

ULTRASTRUCTURAL FEATURES OF MAST CELLS IN SYSTEMIC MASTOCYTOSIS

Y. Naveh, R. Ludatscher, B. Gellei, S. Haim and A. Friedman

From the Rambam University Hospital and Aba Khoushy Medical School, Haifa, Israel

Abstract. A 3-year-old boy with systemic mastocytosis has been observed since the age of 4 months when he was first diagnosed as suffering from urticaria pigmentosa. Involvement of skin, liver, spleen and bones was observed. The electron microscopy of skin and liver revealed varied alterations in the morphology of mast cells. The most important findings were irregularly-shaped cells and unusual long and interdigitated cytoplasmic villi, with consequent aggregation of mast cells which was more prominent in the dermis. Proliferation and accumulation of mitochondria in one part of the cell and deeply indented nuclei were frequent. The problem, whether the morphological changes encountered—especially the complex interdigitation of villi—should be interpreted as a sign of expected neoplastic development, is discussed.

Key words: Mast cell; Mastocytosis; Ultrastructure

Cutaneous mastocytosis is not a rare disease in comparison with systemic mastocytosis (SM). Its incidence is about 1:2 500 new attendances in clinics of dermatology (7, 11). Similar to the number reported by Demis (4), systemic involvement was found in 1 of every 20 cases of mastocytosis in our dermatology clinic.

The mast cell of human skin and gingiva has been intensively investigated in healthy and sick men. Electron microscopy has contributed to our knowledge of mast cell degranulation in patients suffering from urticaria pigmentosa (UP) (8). However, there is little information regarding the ultrastructure of mast cells in different organs involved in SM. In our present study, the ultrastructure of mast cells in the liver and skin of a child is described.

CASE REPORT

A 4-month-old infant was admitted because of brown maculo-papulo-vesicular skin lesions and flushing attacks.

Family history is unremarkable except for a grandmother suffering from hypertension.

His present illness was first observed at the age of 2 weeks,

when an erythematous skin area appeared on the left cheek. A few days later, a number of small vesicles appeared on that area. On the subsequent days the vesicles dried up, leaving a rosy-brown skin lesion. At the age of one month, additional erythematous skin areas and vesicles were observed all over the body. At the age of 3 months he began to have flushing attacks which continued for about 5 minutes and spontaneously disappeared. During the last few days preceding his admission, he had more frequent flushing attacks which continued for about half an hour.

Physical examination, on admission, revealed an active, alert, well-nourished infant. His weight was 7 500 g, length 69 cm and head circumference 41 cm. His temperature was 37.2°C. All over the body, a rosy-brown maculo-papulo-vesicular eruption was seen. Liver was palpable 2 cm below the right costal margin and spleen tip was palpable 3 cm below the left costal margin. The rest of the examination including funduscopy was negative.

A presumptive diagnosis of urticaria pigmentosa was made.

Laboratory investigations showed a hemoglobin value of 11 g/100 ml, and white blood cell count of 11 800/cu mm, of which 5 % eosinophils. Urinalysis and stool examination were negative. Erythrocyte sedimentation rate was 45 mm in one hour (Westergren). Serum total proteins were 6.3 g/100 ml of which albumin 3.5 g/100 ml and globulin 2.8 g/100 ml. Thymol turbidity and cephalin flocculation tests were negative. Transaminase (SGOT) 19 units. Alkaline phosphatase 3.4 Bessy-Lowry (B. L.) units. Total lipids 520 mg/100 ml, cholesterol 119 mg/100 ml, and triglycerides 177 mg/100 ml, sugar 100 mg/100 ml blood urea nitrogen 30 mg/100 ml, creatinine 0.7 mg/100 ml, uric acid 4 mg/100 ml. Serum sodium 137 mEq/l, potassium 4.9 mEq/l, chloride 108 mEq/l, bicarbonate 20 mEq/l, calcium 10.6 mg/100 ml, phosphorus 5.2 mg/100 ml. Prothrombin time, thrombin time and bleeding time, were normal. Daily excretion of 5-hydroxyindole acetic acid (5-HIAA) was normal. Urine mucopolysaccharides showed normal values on two occasions and high values on two other occasions. Urine histamine was very high. Gastric juice values were normal. Electroencephalogram during a flushing episode was normal. Bone marrow puncture did not show mast cells. Rectal biopsy revealed a normal number of mast cells.

X-ray findings: X-ray films of chest, skull, spine, upper and lower limbs were normal.

During his hospitalization, several flushing attacks were noted, especially when the infant was exposed to minor surgical procedures.

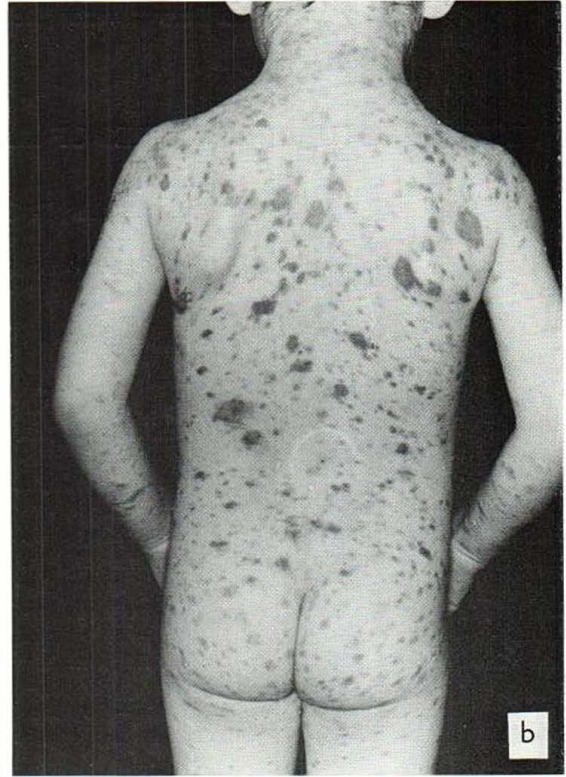
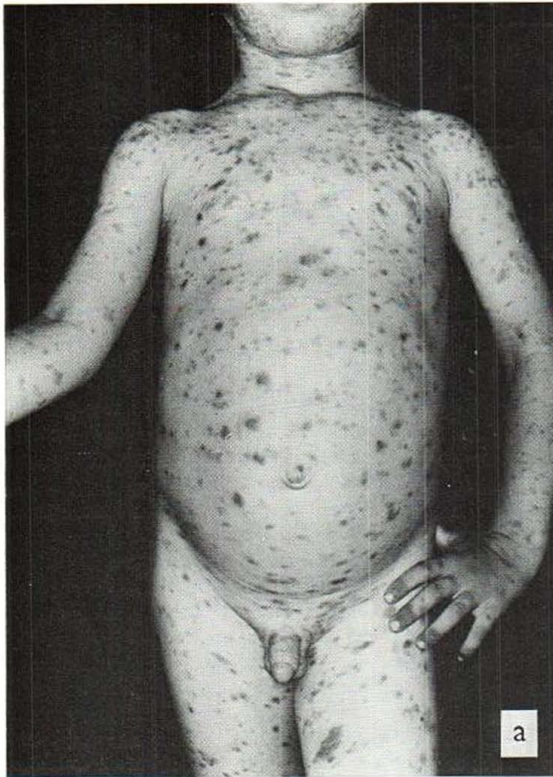


Fig. 1a, b. Macular and maculopapular skin lesions in mastocytosis. Some of these lesions have coalesced to produce large brown areas.

The infant was sent home and was followed up in the out-patient clinic. Over the subsequent years, gradual progressive enlargement of the liver and the spleen was noted, as well as less frequent flushing episodes. At the age of 2 years 5 months the child broke his left femur following relatively mild trauma. Systemic mastocytosis was suspected and the child was re-admitted for reevaluation on March 18, 1974, at the age of 3 years 3 months.

Physical examination, on admission, revealed a thin child with normal motor and mental development. His head circumference was 49.8 cm. His blood pressure was 120/80 mmHg. All over his body brown macular and maculopapular skin lesions were noted (Fig. 1a, b). Enlarged cervical and submandibular lymph nodes were palpated. Some carious teeth were observed. The liver edge was palpated 7 cm below the right costal margin and the spleen tip was palpated 7 cm below the left costal margin. Both were hard. The rest of the physical examination, including fundoscopic examination, was normal. Laboratory investigations showed a hemoglobin value of 11.2 g/100 ml, red blood cells 4.340000/cu mm and hematocrit 35.7%. Platelets 72 000/cu mm. White blood cell count 10 200/cu mm of which 6% eosinophils. Urine analysis normal. Serum total proteins were 5.7 g/100 ml of which albumin 3.6 g/100 ml and globulin 2.1 g/100 ml. Protein electrophoresis was normal. Fibrinogen 328 mg/100 ml. Bromsulphalein retention 1% after 45 min. SGOT 11 units,

amylase 12 units, alkaline phosphatase 2 units (B.L.). Acid phosphatase 0.18 B.L. units. Total lipids 555 mg/100 ml, cholesterol 110 mg/100 ml. Small lipoprotein particles were 260 mg/100 ml, medium lipoprotein particles were 40 mg/100 ml, and large lipoprotein particles were 3.5 mg/100 ml. Carotene 72 mg/100 ml. Sugar 100 mg/100 ml, blood urea nitrogen 20 mg/100 ml, creatinine 0.8 mg/100 ml, uric acid 5.2 mg/100 ml. Serum sodium 139 mEq/l, potassium 4.1 mEq/l, chloride 100 mEq/l, bicarbonate 25 mEq/l, calcium 10 mg/100 ml, phosphorus 4.7 mg/100 ml. Prothrombin time, bleeding time and clotting time were normal. Daily excretion of 5-HIAA was normal. Urine mucopolysaccharides were 9.4 mg/lg creatinine. Bone marrow puncture showed an increase in eosinophils but no mast cell increase was noted.

X-ray findings: Chest film was normal except for mild diffused sclerosis of ribs. Skull film showed advanced closure of sutures relative to the patient's age. Dorsal and lumbar spine film was normal. Upper limb film showed mild sclerosis and coarse trabeculation, especially of the ulna and radius. Hand film showed coarse sclerotic trabeculation and osteoporosis of the metacarpals and phalanges. Pelvis and lower limb films showed coarse sclerotic trabeculation with osteoporosis of the epiphyses, metaphyses and the peripheral parts of the diaphyses. These changes were prominent in the proximal thirds of the femora and the distal parts of the tibias (Figs. 2, 3).

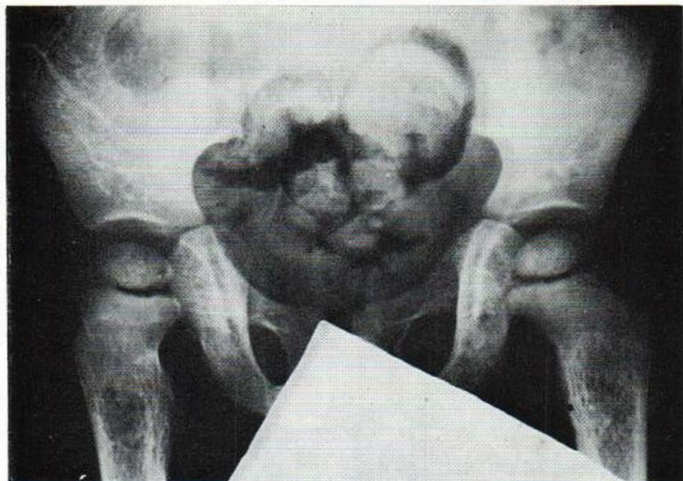


Fig. 2. The pelvic bones and femora show coarse sclerotic trabeculation with osteoporosis.

Microscopic Study

Methods

Skin biopsy was obtained from the left thigh after local infiltration with 1% procaine solution. Percutaneous liver needle biopsy was obtained by Menghini needle. The samples of both skin and liver were divided into two parts. The larger part was fixed in 10% formaldehyde and stained with hematoxylin-eosin and toluidine blue for light microscopy. The smaller part was immediately cut into small pieces and fixed for one hour with cold 3% glutaraldehyde, post-fixed with 2% osmium tetroxide, dehydrated by alcohols and embedded in Epon. Sections $1\mu\text{m}$ thick were stained with toluidine blue for light microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss 9S electron microscope.

Skin

Light Microscopy. Mast cells were seen in the dermis around small blood vessels. The cytoplasmic granules appeared purple in toluidine blue stained sections.

Electron Microscopy. The dermal mast cells were located in the vicinity of blood vessels or were dispersed between collagen fibers. Few cells were elongated. Most mast cells were of irregular-rhomboidal shape with sharp, tail-like angles (Fig. 4). The cell membrane had numerous, long, cellular protrusions that measured up to $3.5\mu\text{m}$. These extensions were often curled and interdigitated in a complicated manner sometimes forming an array of villous cords (Fig. 5). In some areas interdigitation between villi of neighboring cells was encountered and in this way a conglomerate of several mast cells was formed. In the regular-shaped cells the cytoplasmic granules were spherical and had a diameter of $0.3\text{--}0.5\mu\text{m}$. In other cells the granules were much smaller. Sometimes the cell granules were arranged at the periphery of the mast cell, and clusters of mitochondria occupied the center of the cell (Fig. 4). Many membrane bounded vacuoles contained coarse granular material of less density, which could be interpreted as stages of disintegration of the mast cell granules. There was strong evidence of parallelism between the bizarre shape of the mast cell and the unusual, long and interdigitated protrusions. These changes were associated

with multiplication and concentration of mitochondria in one part of the cell (Fig. 4, 5). In the cytoplasm of some mast cells, bundles of parallel membranous structures were evident. These membranes were about $120\text{ \AA}$ in diameter and were tightly grouped in bundles (Fig. 6). The nuclei were either oval-shaped or indented. The dermal collagen fibrils appeared unchanged and were often closely related to the mast cell membrane.

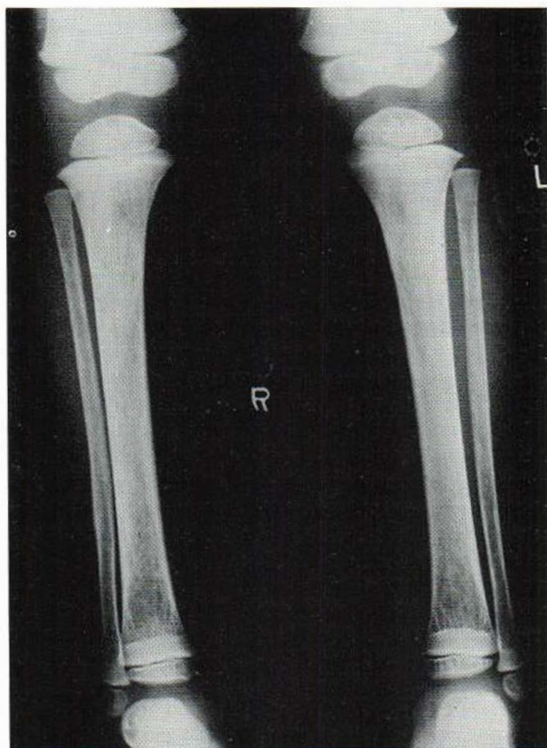


Fig. 3. The bone changes described in Fig. 2 are prominent in the distal parts of the tibiae.

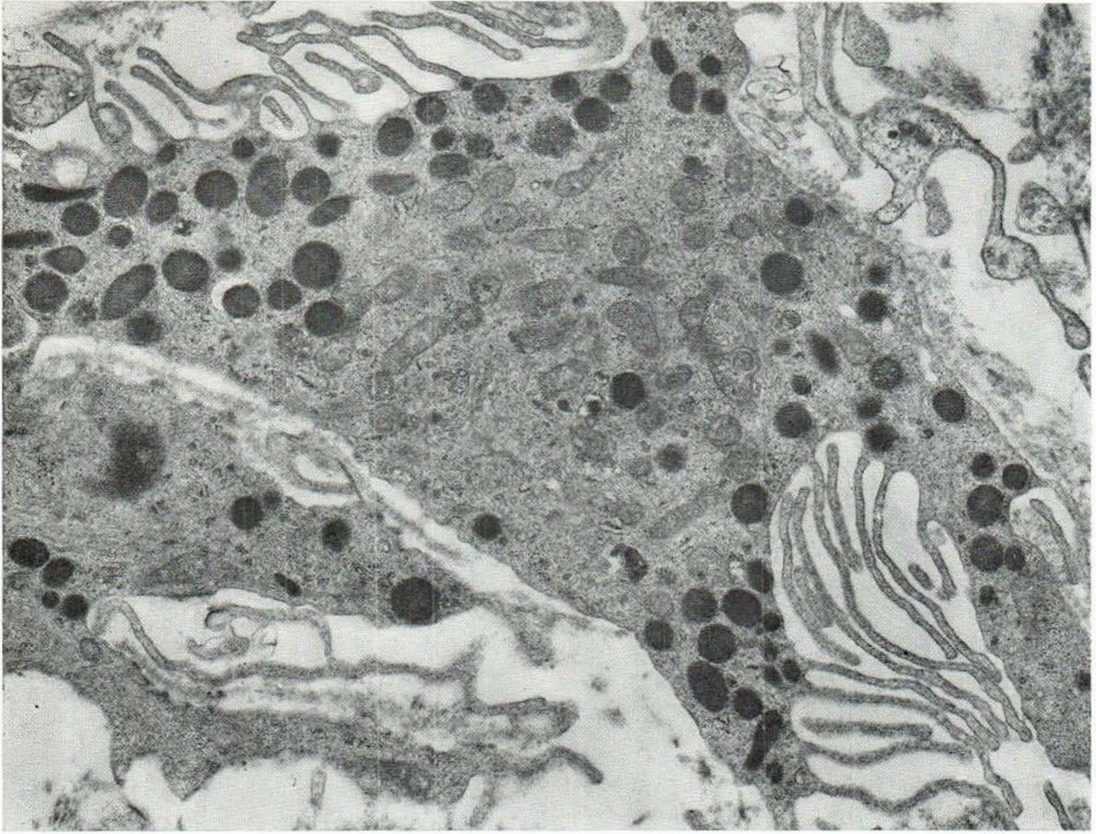


Fig. 4. Electron micrograph showing two skin mast cells of rhomboidal shape and with tail-like angles. Long, cytoplasmic villi extend outward from the cell membrane. Electron-dense

granules are dispersed at the periphery of the cell. The mitochondria are grouped in the center of the cell. $\times 14\,700$.

Liver

Light Microscopy. Small groups of mast cells with meta-chromatic granules were seen in the portal spaces.

Electron Microscopy. The mast cells were dispersed among fibroblasts in the collagen tissue of the portal spaces (Fig. 7). The cells were oval, rhomboidal, or spindle-shaped. The oval cells measured $8\,\mu\text{m}$ in length and $5\,\mu\text{m}$ across; the elongated cells measured $12\text{--}14\,\mu\text{m}$. Villous processes up to $1\,\mu\text{m}$ long extended outward from the cell membrane. They were either parallel or oriented perpendicular to the cell surface. Most nuclei were irregular shaped and featured deep indentations. The cytoplasmic granules varied greatly in shape. Some granules were spherical and had a diameter of $0.3\text{--}0.5\,\mu\text{m}$, but most of them were semilunar shaped. The latter were usually related to an empty vacuole which seemed to compress the granule, or suggested that part of the granular content had been evacuated, thus leaving a "crescent"-like appearance (Figs. 8, 9). A sharp membrane enclosed the granules (Fig. 8). The electron-dense granular material appeared either homogeneous, or as aggregates of fine particles (Fig. 8). Lamellated or beaded structures could be demonstrated in many granules (Figs. 8, 9). Groups of small mitochondria and dilated tubules of smooth endoplasmic reticulum were com-

mon. A few lipid droplets, ribosomes, and fine filaments were usually seen in the cytoplasm. Some mast cells were of a peculiar appearance due to the fact that their cell membrane had few villi and featured dense, coated vesicles and caveolae similar to those usually encountered in fibroblasts or endothelial cells (Fig. 9). In such cells the rough endoplasmic reticulum was better represented than in the mast cells with numerous cytoplasmic villi.

DISCUSSION

According to Sagher & Even-Paz (11), SM is more likely to develop when the disease first appears in adult life than when the onset is in childhood. Nixon (10) reported that only 21% of systemic cases start before 10 years of age.

Varied alterations in the morphology of mast cells were encountered in our case of systemic mastocytosis. In the skin, the villous cytoplasmic protrusions of the mast cells were much longer than those de-

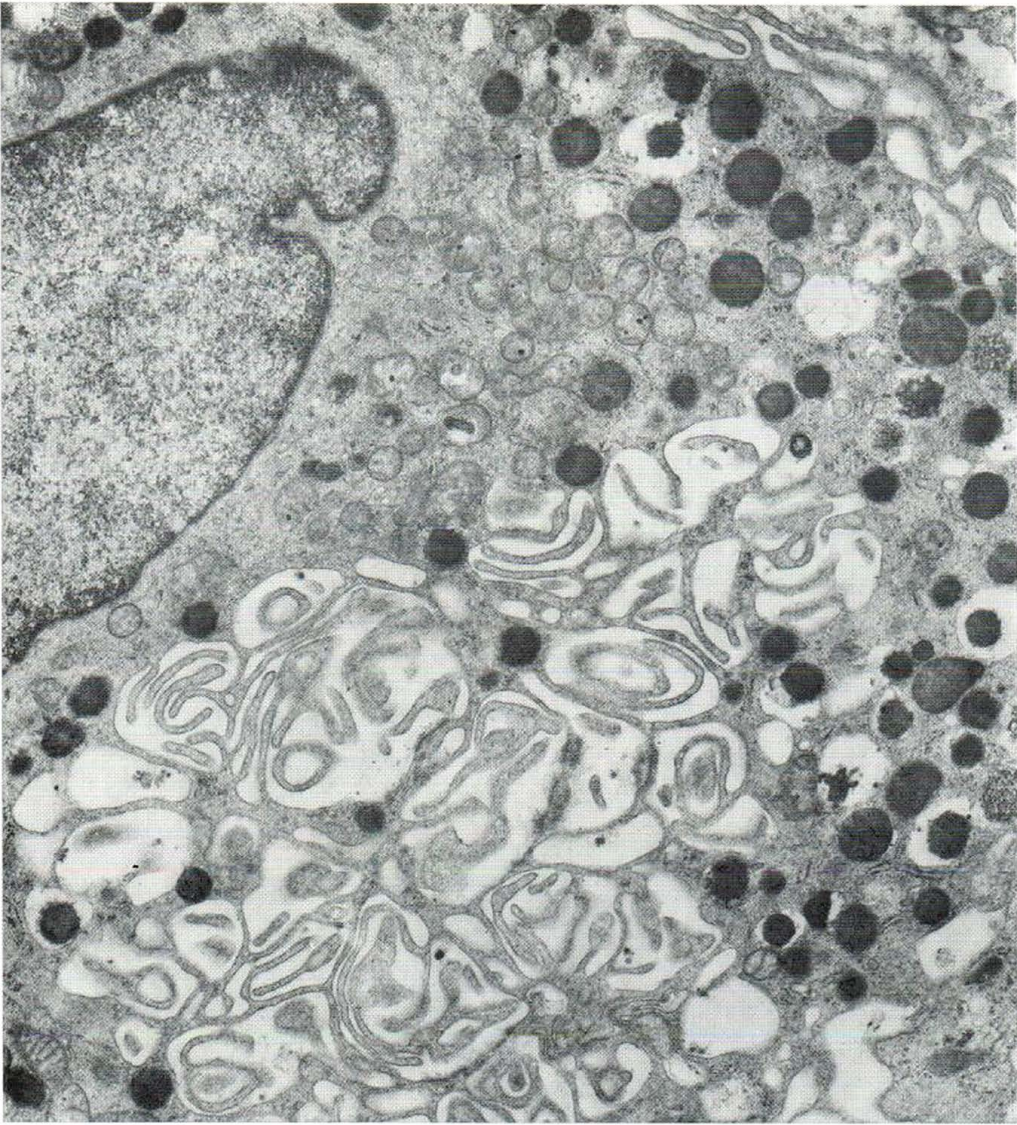


Fig. 5. Electron micrograph of a skin mast cell showing massive interdigitation of twisted cytoplasmic protrusions. The granules are round and of homogeneous appearance.

The nucleus at the left is slightly indented. The mitochondria are packed together in the vicinity of the nucleus. $\times 14\ 700$.

scribed in normal mast cells (1, 12). There was also marked and bizarre interdigitation of villous processes in single mast cells and between adjoining mast cells as well. Interdigitated villi were described in urticaria pigmentosa (6), but the process of interdigitation seems to be much more elaborate in the present case. Crescent-shaped granules were predominant in the mast cells of the liver. We did not see them in the skin, nor were they described in normal mast cells. Bloom (2, 3) described semilunar-

shaped granules in the neoplastic mast cells of mouse and dog mastocytoma. We do not know if the semilunar granules in liver mast cells have a special significance, but it seems that they are not related to the process of mast cell degranulation, since experimental work in this direction did not reveal such granules (6, 8).

It is known that mast cells increase in number with the advance of liver cirrhosis, where they appear around proliferated fibroblasts and seem to originate



from perivascular connective tissue (9). In our material there were cells featuring few cytoplasmic granules and that on the other hand exhibited vesicles and caveolae which are characteristic of fibroblastic cells. Such cells could be interpreted as a stage in the development of the mast cell from a precursor fibroblast (1). The numerous mitochondria in the mast cells of both liver and skin indicate that we are dealing with young developing cells (5).

Though the Golgi apparatus is described as being well developed in cases of mastocytoma as well as in normal mast cells, it is surprising that no characteristic Golgi tubules were evident in our material. Hashimoto et al. (6) believe that abundant Golgi complexes could be due to the effect of corticosteroids, though these were not administered to our patient. Well developed tubules of endoplasmic reticulum were encountered in some mast cells in our

Fig. 6. Skin mast cell. In the center there is a bundle of parallel membranes closely packed together. In the upper right side of the picture there are two granules containing coarse granular material. $\times 19\,000$.



Fig. 7. Low power electron micrograph of the liver portal area. The vein with an erythrocyte in the lumen is seen at upper right. A group of four mast cells containing electron-dense granules is present. Fibroblasts, mononuclear cells and collagen fibrils occupy the area around mast cells. $\times 3\,500$.



Fig. 8. Mast cell of the portal area. The nucleus is seen in the left corner. The granules are enclosed by a sharply demarcated membrane. Several granules are semilunar shaped (G).

In one of them, the granular material has a beaded appearance (arrow). $\times 38\ 000$.

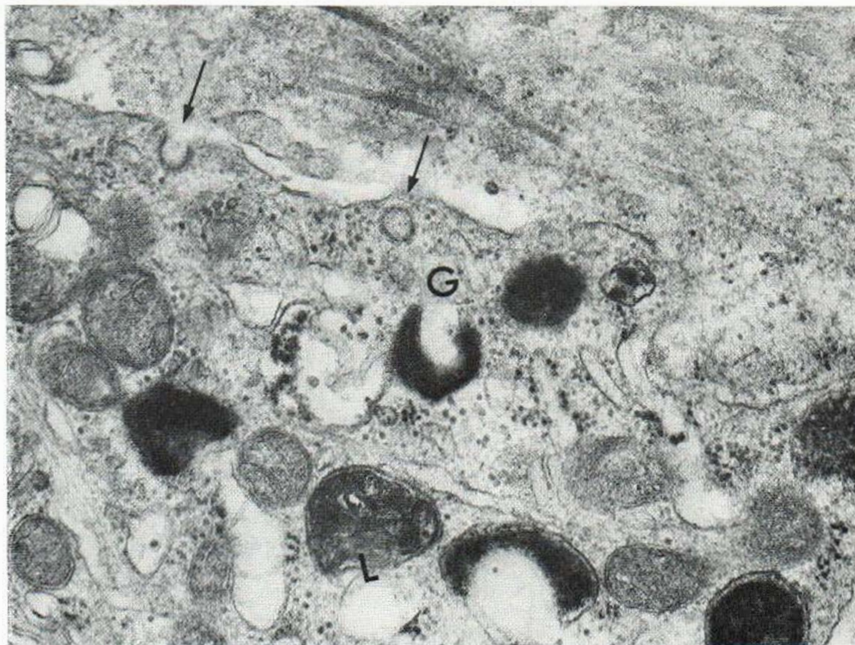


Fig. 9. Mast cell of the portal area. Semilunar shaped granules (G) seem to be compressed by an empty vacuole. A lamellated granule is seen at L. A dense, coated vesicle and a caveola

(arrows) are evident on the outer cell membrane. Note the well developed tubules of endoplasmic reticulum. Collagen fibrils are seen outside the cell. $\times 38\ 000$.

case. They are known to appear in neoplastic mast cells (2).

According to Sagher & Even-Paz (11), hepatosplenomegaly is an unfavorable prognostic sign in systemic mastocytosis and the disease terminated fatally in 50% of these patients. The question is, whether the morphological changes encountered, especially the complex interdigitation of villi, should be interpreted as a sign of expected neoplastic development.

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Y. Naveh, M.D.
Department of Pediatrics B
Rambam University Hospital
Haifa
Israel