Extravascular Cells Within the Perisinusoidal Space of the Avian Liver

M. D. Purton

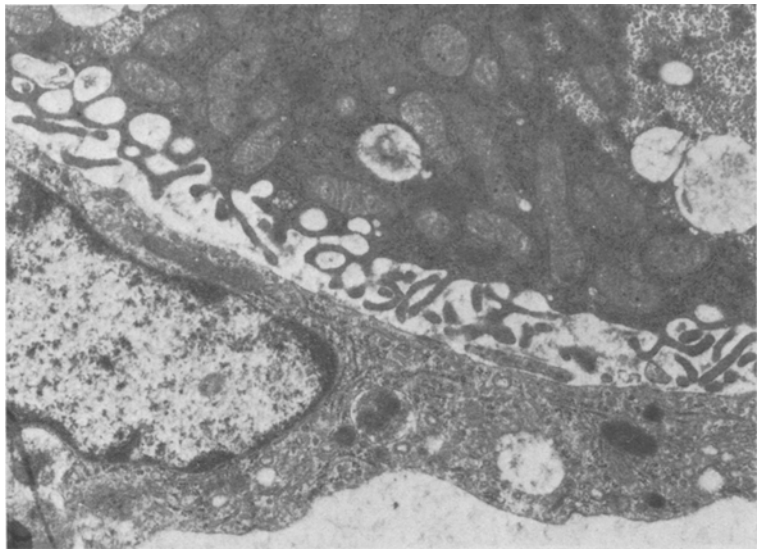
Department of Veterinary Anatomy, University of Nairobi, P.O. Box 30197, Nairobi (Kenya), 6 October 1975.

Summary. Ultrastructural studies of the perisinusoidal space in the avian liver have demonstrated the presence of 2 extravascular cell types – a fat-storing cell and a free mesenchyme cell or histiocyte. This latter cell type is capable of participating in the formation of a bile canaliculus with the hepatic parenchymal cell. The possibility of the fat-storing cell differentiating from the histiocyte is suggested.

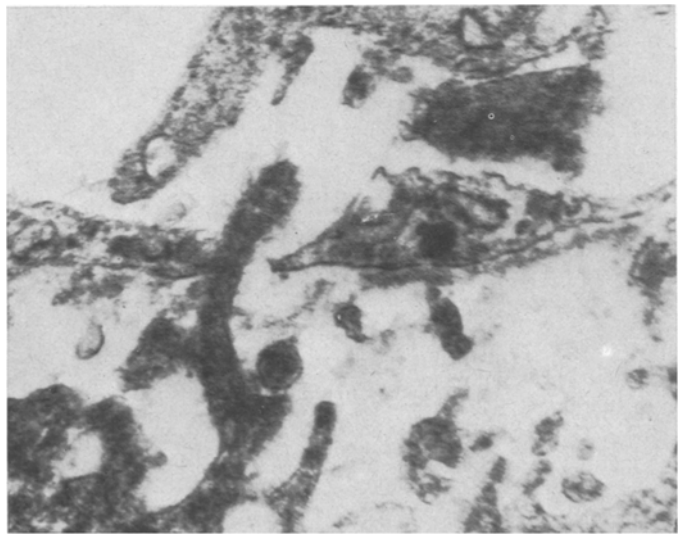
There is at present no agreement about the existence of cells outside the hepatic sinusoidal wall. Many workers deny their existence[1,2], while others make only rare reference to cells within the perisinusoidal space[3]. All these studies, however, have dealt with the mammalian liver; no observations have been reported for the avian liver. The present ultrastructural study has demonstrated the presence of at least 2 extravascular cell types localized within the perisinusoidal space in the liver of the adult fowl.

Materials and methods. The livers of 12 healthy mature fowl of various breeds and both sexes were perfused for 30 min via the hepatic artery with a primary fixative of 2% glutaraldehyde; paraformaldehyde solution buffered at pH 7.2 with 0.1 *M* sodium cacodylate. Tissue blocks not larger than 1 mm³ were excised and fixed for a further

[1] H. Braunsteiner, Z. ges. exp. Med. *121*, 254 (1953).
[2] J. W. Steiner, Am. J. Path. *38*, 411 (1961).
[3] F. Wassermann, Z. Zellforsch. mikrosk. Anat. *49*, 13 (1958).



a



b

Fig. 1. a) The perisinusoidal space, bounded by an endothelial cell (upper right) and an hepatic parenchymal cell. Microvilli arizing from the latter project into this space. × 7,000. b) Microvillus traversing the perisinusoidal space, and projecting through one of the fenestrations in the sinusoidal wall. × 20,000.

2 h. After washing for 24 h in 0.1 *M* sodium cacodylate buffer, the tissue blocks were post-fixed for $1^1/_2$ h in buffered 1% osmium tetroxide at pH 7.1. Following dehydration in ascending grades of alcohol, the tissues were cleared in propylene oxide and embedded in Araldite. Sections were cut and stained with uranyl acetate and lead citrate, and examined on an AEI EM6B electron microscope.

Results and discussion. At electron microscope level, the perisinusoidal space is clearly demarcated. It is bounded on the one side by the hepatic parenchymal cells, and on the other by the fenestrated sinusoidal endothelium. The cell membrane at the vascular pole of the hepatic parenchymal cell is drawn out to form numerous anastomosing and branching microvilli (Figure 1a). These project into the perisinusoidal space, often contacting the endothelial cells lining the sinusoid, and occasionally protruding through the fenestrations in the sinusoidal wall to enter the lumen (Figure 1b), an observation also made in the mammalian liver[4–6]. The existence of such direct communications between the sinusoidal lumen and the perisinusoidal space suggests that the perisinusoidal space is in fact a plasma space[7].

The perisinusoidal space is continued between the cell membranes of adjacent hepatic parenchymal cells as the perisinusoidal recess, which penetrates up to the tight junctions involved in bile canaliculus formation. Measurements of the width of the perisinusoidal space would seem to be of little value, as it varies with the functional state of the liver[4, 8]. In the embryonic chick liver, the endothelial cells, which at this stage constitute a continuous lining, are in direct contact with the hepatic parenchymal cells, and a definite perisinusoidal space is absent[9].

It has been possible to identify 2 extravascular cell types. The first of these is the fat storing cell, a cell type previously observed in several mammalian species[10–13]. This cell type is localized within the perisinusoidal space, beneath the endothelial lining cells of the sinusoid. It is characterized by its position, and by the large and numerous fat droplets carried within the cytoplasm (Figure 2); the cytoplasm is also drawn out into long processes, characteristic of mesenchymal cells. Indeed, it is possible that this cell type may be derived from the second cell type.

[4] F. C. SCHMIDT, Anat. Anz. *108*, 376 (1960).
[5] F. SCHAFFNER and H. POPPER, Am. J. Path. *38*, 393 (1961).
[6] R. L. WOOD, Z. Zellforsch. mikrosk. Anat. *58*, 679 (1963).
[7] M. D. PURTON, J. Zool., Lond. *159*, 273 (1969).
[8] J. R. RUTTNER and A. VOGEL, Verh. dt. Ges. Path. *41*, 314 (1958).
[9] H. E. KARRER, J. Ultrastruct. Res. *5*, 116 (1961).
[10] T. ITO and M. NEMOTO, Okajimas Folia anat. jap. *24*, 243 (1952).
[11] M. YAMAGISHI, Arch. histol. jap. *18*, 223 (1959).
[12] T. ITO and S. SHIBASAKI, Arch. histol. jap. *29*, 137 (1968).
[13] E. WISSE, J. Ultrastruct. Res. *31*, 125 (1970).

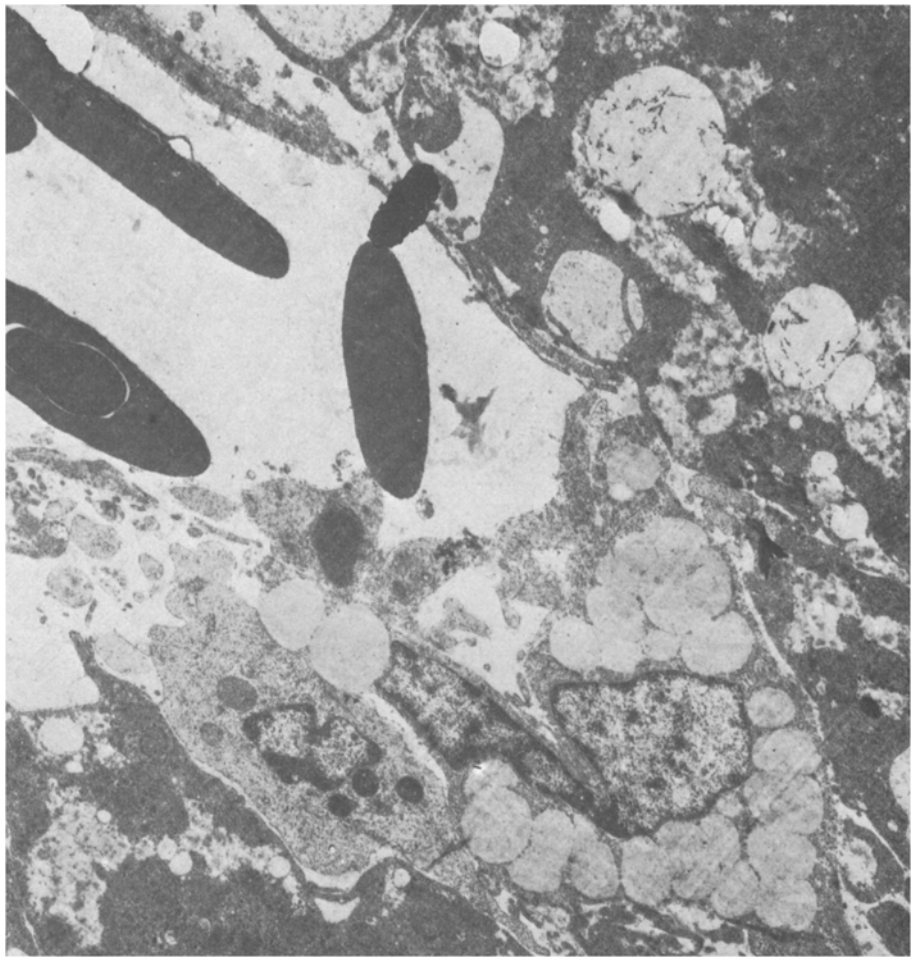


Fig. 2. A fat-storing cell situated within the perisinusoidal space, and lying beneath processes of a Kupffer cell incorporated into the sinusoidal wall. Note the presence of numerous large fat droplets within the cytoplasm of this cell type. × 3,000.

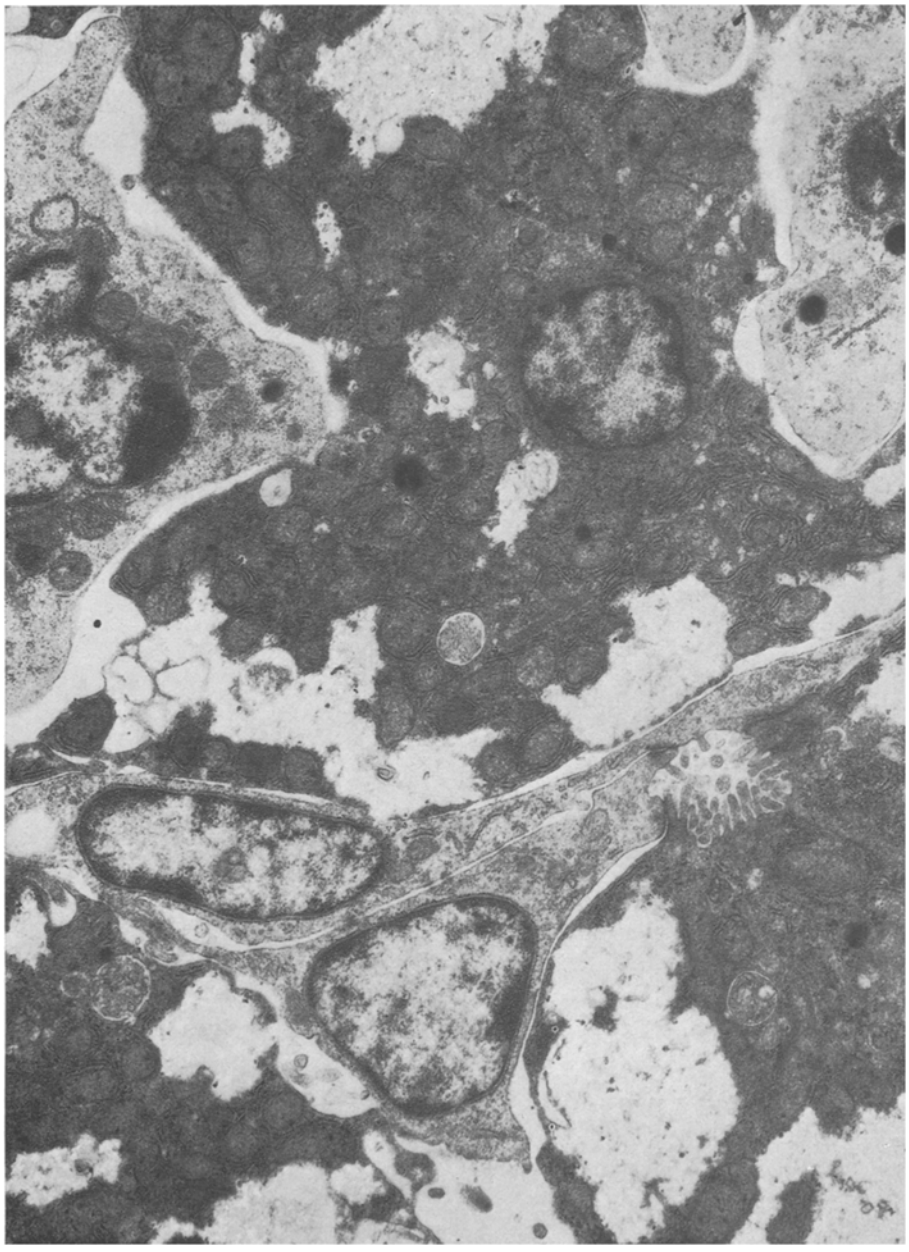


Fig. 3. Free mesenchymal cells, or histiocytes, (upper right, and upper left) within the perisinusoidal space and recess. Two such cells (bottom) have entered into the formation of a bile canaliculus. Tight junctions have formed between themselves, and between each cell and the adjacent hepatic parenchymal cell. These cells can be seen to be forming microvilli which project into the lumen of the canaliculus. × 7,000.

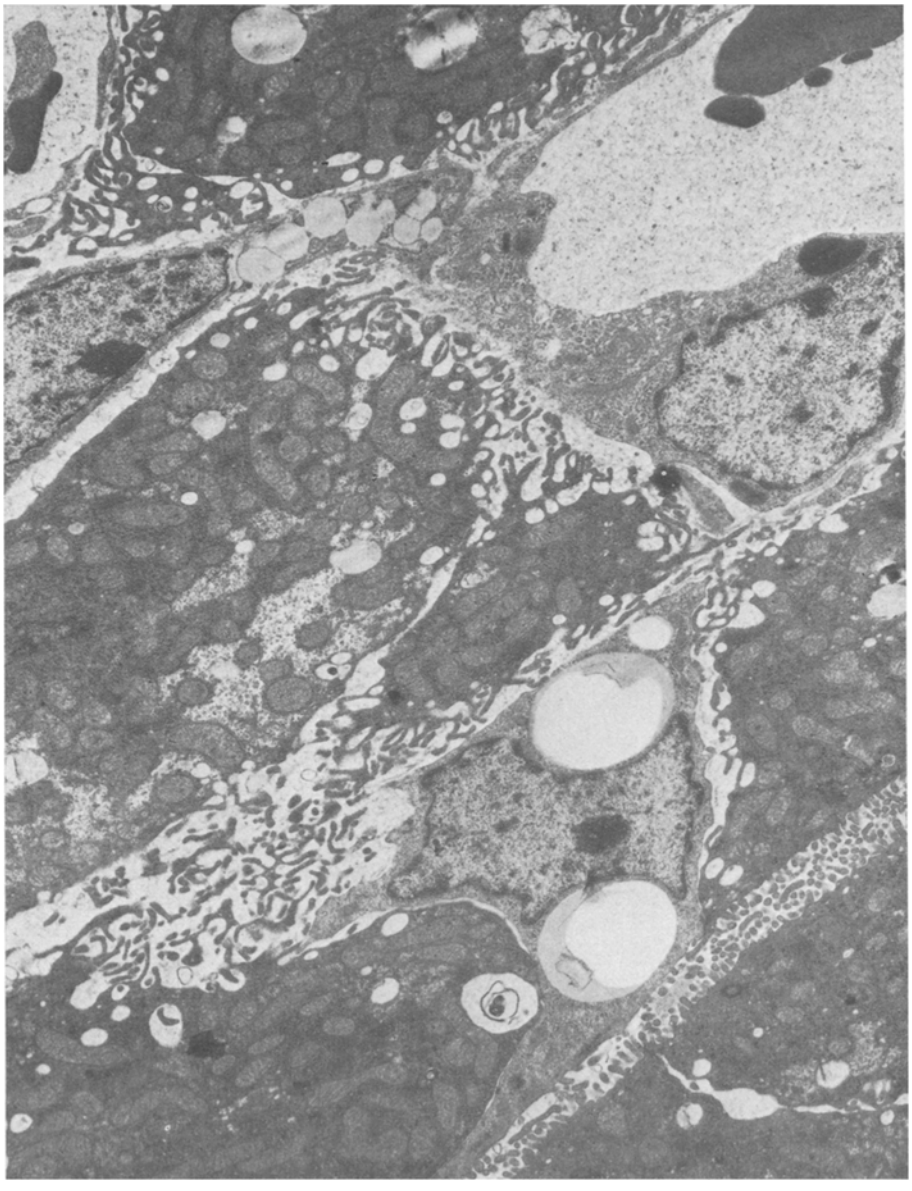


Fig. 4. Both cell types are visible. At upper left is a fat storing cell with numerous fat droplets within its cytoplasm. Lying within the perisinusoidal space (lower right) is a histocyte; this cell is also involved in bile canaliculus formation. Note the large fat vacuoles within the cytoplasm of this cell. ×3,000.

The second cell type can, at this stage, only be described as a free mesenchymal cell, or histiocyte. It is most frequently localized within the perisinusoidal space or recess (Figure 3). It has a typical irregular appearance, the cytoplasm often being drawn out into long processes. It appears to have the ability to take part in the formation of a bile canaliculus with the hepatic parenchymal cells (Figures 3 and 4). In this context, it has been shown that these cells show characteristic and significant differences from the bile duct cells found in the junction zone linking the bile canaliculi with the bile ducts in the portal triads[14]. Although bearing a strong morphological resemblance to a fibrocyte, the fact that this cell type is capable of entering into such an arrangement with the hepatic parenchymal cell suggests that it is a less differentiated cell altogether. It is often found to contain large fat vacuoles, even when involved in bile canaliculus formation (Figure 4), suggesting that it may be able to differentiate into, or from, a fat storing cell.

It appears that both cell types are of free mesenchymal cell origin, although the fat-storing cell may be more highly differentiated. The occurrence of extravascular cells within the mammalian liver has led to them being described as fat-storing cells; stellate, reticular or mesenchyme-like cells in embryonic livers[15]; or reticular cells or fibroblasts[6]. In the present study, fibrocytes or fibroblasts were extremely difficult to find; this may be related to the extremely sparse reticular framework supporting the hepatic parenchymal cells[7, 14].

[14] M. D. PURTON, Ph. D. Thesis, University of Cambridge (1971).
[15] G. A. ACKERMAN, Lab. Invest. *10*, 787 (1961).