*Biochem. J.* (1979) **181**, 245–246
*Printed in Great Britain*



# Localization of the Heparin-Releasable Lipase *in situ* in the Rat Liver

By Timo KUUSI,* Esko A. NIKKILÄ,* Ismo VIRTANEN† and Paavo K. J. KINNUNEN‡
**Third Department of Medicine, †Department of Pathology and*
*‡Department of Medical Chemistry, University of Helsinki, Helsinki, Finland*

(*Received* 26 *March* 1979)

Immunofluorescence and immuno-electron microscopy were used for the localization of the heparin-releasable lipase *in situ* in the rat liver. The lipase is located exclusively on the liver endothelial cells. No labelling could be detected on the parenchymal or Kupffer cells, or in the livers of heparin-pretreated animals. The physiological significance of the endothelial localization of the hepatic lipase is discussed.

The presence of a heparin-releasable lipase in the liver is well documented (Hamilton, 1964; Felts & Mayes, 1967; LaRosa *et al.*, 1972). In contrast with the lipoprotein lipase of extrahepatic tissues, the function of the hepatic lipase in plasma lipoprotein metabolism is still in dispute (Huttunen *et al.*, 1976; El-Maghrabi *et al.*, 1978; Assmann *et al.*, 1973). The liver is composed of a number of different cell types, which evidently catabolize different types of lipoproteins (van Berkel & van Tol, 1978). In the present study, indirect immunofluorescence and immuno-electron microscopy have been used to determine the localization of the hepatic lipase *in situ* in rat liver.

## Materials and Methods

### *Studies with immunofluorescence*

Male Sprague–Dawley rats (300–350g) were anaesthetized with Nembutal (25mg/kg body wt). They were then injected into the exposed jugular vein with 1.0ml of rabbit antiserum raised against purified rat hepatic lipase (Kuusi *et al.*, 1979). Control rats were injected with heparin (500units/kg body wt.) 5min before the antiserum. After 5min the livers were perfused through their portal veins with 250ml of Krebs–Ringer bicarbonate buffer, pH7.4, equilibrated with $O_2/CO_2$ (19:1, v/v) and thereafter with 3.5% (w/v) paraformaldehyde in 0.1M-potassium phosphate buffer, pH7.2, to fix the livers for immunofluorescence studies. Thereafter the livers were removed, cut into small pieces and further fixed in 3.5% (w/v) paraformaldehyde buffered with 0.1M-potassium phosphate, pH7.2. The small pieces of the liver were frozen and cut into sections. These were labelled with fluorescein isothiocyanate–sheep anti-(rabbit immunoglobulin G) (Meloy, Springfield, VA, U.S.A.), washed thoroughly in 0.9% (w/v) NaCl in 0.02M-potassium phosphate buffer, pH7.2, embedded in 50mM-sodium veronal buffer, pH8.6, containing 50% (v/v) glycerol, and examined in a Zeiss Universal microscope equipped with epi-illuminator and with filters for fluorescein isothiocyanate fluorescence.

### *Studies with immuno-electron microscopy*

For electron microscopy the livers were perfused with Krebs–Ringer bicarbonate buffer and then with 20ml of Krebs–Ringer bicarbonate buffer containing 0.05ml of sheep anti-(rabbit immunoglobulin G)–ferritin conjugate/ml (Miles–Yeda, Elkhart, IN, U.S.A.) for 5min. The livers were washed by perfusion with 250ml of Krebs–Ringer bicarbonate buffer (rate 50ml/min) and subsequently fixed by perfusion with 2.5% (v/v) glutaraldehyde in 0.1M-sodium cacodylate buffer, pH7.0. Thereafter, the livers were removed, cut into small pieces, further fixed with glutaraldehyde, and post-fixed for 60min in 1% (w/v) $OsO_4$ buffered with 0.1M-potassium phosphate buffer, pH7.2, dehydrated and embedded in Epon S12. Thin sections were examined, usually unstained to improve the resolution of the ferritin particles, in a JEOL 100 B electron microscope.

## Results and Discussion

In indirect immunofluorescence a bright sinusoidal type of fluorescence was seen (Plate 1). In electron microscopy ferritin particles were seen on the surface of endothelial cells as discrete patches (Plate 2*a*), on the surface of forming endocytotic vesicles (Plate 2*a*), on the surface of forming endocytotic vesicles (Plate 2*b*) and in endocytotic vesicles inside the endothelial cells (Plate 2*c*). It is noteworthy that no ferritin was present on the microvillus surfaces of liver parenchymal cells or of Kupffer cells. The lack of staining of the parenchymal cells cannot be due to the poor penetration of immunoglobulins, because macromolecules enter rapidly into the space of Disse (Wisse, 1977). No ferritin granules were visible in

the livers from control rats injected intravenously with heparin before the administration of the antiserum.

It has been suggested that hepatic lipase is located in or near the plasma membranes of the liver. This assumption is supported by the rapid release of the enzyme in plasma after the injection of heparin. Hamilton (1964) injected dye and heparin, or radioactive heparin alone, into the portal vein of anaesthetized dogs and observed a parallel appearance of the injected substance and the lipase activity in the hepatic vein. He suggested that the hepatic lipase is located on the endothelial cell surface.

Direct evidence for the association of heparin-releasable lipase with the liver plasma membranes has been obtained from studies on the subcellular distribution of the lipase activity. These studies have shown that the enzyme is bound to the plasma membranes of the liver and is released by heparin (Assmann *et al.*, 1973; Waite & Sisson, 1973). No distinction, however, was made in these studies between different cell types in the liver. This distinction between parenchymal and non-parenchymal cells was made in the experiments of Jansen *et al.* (1978), who studied the binding *in vitro* of hepatic lipase to these cells. The indirect evidence thus obtained supported the non-parenchymal location of the enzyme in liver.

About 10% of the liver cell mass is composed of non-parenchymal cells that, because of their small size, contribute about 35% of the total number of cells in this organ (Weibel *et al.*, 1969). It is noteworthy that hepatic lipase was detected only on the endothelial cells and not on the parenchymal or the Kupffer cells. The functional significance of this finding is important. The different cell types in liver apparently metabolize different classes of lipoproteins (Stein & Stein, 1969; Stein *et al.*, 1974; van Berkel *et al.*, 1977; Andersen *et al.*, 1977; van Berkel & van Tol, 1978).

In radioautographic studies, chylomicron cholesteryl esters accumulate in parenchymal cell membrane, followed by a slow intracellular distribution (Stein & Stein, 1969). Therefore it is clear that the hepatic lipase is not taking part in the uptake of chylomicron remnants, since the enzyme is not located on parenchymal cells. Also the apoprotein moiety of very-low-density lipoprotein accumulates mainly in parenchymal cells (Stein *et al.*, 1974), suggesting a similar catabolic pathway for these lipoproteins and for chylomicrons.

The function of the liver in the catabolism of low-density and high-density lipoprotein has not yet been elucidated. When radioactively labelled low-density and high-density lipoprotein is injected into rats the label is found predominantly in non-parenchymal cells. This suggests that these cells are involved in some way in the metabolism of these lipoproteins (van Berkel *et al.*, 1977; van Berkel & van Tol, 1978). Low-density and high-density lipoproteins both contain phospholipids, which are good substrates for hepatic lipase, and also cholesteryl esters, which are known to be taken up in the liver (Glomset, 1968; Andersen *et al.*, 1977). In the present studies with hepatic lipase antiserum to inactivate rat hepatic lipase *in vivo*, increased concentrations of both low- and high-density lipoproteins, phospholipids and cholesteryl esters could be demonstrated (T. Kuusi, P. K. J. Kinnunen & E. A. Nikkilä, unpublished work). This finding is compatible with the observed association of low- and high-density $^{125}$I-labelled lipoproteins with non-parenchymal cells, and with the localization *in situ* of the hepatic lipase on the liver endothelial cell plasma membranes.

This work was supported by the State Medical Research Council (Academy of Finland) and by the Finnish Foundation for Cardiovascular Research.

## References

Andersen, J. M., Nervi, F. O. & Dietschy, J. M. (1977) *Biochim. Biophys. Acta* **486**, 298–307

Assmann, G., Krauss, R. M., Fredrickson, D. S. & Levy, R. I. (1973) *J. Biol. Chem.* **248**, 1992–1999

El-Maghrabi, R. M., Waite, M., Rudel, L. L. & Sisson, P. (1978) *J. Biol. Chem.* **253**, 974–981

Felts, J. M. & Mayes, P. A. (1967) *Nature (London)* **214**, 620–621

Glomset, J. (1968) *J. Lipid Res.* **9**, 155–167

Hamilton, R. L. (1964) Ph.D. Thesis, University Microfilms, Ann Arbor, MI

Huttunen, J. K., Ehnholm, C., Kekki, M. & Nikkilä, E. A. (1976) *Clin. Sci. Mol. Med.* **50**, 249–260

Jansen, H., van Berkel, T. J. C. & Hülsmann, W. C. (1978) *Biochem. Biophys. Res. Commun.* **85**, 148–152

Kuusi, T., Kinnunen, P. K. J., Ehnholm, C. & Nikkilä, E. A. (1979) *FEBS Lett.* **98**, 314–318

LaRosa, J. C., Levy, R. I., Windmueller, H. G. & Fredrickson, D. S. (1972) *J. Lipid Res.* **13**, 356–363

Stein, O. & Stein, Y. (1969) *J. Cell Biol.* **43**, 410–431

Stein, O., Rachmilevitz, D., Sanger, L., Eisenberg, S. & Stein, Y. (1974) *Biochim. Biophys. Acta* **360**, 205–216

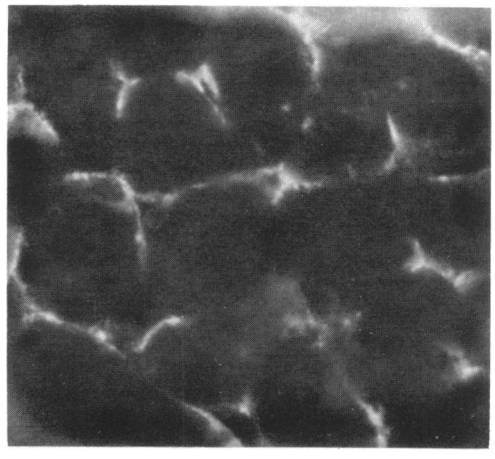
van Berkel, T. J. C. & van Tol, A. (1978) *Biochim. Biophys. Acta* **530**, 299–304

van Berkel, T. J. C., Koster, J. F. & Hülsmann, W. (1977) *Biochim. Biophys. Acta* **486**, 586–589

Waite, M. & Sisson, P. J. (1973) *J. Biol. Chem.* **248**, 7201–7206

Weibel, E. R., Stäubli, W., Gnägi, H. R. & Hess, F. A. (1969) *J. Cell Biol.* **42**, 68–91

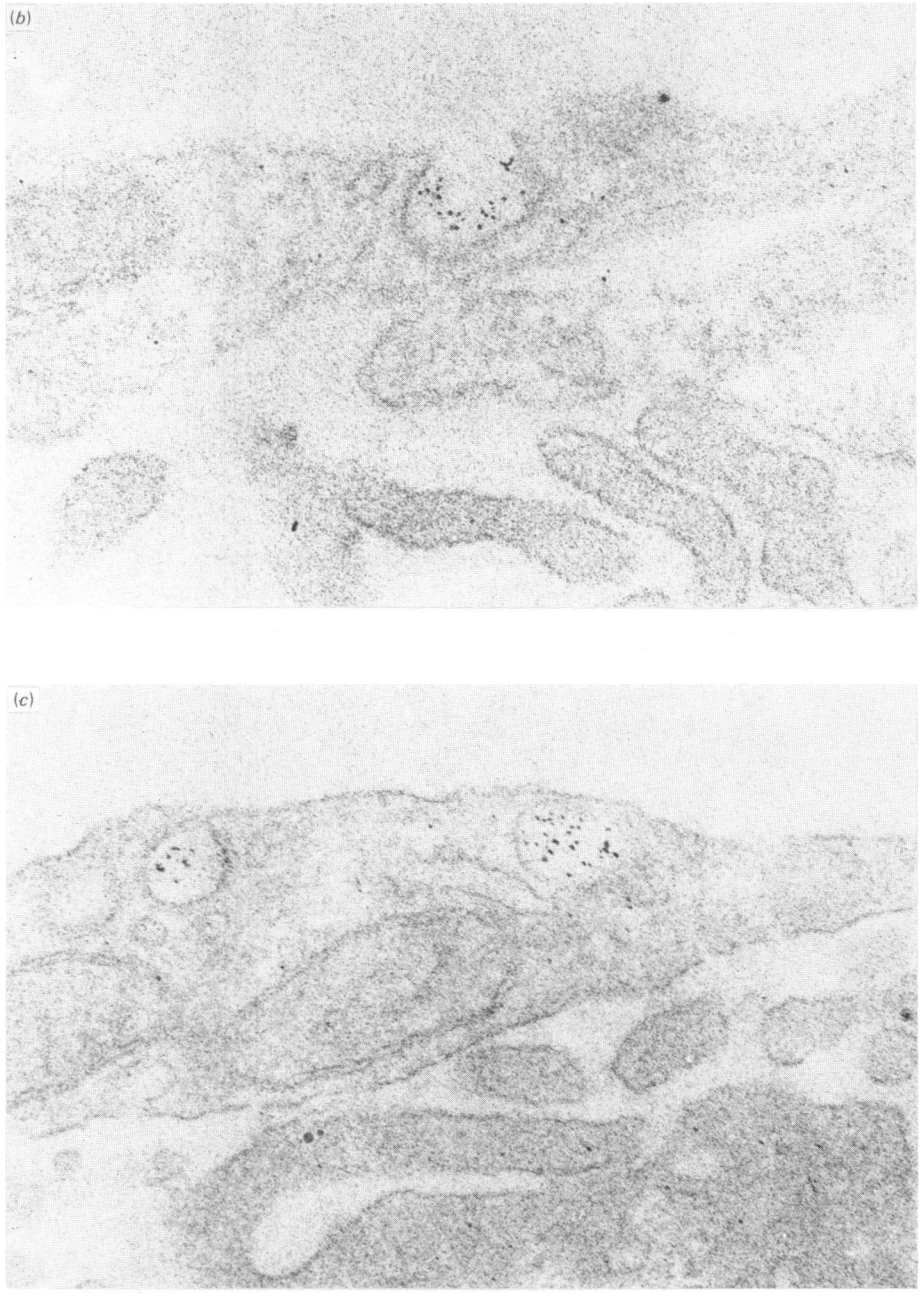
Wisse, E. (1977) in *Kupffer Cells and Other Liver Sinusoidal Cells* (Wisse, E. & Knock, D. L., eds.), pp. 33–60, Elsevier, Amsterdam



EXPLANATION OF PLATE 1

*Immunofluorescence micrographs of rat liver after the injection of anti-(rat hepatic lipase) serum*
A bright sinusoidal-type fluorescence is seen in the antiserum-treated livers. Livers from rats injected intravenously with heparin before administration of the antiserum completely lacked the fluorescence. Magnification, 300×.


PLATE 2




## EXPLANATION OF PLATE 2

*Electron micrographs of normal rat livers injected with anti-(rat hepatic lipase) serum and perfused with ferritin–anti-(rabbit immunoglobulin G)*

In all sections studied ferritin particles were seen only on the surface of liver endothelial cells (*a–c*). Liver parenchymal cells and Kupffer cells were completely devoid of ferritin granules. In endothelial cells the particles were either seen as discrete patches on the surface of the cells with an average distance of 10–12nm from the plasma membrane (*a*), on the surface of developing endocytotic vesicles (*b*) or in intracellular vesicles (*c*). Magnifications were: *a*, 73000 ×; *b*, 60000 ×; *c*, 57000 ×.

T. KUUSI, E. A. NIKKILÄ, I. VIRTANEN AND P. K. J. KINNUNEN