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The Pit Cell: Description of a New Type of Cell Occurring in Rat Liver Sinusoids and Peripheral Blood

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Summary. Pit cells – a new type of cell first described here and so named because they contain highly characteristic granules – are situated in the wall of rat liver sinusoids, and have hyaloplasmic pseudopodia intermingling with the microvilli of the parenchymal cells. The characteristic granules are mainly situated at one side of the nucleus, the other side showing organelle-free hyaloplasm. Pit cells are also found in portal tracts and in granuloma-like cellular aggregates. They also occur in rat peripheral blood, although there are morphological differences between cells in these two sites. Pit cells can be regarded as regular inhabitants of the sinusoidal wall, and therefore belong to the series of sinusoidal cells, i.e., the endothelial (Wisse, 1972), Kupffer (Widmann et al., 1972; Wisse and Daems, 1970; Wisse, 1974a, b), and fat-storing cells (Ito, 1973). Pit cells do not phagocytose and do not react to a great number of experimental conditions, to which endothelial and Kupffer cells do react (Wisse, 1972, 1974b). Mitosis has been observed in a pit cell.

The function of pit cells remains obscure, but an endocrine function is suggested by the morphology of their highly characteristic granules.

Key words: Liver – Rat – Sinusoid – Pit cell – Ultrastructure.

Introduction

During an ultrastructural investigation on the sinusoidal lining cells of rat liver, it was found that in addition to endothelial cells (Wisse, 1970, 1972), Kupffer cells (Widmann et al., 1972; Wisse and Daems, 1970; Wisse, 1974a, b), and fat-storing cells (Ito, 1973), a fourth type of cell is present (Wisse and Daems, 1970). The present paper gives a description of this cell type, to which we have given the

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name pit cell, since this cell is recognized by the presence of highly characteristic granules. Pit cells are unreactive under a number of experimental conditions and also occur in buffy coat preparations from peripheral blood.

Material and Methods

Rat (male, Wistar, specific pathogen free, 200 g) livers were fixed by perfusion, via the portal vein, with a fixative solution containing 1.5% glutaraldehyde (Fluka), 0.067 M cacodylate (pH 7.4), and 1% sucrose (mOsmol 310), at a flow rate of about 10 ml/min at room temperature for 120 s, during which the liver becomes pale and solid. Tissue blocks from the right lobe were postfixed, without rinsing in buffer, by immersion in a 1% OsO_4 solution in phosphate buffer (pH 7.4) at 4° C for 1 h (mOsmol 310), dehydrated in an ethanol series (70–100%), and embedded in Epon. Ultrathin sections were cut on Reichert ultramicrotomes and stained with saturated uranyl acetate and lead hydroxide. Photographs were taken with the Philips EM 200, EM 300, and EM 201 electron microscopes. For the morphological study of the pit cells, only photographs showing two or more of the characteristic granules were used.

Photographs of pit cells were collected over a period of about nine years, during investigations on the structure and function of sinusoidal lining cells (endothelial and Kupffer cells) performed in normal and experimental animals (Wisse, 1970–1974). Observations on pit cells were routinely made in liver tissue from animals subjected to the following experimental procedures: Thorotrast i.v. injections (1 min to 7 days); latex i.v. injections (0.1–7.0 μ; 3 min to 7 days), and horseradish peroxidase i.v. injections (1 min to 7 days); 10 to 60 days after splenectomy; occlusion of the bilde duct; 50 to 66 h after partial hepatectomy; sham operation; and RES-stimulation by Zymosan (Wisse, 1972, 1974b). Also the livers from germfree and Gunn rats as well as from mice, dogs, hamsters and patients were studied. Peroxidase incubations (Wisse, 1974a) were applied to 20–100 μ Vibratome sections of livers fixed by perfusion for 40 s. Silver methenamine was used for staining (Rambourg, 1967).

Sinusoidal lining cells were routinely isolated (Mills and Zucker-Franklin, 1969) to obtain an enriched population of sinusoidal lining cells including pit cells.

Because pit cells are also found outside the liver sinusoids, and have a morphology suggesting a capacity to migrate, buffy coat preparations of human and rat peripheral blood were screened for the presence of pit cells. Buffy coat preparations were made from heparinized, dextran-sedimented blood samples.

Observations

In the liver, pit cells can be found in the sinusoids and terminal branches of the portal vein. Their frequency is much lower than that of endothelial or Kupffer cells. In the sinusoids, the pit cells occur singly and like endothelial and Kupffer cells, are directly exposed on one side to the blood stream (Fig. 1). Pit cells are almost always in contact with the endothelial lining (Figs. 1–3, 5, 9, 11). They are seldom seen beneath the endothelial lining within the space of Disse. The pit cell may replace the endothelial lining completely or over variable distances (Fig. 2), but no interruptions or gaps are seen between the two types of cell. Contact also occurs between pit cells and fat-storing cells and between pit cells and Kupffer cells (Fig. 10).

The shape of the pit cell usually varies widely, like that of the Kupffer cell (cf. Figs. 1, 2, 9, 10). In elongated cells, the nucleus often gives the cell a high degree of polarity by dividing it into two parts, one part containing all of the organelles, the other consisting of organelle-free hyaloplasm (Fig. 2). The hyaloplasm forms pseudopodia which penetrate the endothelium and intermingle with microvilli of the parenchymal cells (Fig. 5).

Table 1. Quantitative data on pit cells isolated from rat liver and rat blood

	Diameter of the cell ($n=45$)	Number of granules per cell section	Diameter of the dense inclusion	Number of mitochondria per cell section	Diameter of the mitochondria
Sinusoidal cell isolates[a]	7.20 μ ± 0.54	12.9 ± 4.9	0.28 μ ± 0.05	5.4 ± 2.9	0.41 μ ± 0.08
Buffy coat from peripheral blood	6.71 μ ± 0.84	6.6 ± 2.5	0.43 μ ± 0.10	7.0 ± 4.6	0.30 μ ± 0.04
Level of significance of the differences	$P > 0.05$	$P < 0.05$	$P < 0.05$	$P > 0.05$	$P < 0.05$

[a] Sinusoidal isolates were used for measurements, because, this material contained pit cells in higher frequency and for that reason allowed the collection of data with one microscope with a calibrated magnification. Photographs of pit cells in situ did not allow such measurements because of the use of several uncalibrated microscopes. It is assumed that this isolated population represents the sinusoidal pit cells

The organelles are grouped around the cytocentre, in the non-hyaloplasmic part of the cytoplasm (Figs. 1, 3). Mitochondria have a diameter of 0.4 μ (see also Table 1), and about 5 are seen per cell section.

The nucleus contains densely staining chromatin. Nuclear pores are easily distinguished as well as the lucent channels within the chromatin leading to these pores. A nucleolus can hardly be distinguished from the surrounding chromatin; on a few occasions a small filamentous sphaeridium or nuclear body was observed.

The granules, which have a diameter of about 0.3 μ (see also Table 1), form one of the most characteristic features of the pit cell (Figs. 3, 6–8). Several types can be discerned. One type has a smooth membrane enclosing a round, electron-dense inclusion (diameter 0.28 μ; see Table 1), separated from the membrane by a narrow lucent halo. The dense inclusion may be round or hemispherical in a slightly less dense matrix (Fig. 8). Other granules have a sinuous membrane enclosing the same type of inclusion but showing a wider halo, which may also vary in density. Tubular attachments are seldom seen; occasionally, transitional stages to multivesicular bodies containing a dense inclusion are observed (Fig. 3). The average number of granules per cell section (after the selection of cells with two or more granules) is about 13 (Table 1).

The Golgi apparatus consists of a few cisternae, one or two of which may be swollen on one side and give rise to vesicles with an electron-lucent content (Figs. 3, 4). These vesicles seem to spread through the cytoplasm and can also be seen in the hyaloplasmic part of the cell. More than one Golgi apparatus may occur in one cell; in that case they always lie close together, sometimes separated by a centriole. The usual small bristle-coated Golgi vesicles can be seen to pinch-off from the cisternae (Fig. 4). Larger bristle-coated vesicles pinching off from the cell membrane are seldom observed. Microtubules radiate from the centriole throughout the organelle-containing cytoplasm; none are seen in the hyaloplasm. A few single cisternae of rough endoplasmic reticulum are scattered through the cytoplasm, and smooth endoplasmic reticulum cannot be distinguished. Free ribosomes arranged in rosettes or small clusters are abundant, predominantly in

the organelle-containing cytoplasm (Figs. 3, 4). Multivesicular bodies are sometimes present (Fig. 3), as are bundles of filaments.

No signs of endocytotic activity of the pit cells are seen after i.v. injection of Thorotrast (Figs. 9, 10), Latex, Zymosan, or horseradish peroxidase. Pit cells take part in the formation of granuloma, which appear 2 to 7 days after the injection of Thorotrast or Zymosan (Wisse, 1974b). They can also be found in comparable cell accumulations, rarely observed in livers of normal animals (Fig. 12), usually in the areas around the portal tract.

Key to abbreviations: *L* lumen of the sinusoid, *end* endothelial cell (process), *SD* space of Disse, *N* nucleus of the pit cell, *h* hyaloplasm of the pit cell, *Ga* Golgi apparatus of the pit cell, *Pc* parenchymal cell

Fig. 1. Pit cell anchored to the sinusoidal wall. The organelle-rich part, which contains many characteristic granules, faces the lumen of the sinusoid, the part occupied by hyaloplasm is in contact with the endothelial lining. *ery* red blood cell. × 13,000.

Fig. 2. Pit cell showing the grouping of organelles to one side of the nucleus, hyaloplasm is present on the other side. This gives the cell a high degree of polarity. The endothelial lining can be replaced by pit cells over considerable distances (arrow); fat-storing cell processes (*f*) underly the endothelium. × 8,000

Fig. 3. Higher magnification of the organelle-rich part of the pit cell cytoplasm. The granules vary in appearance. A multivesicular body (*mvb*) contains a dense inclusion. × 27,600

Fig. 4. The Golgi apparatus of a pit cell consists of parallel, perforated cisternae, here cut transversely and longitudinally. Small vesicles may pinch off at the margins (arrow). × 38,400

Fig. 5. The hyaloplasmic part of the pit cell may send out microvilli which intermingle with the microvilli of the parenchymal cell, suggesting attachment of the pit cell. × 19,000

Figs. 6–8. Variations in the appearance of the characteristic dense granules include the degree of straightness of the limiting membrane (Fig. 6), the density of the matrix, the shape of the dense core (Fig. 8), and the presence of small vesicles inside the granules (Figs. 7, 8, arrows), also suggesting transitional stages to multivesicular bodies. × 47,100; × 47,100; × 41,600, respectively

Fig. 9. Pit cell surrounded by Thorotrast, 30 min after injection of a RES blockading dose (0.3 ml/100 g body weight). Although the pit cell is able to form pseudopodia, there is no phagocytosis of the aggregated particulate matter. × 13,000

Fig. 10. Pit cell on top of a Kupffer cell, 3 h after injection of Thorotrast, showing the marked difference in endocytotic capacity. The Kupffer cell is loaded with Thorotrast (*Th*), but none is seen in the pit cell. × 12,000

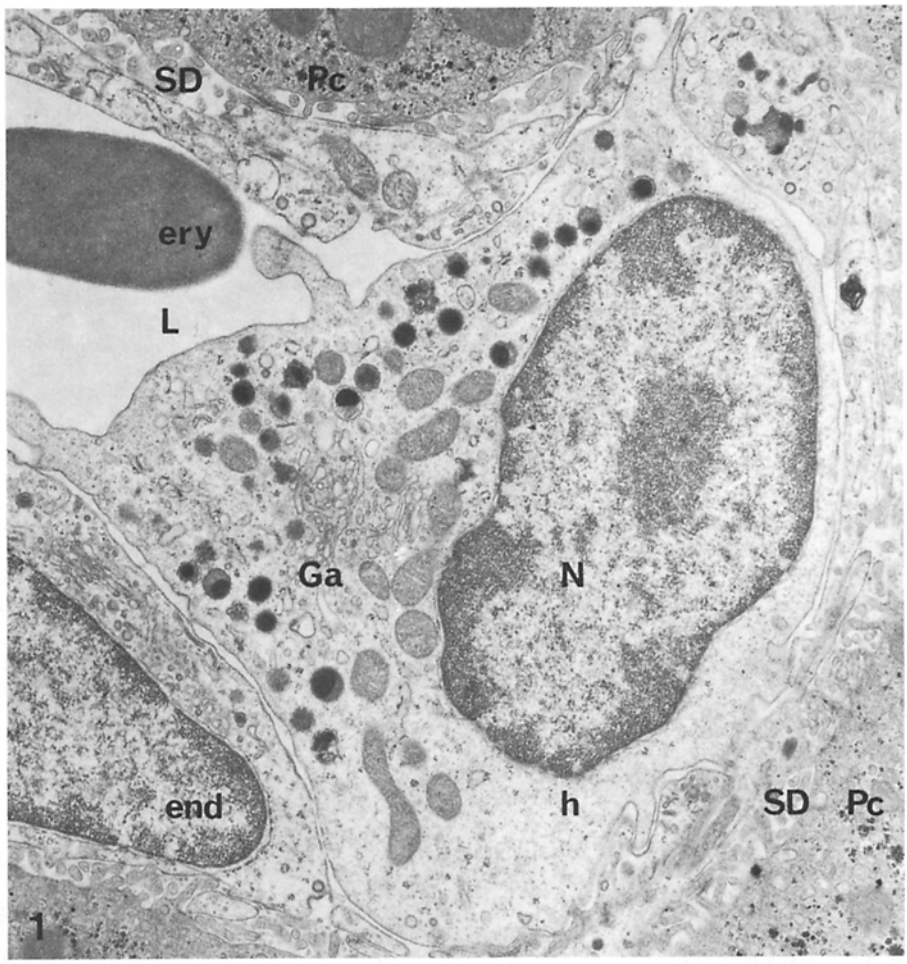
Fig. 11. Pit cell in mitosis, showing the presence of chromosomes (asterisk). The pit cell was recognized by the presence of the characteristic granules. This picture derives from a normal untreated control liver. × 9,300

Fig. 12. A cluster of pit cells and probably other cell types, as found infrequently, mostly in the vicinity of portal vein branches. This section is from a normal, control liver. × 3,300

Fig. 13. Illustration of the intense silver methenamine staining of the characteristic granules. The reticulin in the space of Disse also stains intensely (arrow). × 17,125

Fig. 14. Cells isolated from rat liver by pronase perfusion show a high proportion of sinusoidal lining cells, including pit cells, Kupffer cells (*K*), and endothelial cells (*end*). × 3,000

Figs. 15 and 16. Pit cells isolated from rat liver (Fig. 15) and peripheral rat blood (Fig. 16). Those from rat liver have more granules, but of smaller diameter and larger mitochondria (see also Table 1). × 29,500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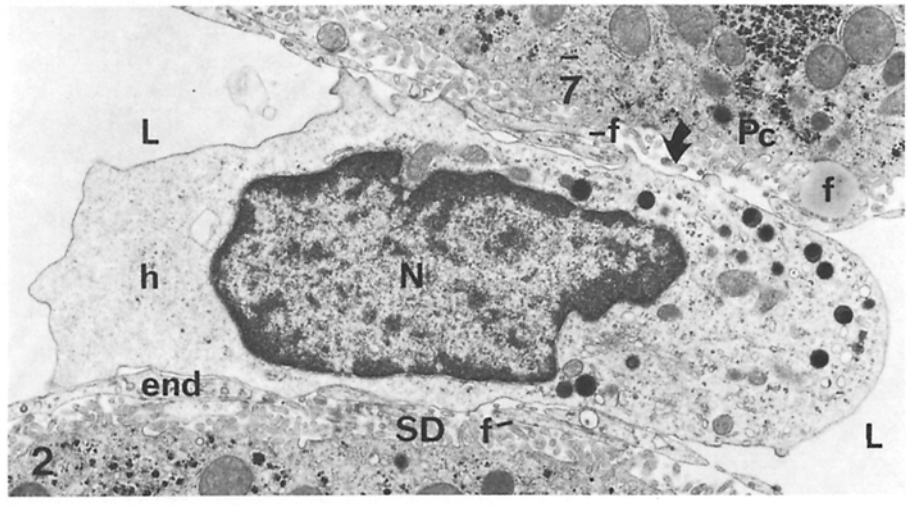
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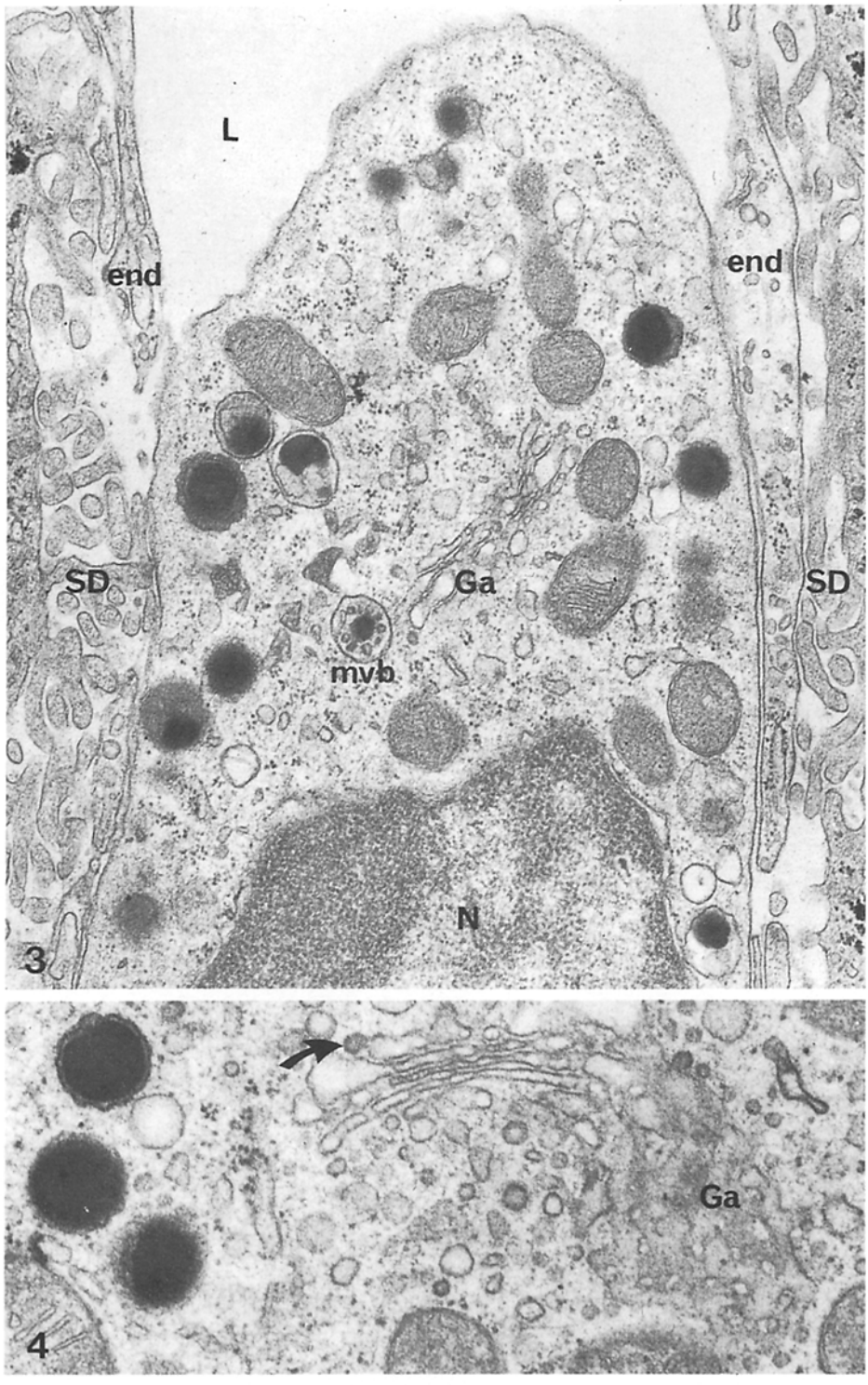

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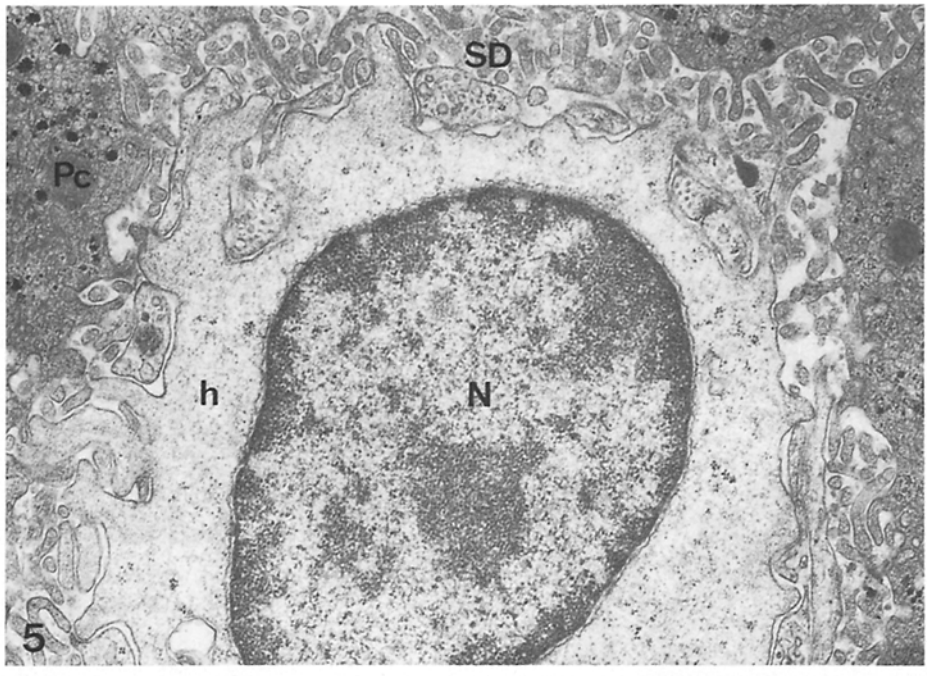

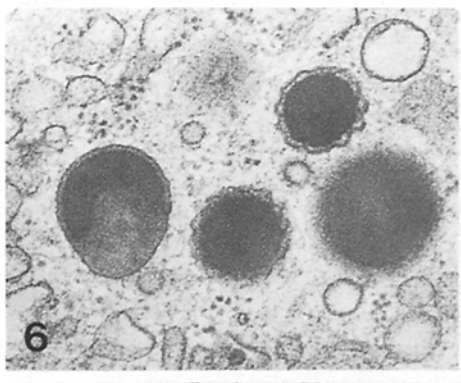

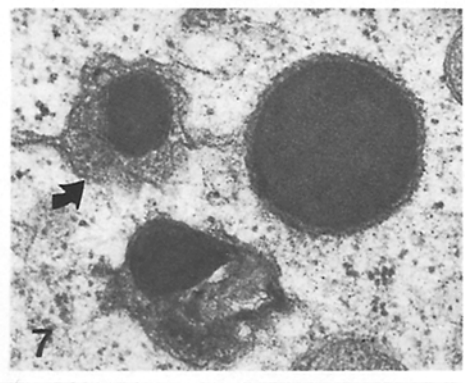

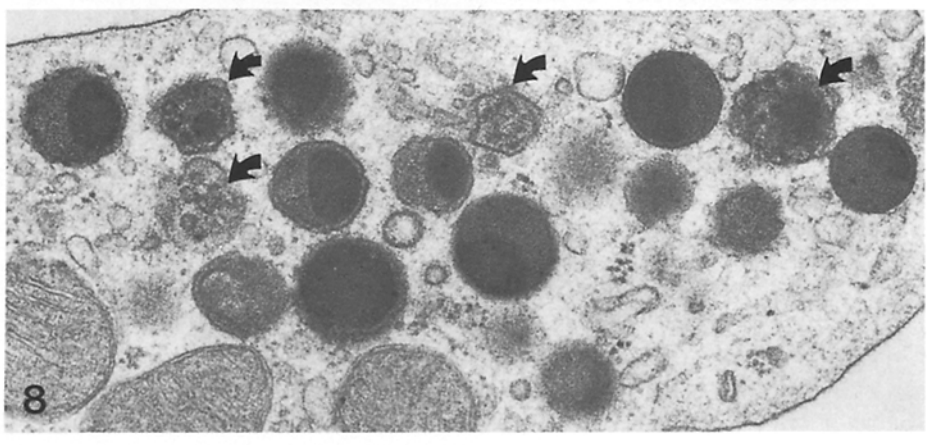

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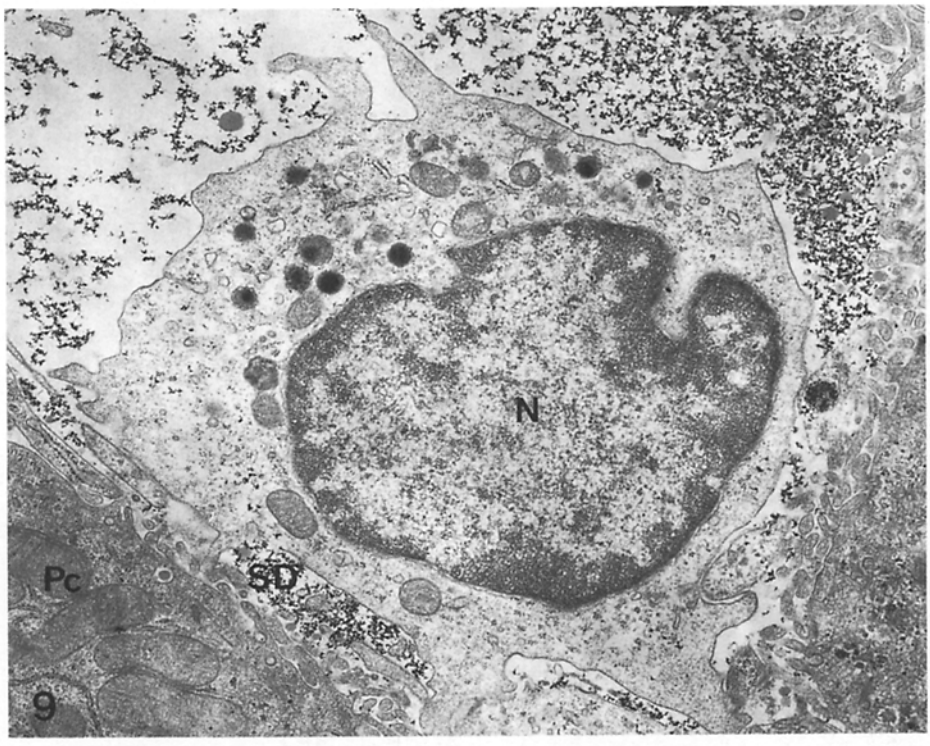

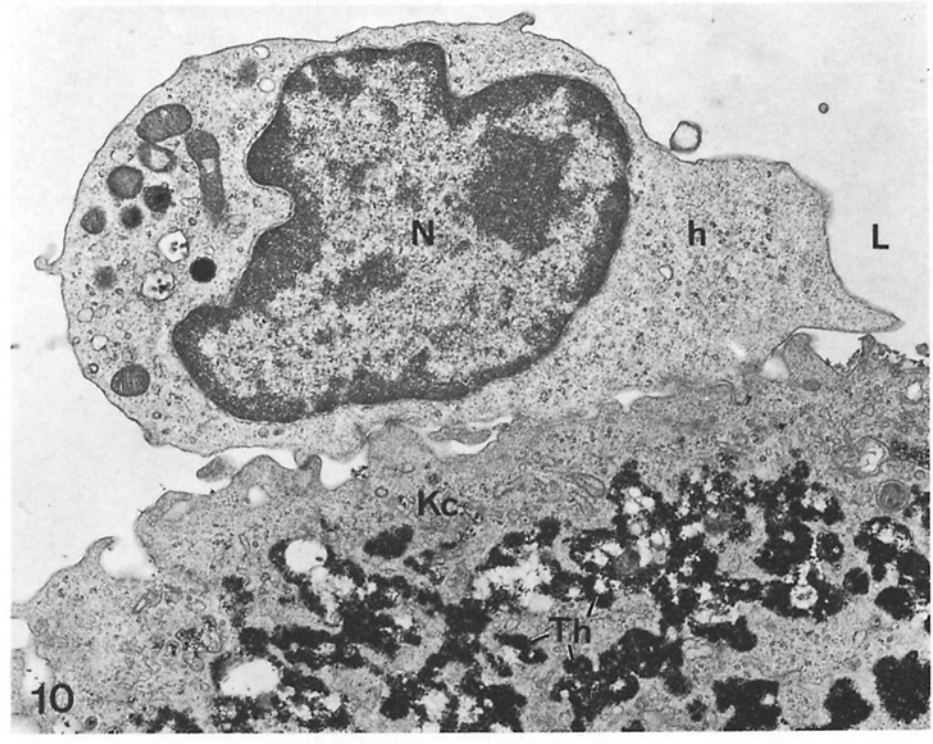

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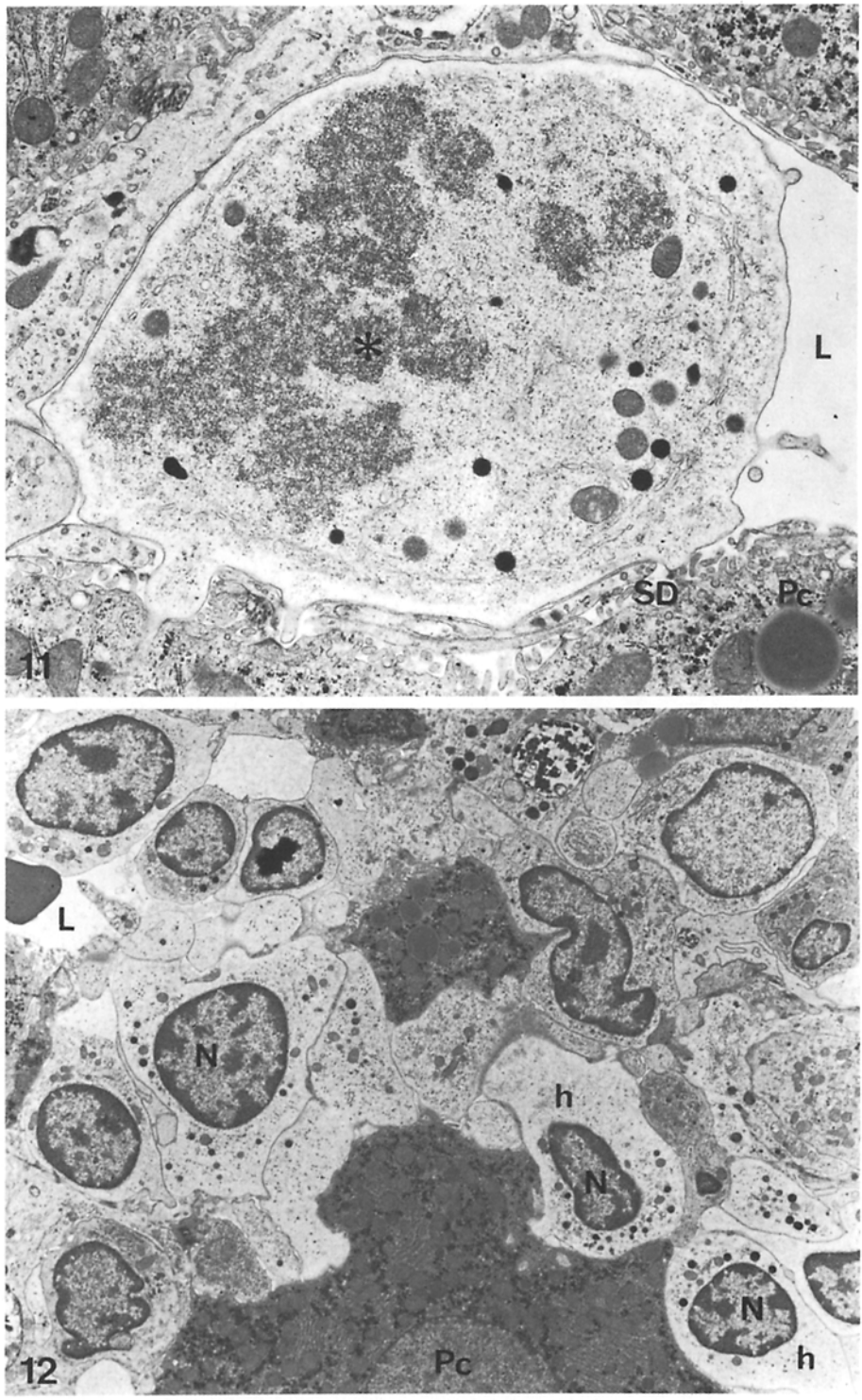

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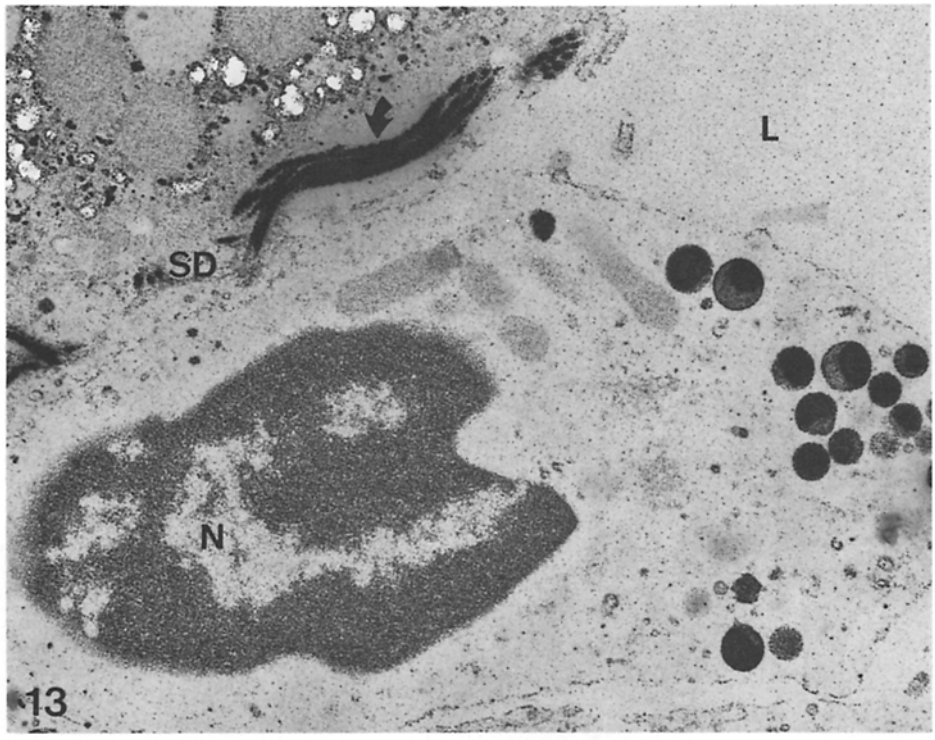

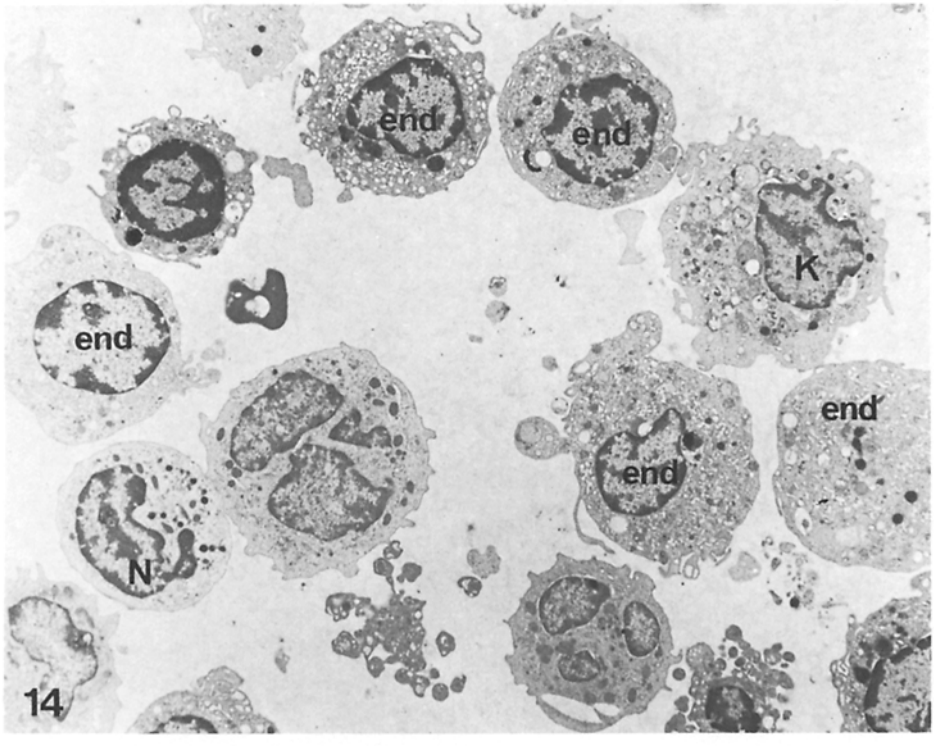

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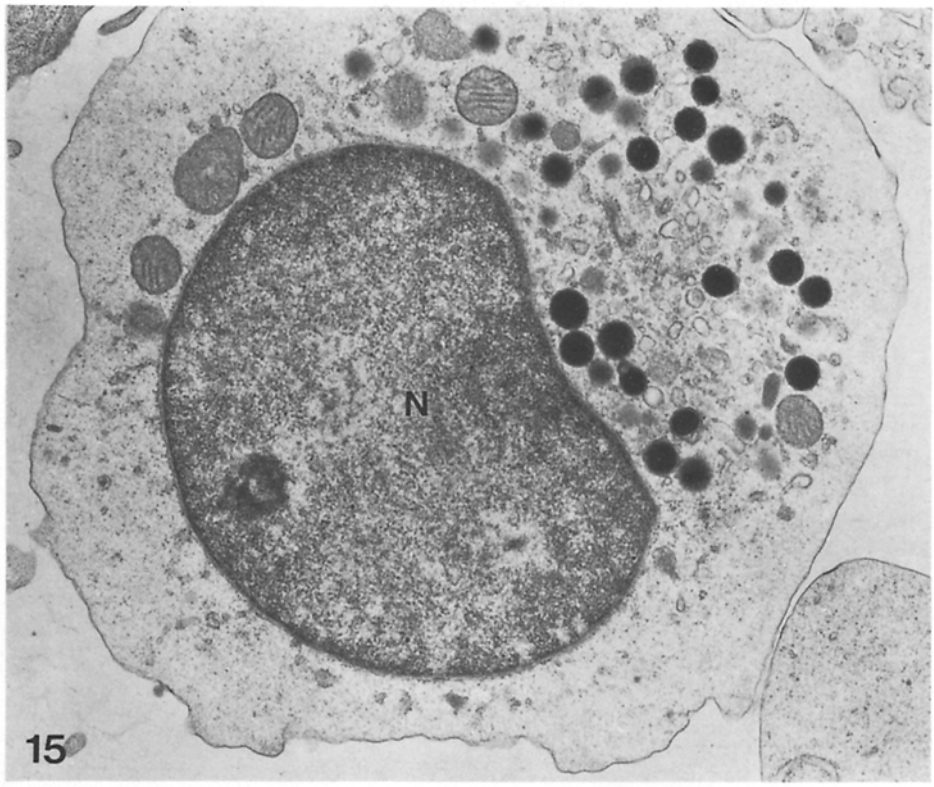

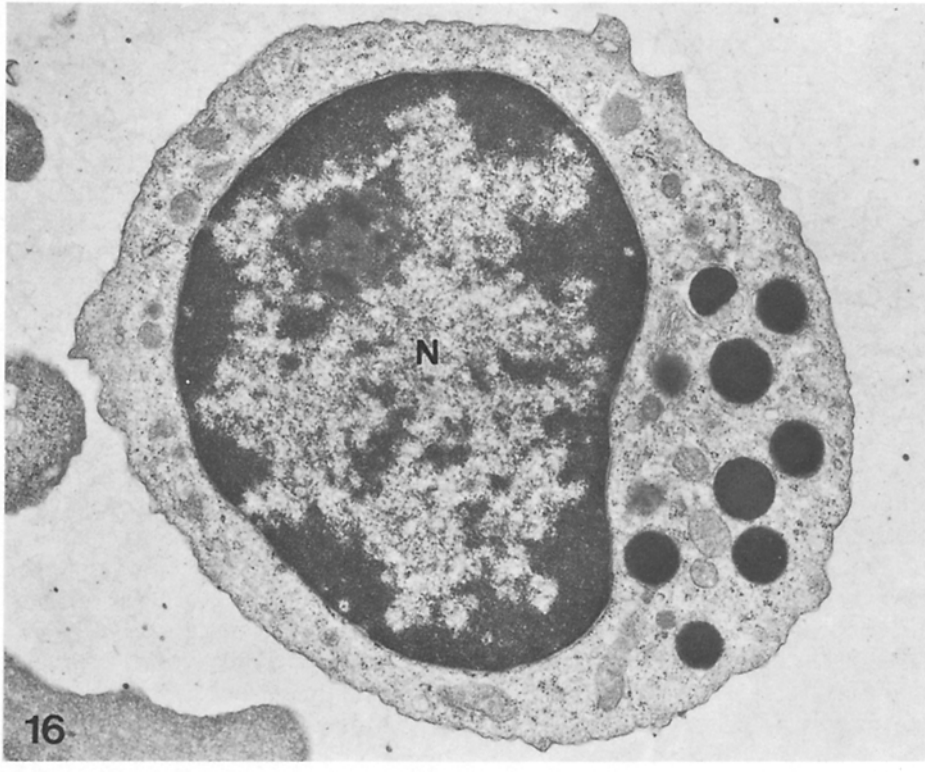

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No alterations are found in pit cells after ligation of the bile duct or following splenectomy. After partial hepatectomy, some of the pit cells contain many free ribosomes, which are indicative of mitosis-linked RNA synthesis. A single example of a pit cell mitosis was found in normal liver (Fig. 11).

Incubation for the demonstration of peroxidase does not reveal positive organelles within the pit cells. Silver methenamine stains the characteristic granules of these cells very intensely (Fig. 13).

Pit cells can be distinguished morphologically from monocytes, eosinophils, basophils, mast cells, and plasma cells, a few of which are still present in the sinusoids in spite of the rinsing effect of perfusion fixation.

Pit cells are found in the livers of Gunn rats and germfree rats as well as in other experimental animals such as dogs, mice, and hamsters. They were also found in rat and mouse spleen by one of us (W.Th.D.).

In biopsy specimens of human liver as well as in human buffy coat preparations pit cells cannot be identified with certainty.

The morphology of pit cells, their presence in portal areas, and the granuloma-like cell configurations led to the assumption that pit cells have the ability to migrate, which prompted us to look for these cells in the peripheral blood. Pit cells were indeed found in buffy coat preparations (Fig. 16), but these cells differ significantly in some respects from pit cells in isolated sinusoidal cell populations (Figs. 14, 15; Table 1). The granules are larger but less numerous and the mitochondria are smaller (cf. Figs. 15, 16). In sinusoidal isolates far more pit cells are found than in ultrathin sections of normal rat liver.

Discussion

The sinusoidal wall of the liver is known to accomodate three morphologically, functionally, and cytochemically distinct cell types, i.e., endothelial cells (Wisse, 1972), Kupffer cells (Wisse, 1974a), and fat-storing cells (Ito, 1973). A fourth type can now be added: the pit cell, which may also be regarded as a consistent inhabitant of the sinusoidal wall. The fact that a pit cell mitosis was observed, confirms this conclusion.

The function of the pit cells is obscure. The characteristic granules resemble those seen in endocrine cells present in other gastrointestinal organs (Forssmann et al., 1969). The presence of chromaffin cells in sinusoids and portal tracts of rat liver has also been reported (Martinez, 1974).

The inertia of pit cells under conditions to which the endothelial and Kupffer cells react (Wisse, 1972, 1974b) indicates a difference in function without providing any indication of what that function might be. Their consistent presence in our material suggests, however, that the pit cells have a function in the normal liver.

The irregularity of the cell shape as well as the formation by the cytoplasm of hyaloplasmic portions bearing pseudopodia that penetrate endothelial cells, are phenomena seen in Kupffer cells as well (Wisse, 1974a, b). In the latter cells, however, hyaloplasm formation occurs in response to the adherence of particles to the cell surface as an early stage in the process of phagocytosis. In the case of the pit cells, no phagocytosis was observed; the hyaloplasm is present as a normal

component of the cell. This hyaloplasm may have a function in the movement and attachment of the cell. The fact that pit cells are attached to the sinusoidal wall by means of hyaloplasm and remain so after perfusion with fixative or pre-fixation perfusion with physiological saline, suggests an attachment function of the hyaloplasmic pseudopodia intermingled with the parenchymal cell microvilli.

Although there are significant morphological differences between the pit cells present in the blood (see also Hoffer et al., 1973) and those found in sinusoidal isolates, they may nevertheless represent one type of cell. The difference in number and diameter of the granules and mitochondria may reflect different functional or developmental stages of the cell. Support for the migratory capacities of pit cells is possibly offered by the report describing a highly comparable cell occurring in rat epididymis (Hoffer et al., 1973).

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