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Ontogeny of Lysozyme in the Rat[1] (37859)

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(Introduced by J. Furth)

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Studies of the ontogeny of lysozyme (LZM) have shown that the enzyme is already present in human fetuses between 9 and 12 wk of age and that the levels of LZM in sera of newborns are higher than those in the corresponding maternal sera (1, 2). There is no correlation between maternal and newborn serum LZM activity; neither is there evidence for transplacental crossing of LZM (3).

In saline extracts of tissues of newborn mice, LZM activity has been found to manifest significant changes during the first weeks of life, probably in relation to exposure to bacterial flora (4).

In this paper we describe the localization of LZM in rat tissues during fetal and perinatal life, using the immunoperoxidase technique (5, 6).

Materials and Methods. Rats of inbred Wistar/Furth strain were used. Fetal ages were calculated from controlled mating times. Tissues were collected from fetuses 16, 18 and 21 days old, and from newborns at birth and 1, 2, 5, 8, 12, 14, 18, 22 and 28 days after birth. Amniotic fluid was obtained at 16, 18 and 21 days of gestation. Organs were fixed in neutral 10% formalin, embedded in paraffin and sectioned to 5–6 μm thickness. After deparaffinization and rehydration, the sections were washed in 0.01 *M* phosphate buffered saline, pH 7.2 (PBS). The immunoperoxidase bridge method for LZM localization (5, 6) was carried out as follows: tissue sections were incubated with rabbit anti-rat LZM for 30 min. After washing with PBS, the sections were incubated successively with sheep anti-rabbit gamma globulin and rabbit anti-peroxidase. The sections were then covered with horseradish peroxidase (Sigma Type VI) at a concentration of 250 μg/ml. The peroxidase was stained by the method of Graham and Karnowsky (7) using 3,3′-diaminobenzidine and H_2O_2. To test the specificity of the LZM staining, the specific anti-rat LZM antiserum was replaced with either PBS, rabbit anti-hen egg-white LZM or rabbit anti-rat LZM absorbed with purified rat LZM. LZM assays of serum and tissue extracts were performed by the lyso-plate method (8). The cytobacterial demonstration of LZM-containing blood cells was done by the technique of Briggs, Perillie, and Finch (9).

Results. The LZM activity of the amniotic fluid was less than 1 μg/ml in all samples from Days 16 to 21 of gestation. The changes in serum LZM activity are shown in Fig. 1. At Day 18 of gestation, the activity was about 2 μg/ml and at birth, approximately 6 μg/ml. One week after birth the levels of LZM in serum decreased reaching adult levels within 2 wk.

At Day 18 of gestation, the fetal blood contained a very small number of mononuclear cells, presumably monocytes, which

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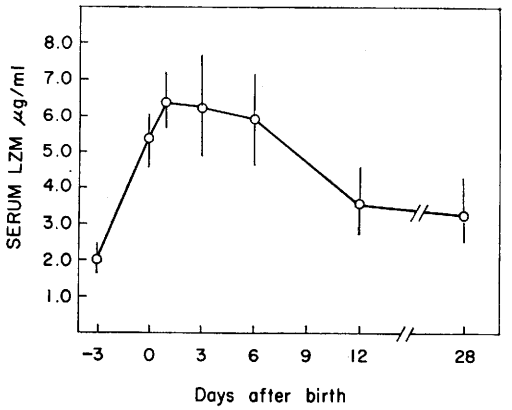

FIG. 1. Serum LZM concentrations in fetal, newborn and postnatal rats up to Day 28. Each point represents the mean of 5 determinations. The range of values at each time point is also indicated.

lysed *Micrococcus lysodeikticus* (Fig. 2).

Using the immunoperoxidase technique, the presence of LZM in tissues was detected as a brown precipitate. The reaction was abrogated when anti-rat LZM was replaced with PBS, anti-hen egg-white LZM or anti-rat LZM previously absorbed with rat LZM.

In the kidneys, LZM was first detected in 18-day-old fetuses associated with the appearance of the proximal tubule cells in the intermedullary area (Fig. 3). Between Days 18 and 21 the number of mature tubules showing LZM increased and, simultaneously, the amount of LZM in each tubule cell was found to increase as judged by the intensity of the specific immunoperoxidase reaction. LZM was absent in the undeveloped tubules of the neogenic zone as well as in the collecting tubules of the medullary zone. After birth, the development of the neogenic zone, characterized by the appearance of numerous mature glomeruli and typical proximal tubules, was associated with increasing LZM activity in tubule cells. In the kidneys of 2-wk-old rats, the distribution of LZM resembled that in adult animals (Fig. 4).

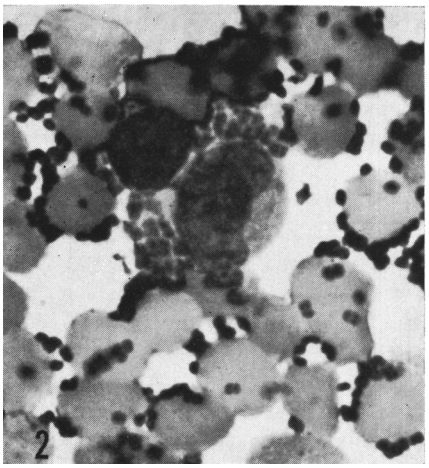

FIG. 2. Demonstration of LZM activity in a peripheral blood monocyte of an 18-day rat fetus. The *M. lysodeikticus* organisms surrounding the monocyte are swollen and more lightly stained. Wright–Giemsa stain (×1240).

In fetal lung, traces of LZM activity were found in sparse alveolar macrophages. A rapid increase in the number of alveolar macrophages, associated with increased LZM activity, was observed in newborn rats between 2 and 5 days of age. Figure 5 shows the LZM staining in alveolar macrophages from an 8-day-old rat. These cells are distributed mainly at the periphery of the lung.

LZM was not detected in the intestinal tissues during fetal life. Paneth cells were observed as single cells in the ileum of 8–12-day-old rats and, at this stage, these cells were found to be strongly LZM-positive (Fig. 6). In the next few days, the number of Paneth cells increased in each crypt of Lieberkühn, reaching, at Day 22, an appearance similar to that in adult rats (Fig. 7).

No LZM was detected in fetal or neonatal rat liver. Keratinized epithelium, collagen and, occasionally, nuclei were found to react nonspecifically with the immunoperoxidase technique. No LZM was detected in cartilage.

Discussion. Major changes in the distribution of LZM were found to occur during fetal and neonatal development in rats. At 18 days of gestation, LZM was detected for the first time in circulating monocytes and, simultaneously, was observed in the developing proximal tubules of the kidneys. The timing of the appearance of LZM in the proximal tubule cells generally coincides with that reported for other enzymes

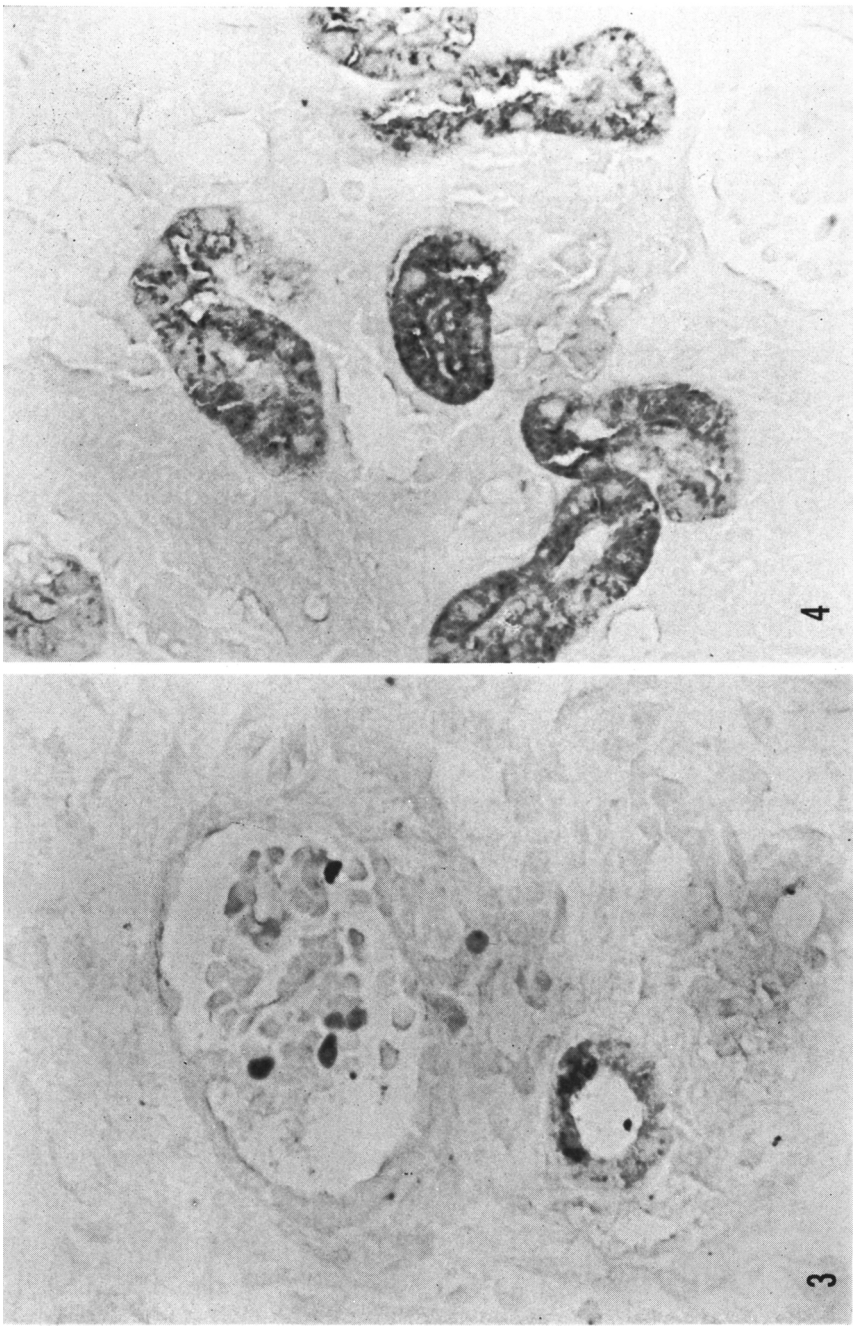

FIG. 3. Immunoperoxidase demonstration of LZM in a proximal tubule of an 18-day-old rat fetus. The staining of scattered glomerular nuclei is nonspecific (×560).

FIG. 4. Kidney of a 2-wk-old rat stained for LZM by the immunoperoxidase method, showing extensive LZM in proximal tubules (×560).

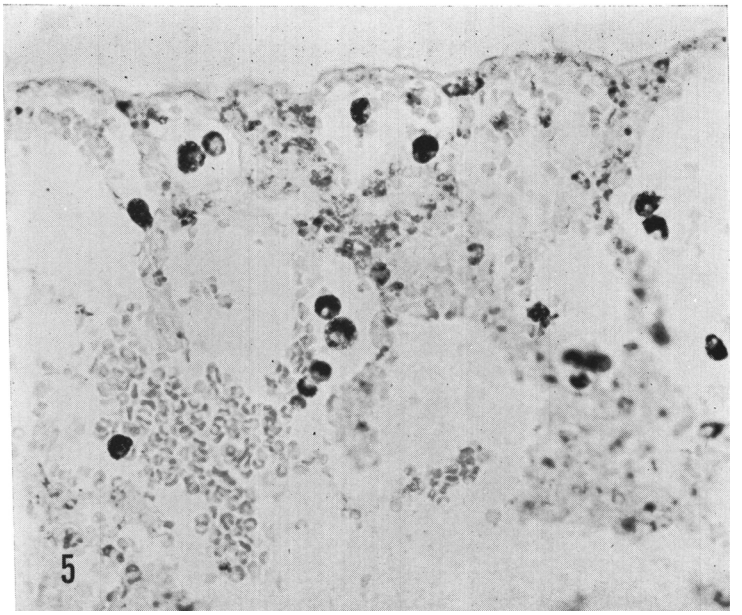

FIG. 5. Lung of an 8-day-old rat showing LZM staining in the alveolar macrophages by the immunoperoxidase technique (×500).

including acid and alkaline phosphates, leucine amino peptidase, cytochrome oxidase and succinic dehydrogenase (10). Since present evidence indicates that the LZM in kidneys of adult animals is derived from the serum via the glomerular filtrate (11), the source of fetal kidney LZM is probably the same.

In newborn rats during the first days of life, serum LZM levels were found to be higher than in adult animals. These LZM levels in newborns possibly reflect the proliferation of monocytes during the first days of life and the development of other cells of the RE system containing LZM, such as the alveolar macrophages. The appearance of LZM-rich alveolar macrophages coincides with exposure of the respiratory tract to environmental microorganisms.

In the present studies, Paneth cells were observed in newborn rats between 8 and 12 days old, earlier than previously reported by Behnke and Moe (12). At the time of their appearance, the Paneth cells were already found to contain LZM. The function of the Paneth cells is still uncertain (13). However, recent studies have shown that rat Paneth cells are capable of phagocytosis and intracellular digestion of the trophozoites of the intestinal flagellate *Hemamita muris* (14) and spiral microorganisms (15).

The present studies are in agreement with previous investigations of LZM ontogeny in man (1–3) and provide additional information regarding the cellular and tissue distribution of the enzyme in fetuses and neonates. The functions of LZM are generally considered to be related to antibacterial defenses although some recent studies have indicated an effect of the enzyme on mammalian cell membranes, both normal and transformed (16).

Summary. Using the immunoperoxidase technique, LZM was detected in the kidney of 18-day rat fetuses. At the same time LZM was found to be present in circulating monocytes. After birth, a marked increase of the number of alveolar macrophages was observed, associated with intense LZM staining. The enzyme was detected in Paneth cells for the first time in 8 to 12 days after birth. These findings are discussed in relation to the known and postulated functions of LZM.

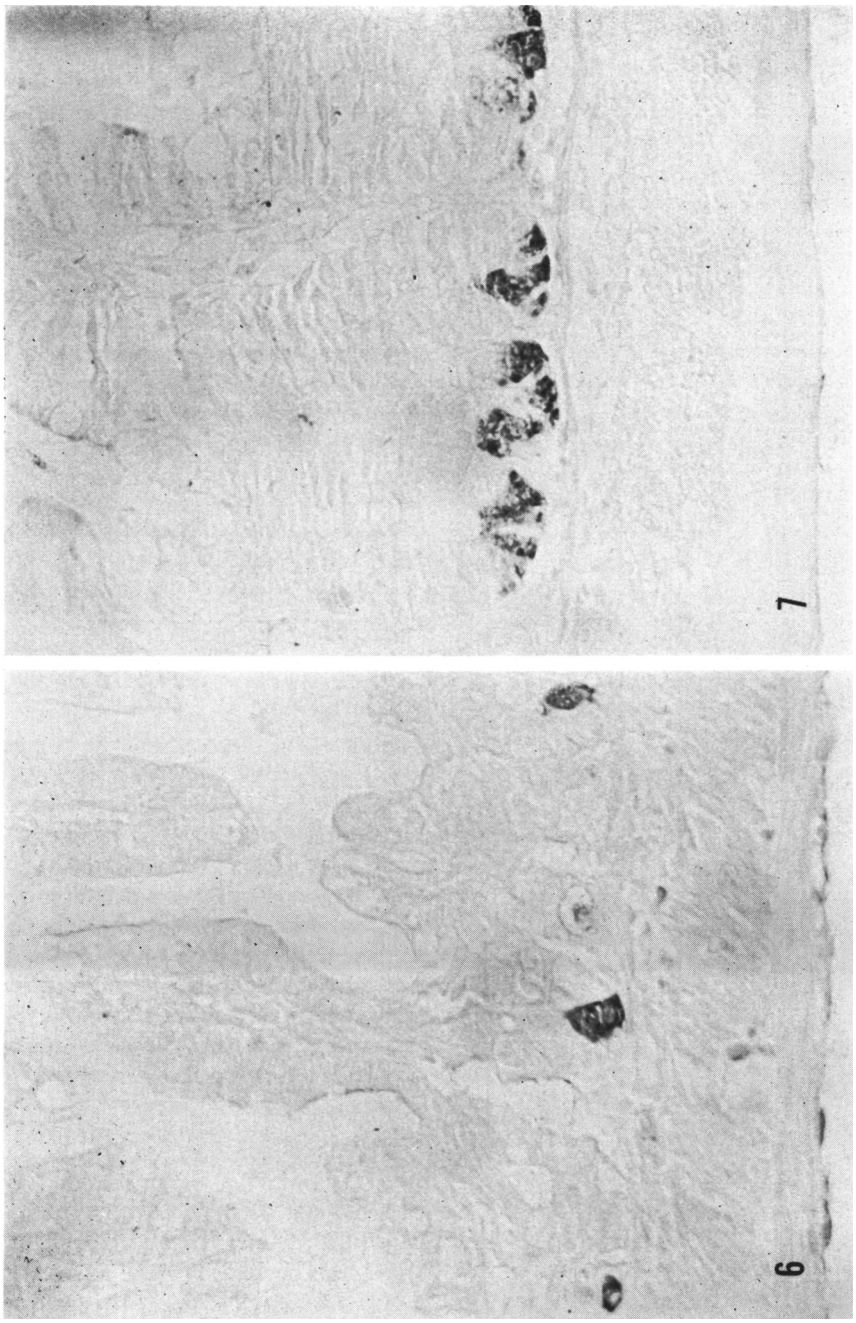

FIG. 6. LZM Localization in Paneth cells of a 12-day-old rat (×500).

FIG. 7. Paneth cells of a 22-day-old rat showing an increase in number and LZM staining compared to day 12 (×500).

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