found in the epithelioid cells. Irrespective of the degree of mycobacterial saturation, a decrease in oxidative enzyme (chiefly G6PDH) and NE activity was observed in the macrophages. Activity of BGL was appreciably increased, whereas that of AP was virtually unchanged. Processes of glycolysis (LDH) and the pentose phosphate shunt (G6PDH) predominated in the epithelioid cells, but activity of the key enzyme of the Krebs' cycle (SHD) was very weak. NE activity in the epithelioid cells was much lower than

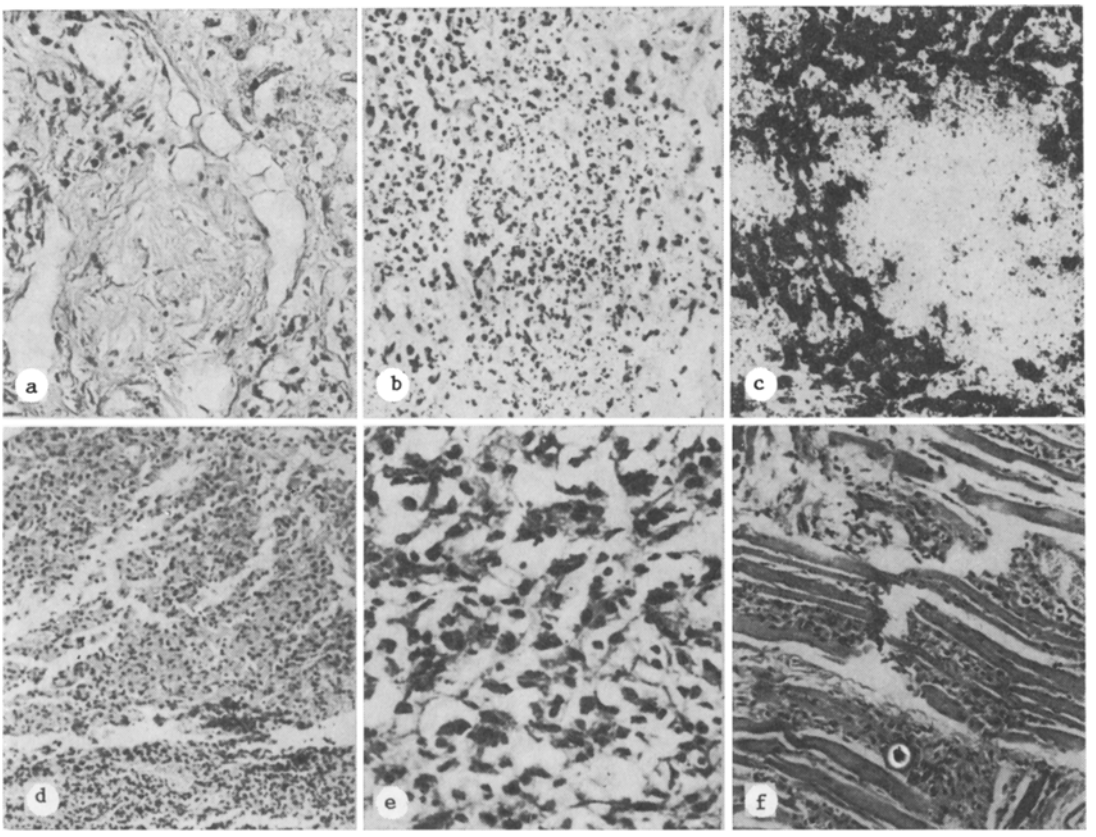

Fig. 3. Internal organs during reversive reaction. a) Fibrosis of granuloma in the liver; b) necrosis of granuloma in the liver. 160 × (Here and in Fig. 3b-d, f); c) absence of esterase activity in granuloma of the liver while activity remains in the hepatocytes. Nachlas and Seligman's stain in Gomori's modification; d) lymph node, granuloma consisting of epithelioid cells; e) spleen. Degenerative changes in granuloma consisting of epithelioid and lymphoid cells. 400 ×. f) Tongue. Zones of lepromatous infiltration between muscle fibers. Hematoxylin-eosin (Fig. 3a, b, d-f).

in the macrophages before reversion, but BGL activity was considerably higher. Uniform activity of the key dehydrogenases (SDH, LDH, G6PDH) was observed in the giant cells, whereas activity of NE, BGL, and AP was similar to that in the epithelioid cells (Table 1).

At autopsy specific changes were the presence of numerous flattened cutaneous lepromas, enlarged and firm inguinal and axillary lymph nodes, and multiple nodules beneath the capsule of the liver and spleen. On histological investigation of the cutaneous lepromas, besides manifestations of reversion described above, massive areas of collagenized connective tissue with immured macrophages, some of them characterized by foamy cytoplasm, with a high degree of mycobacterial saturation and with high dehydrogenase and esterase activity also were found, i.e., the morphological and histochemical parameters of the original granulomas were preserved. When manifestations of reversion were present (a reduction of mycobacterial saturation, epithelioid-cell and giant-cell transformation of macrophages, and lymphoid infiltration of the stroma), the enzyme activity of the macrophages and epithelioid and giant cells was the same as in the biopsy material during reversion.

Leprous granulomas in the spleen and lymph nodes were penetrated by massive bands of lymphocytes and they consisted of macrophages with foamy cytoplasm, containing tiny vacuoles and a few mycobacteria, with large masses of melting macrophages and epithelioid cells without mycobacteria (Fig. 3d, e) and solitary giant cells. Well-defined lepromatous granulomatous zones of infiltration were found in the intermuscular layers of the tongue (Fig. 3f). In the liver many macrophagal-epithelioid-cell granulomas were seen, mainly in a state of destruction and fibrosis (Fig. 3a, b). Absence of NE activity, characteristic of the lepromatous type of leprosy, was observed in the lepromas in the liver, although it was preserved in the hepatocytes (Fig. 3c).

As a result of these investigations the morphological characteristics of development of reversive reactions in nine-banded armadillos was thus obtained for the first time and the time course of enzyme activity of the mononuclear phagocytes was demonstrated during this process.

LITERATURE CITED

1. F. E. Vishnevetskii and A. A. Osipov, Arkh. Patol., No. 1, 22 (1980).
2. F. E. Vishnevetskii and A. A. Yushchenko, Byull. Éksp. Biol. Med., No. 8, 105 (1981).
3. F. E. Vishnevetskii and A. A. Yushchenko, Phagocytosis and Immunity [in Russian], Moscow (1983), pp. 54-55.
4. A. A. Yushchenko, Current Problems in Leprology [in Russian], Astrakhan' (1984), pp. 40-42.
5. A. A. Yushchenko, F. E. Vishnevetskii, and V. A. Evstratova, Great Medical Encyclopedia, 3rd edition [in Russian], Vol. 13, Moscow (1980), pp. 34-50.
6. A. A. Yushchenko, V. A. Evstratova, L. M. Chernysheva, et al., Abstracts of Proceedings of the First Kalmyk Republican Conference of Dermato-Venereologists [in Russian], Élista (1984), pp. 82-84.
7. C. H. Binford, E. E. Storrs, and G. P. Walsh, Int. J. Leprosy, 44, 80 (1976).
8. J. Convit, N. Aranzazu, and M. E. Pinardi, The Armadillo as an Experimental Model in Biomedical Research, Washington (1978), pp. 41-46.
9. R. C. Hastings and C. K. Job, Am. J. Trop. Med. Hyg., 27, 995 (1978).
10. C. K. Job, W. F. Kirchheimer, and R. M. Sanchez, Int. J. Leprosy, 50, 177 (1982).
11. C. K. Jon, W. F. Kirchheimer, and R. M. Sanchez, Int. J. Leprosy, 50, 488 (1982).
12. W. F. Kirchheimer and E. E. Storrs, Int. J. Leprosy, 39, 693 (1971).