MOVEMENT OF ENDOCYTIC SHUTTLE VESICLES FROM THE SINUSOIDAL TO THE BILE CANALICULAR FACE OF HEPATOCYTES DOES NOT DEPEND ON OCCUPATION OF RECEPTOR SITES

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1. Introduction

Three of the major proteins of rat bile, namely IgA, secretory component and the haptoglobin : haemoglobin complex, reach bile by means of specialised endocytic vesicles [1–4]. The route for IgA and secretory component transport is understood. Secretory component is made in hepatocytes [3,5] and transported to the sinusoidal surface of the cells. Here the secretory component remains firmly bound to the surface of the cells but is capable of binding polymeric IgA [3,6]. The IgA-secretory component complex is then taken up into vesicles and transported across hepatocytes to the bile canalicular face of the cell [1,2]. The transport of haptoglobin : haemoglobin complexes is essentially the same [4], although, in this case, the receptor has not been identified.

Although the route of transfer of these proteins across hepatocytes is now understood, the mechanics of the process require further investigation. The simplest explanation for the initiation of formation of the endocytic vesicles is that the binding of IgA to secretory component, or of haptoglobin : haemoglobin complexes to their receptor, triggers the uptake. However, bile contains large amounts of free secretory component [7]. Although this might perhaps arise from dissociation of secretory IgA in the bile or from carriage of some free secretory component molecules adjacent in the plasma membrane to a secretory component molecule which had bound IgA, the large amounts suggested that the receptor might be transferred to bile whether or not IgA was bound. We now report that formation of the endocytic 'shuttle' vesicles does not depend on binding of transported proteins to their receptors since significant amounts of newly made secretory component continue to appear in bile from isolated perfused rat livers although neither IgA nor the haptoglobin : haemoglobin complex are available for transport.

2. Materials and methods

The liver perfusion experiments were performed by the method of Brewster et al. [8]. Wistar albino rats from the University of Surrey Animal Unit were anaesthetised with sodium pentobarbital (Sagatal, 60 mg/kg body wt.; May and Baker, Dagenham, Essex, UK), and the bile duct and hepatic portal vein of each rat cannulated with pp25 and pp60 polypropylene tubing (Portex, Hyte, Kent, UK) respectively. Bile collection was begun immediately. An infusion of the liver in situ (10 ml/min) with 40 ml of tissue-culture Medium 199 without Phenol Red (Difco Laboratories, Detroit, MI, USA) containing 1 g of bovine serum albumin (fraction V; Sigma Chemical Company, St. Louis, MO, USA), 200 I.U. of heparin (BHD Chemicals, Poole, Dorset, UK) and 2 mg of Neomycin (Sigma Chemical Company) was performed during hepatectomy, after which the liver was connected into the recirculating perfusion system. The liver was then perfused for 40 min at a rate of 1 ml/min/g of liver under 30 cm hydrostatic pressure and at a temperature of 37°C with an oxygenated (O_2/CO_2, 19 : 1) bicarbonate-buffered medium comprising 65 ml of tissue-culture Medium 199 without Phenol Red, 1.6 g of bovine serum albumin,

325 I.U. of heparin, 3.25 mg of neomycin and washed goat erythrocytes (35% haematocrit).

L-[1-^{14}C] fucose (0.2 ml, 10 μCi, prepared by dilution with 0.15 M NaCl of a stock solution [59.2 mCi/mmol] obtained from the Radiochemical Centre, Amersham, Bucks., UK) was then added to the perfusion medium in the reservoir and the perfusion continued for 4 h. Perfusate samples (0.5 ml) were collected from the reservoir at various time intervals throughout the perfusion and bile was collected into weighed vessels over accurately timed intervals. Acid-precipitable and acid-soluble radioactivities in the perfusion medium, from which the red cells had been removed by centrifugation, and in the bile samples were determined and immunoelectrophoresis of bile samples against anti-(rat bile) carried out as described previously [9].

3. Results

Under the conditions used in these experiments, the livers steadily secreted glycoproteins into the perfusion medium (fig.1). Although the apparent rate of secretion fell slightly with time of perfusion, a similar fall was observed in intact animals [9] and is probably due to dilution of the fucose pool. Over a 4-h period, 40–55% of the added fucose was incorporated into glycoproteins discharged into the perfusion medium. We therefore conclude that the perfused liver was fully functional. The rate of secretion of glycoproteins into bile was somewhat slower than the rate of secretion observed in intact animals. In both cases, only slow secretion was observed within the first 30 min. With liver in intact animals [9], the secretion rate rose rapidly to a peak just over an hour after injection of the label. In perfused livers, the peak was observed at just over 2 h, although the rate of incorporation of [^{14}C]fucose into glycoprotein was at least as great in the perfused liver as in the intact animals. The difference in peak time is due to differences in the supply of fucose. In the intact animal with a blood volume of about 12 ml and with other tissues competing for [^{14}C]fucose, the half-life of acid-soluble fucose in plasma was about 40 min; in the perfused liver with a total 'blood' volume of 100 ml and no competition for fucose uptake, the half-life of acid-soluble [^{14}C]fucose in the perfusion fluid was about 100 min. We conclude that glycoprotein synthesis and secretion continues normally in the isolated perfused liver.

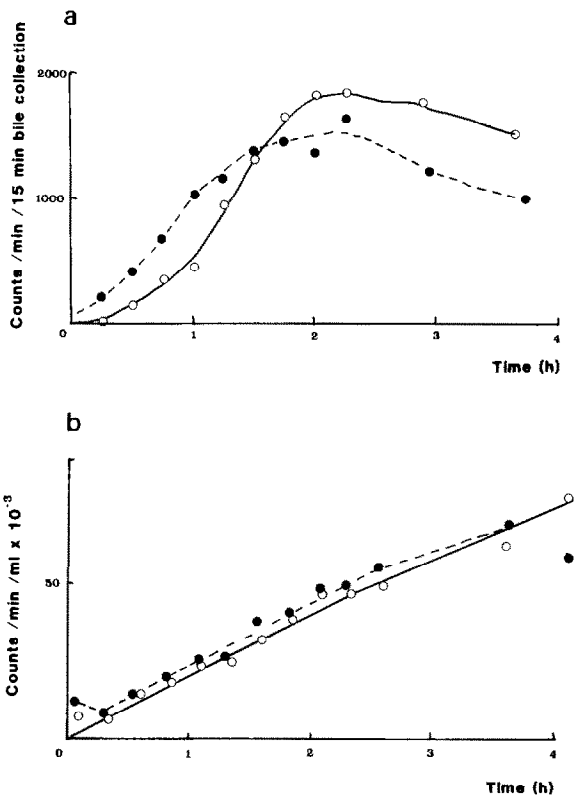

Fig.1. Appearance of total acid-precipitable ^{14}C-labelled glycoproteins in (a) bile and (b) perfusion medium following addition of L-[1-^{14}C]fucose to the recirculating perfusion medium supplied to an isolated rat liver. The results of two independent experiments are illustrated. Acid-precipitable radioactivity is plotted against the time after addition of [^{14}C]fucose to the perfusion medium. The results obtained with liver 1 (•), which gave higher incorporation of [^{14}C]-fucose into glycoproteins than liver 2 (○), have been divided by two to facilitate comparison of the shapes of the incorporation curves.

The nature of the proteins reaching the bile was examined by crossed immunoelectrophoresis of the bile samples collected at different times. Representative examples of the patterns obtained are shown in fig.2. The normal pattern of bile proteins (fig.2a), shown in the sample collected while the liver was still in the rat, had changed relatively little in bile collected during the first 40 min of perfusion in the apparatus (fig.2b), although the haptoglobin : haemoglobin peak had increased in size, presumably as a consequence of haemolysis due to the operative

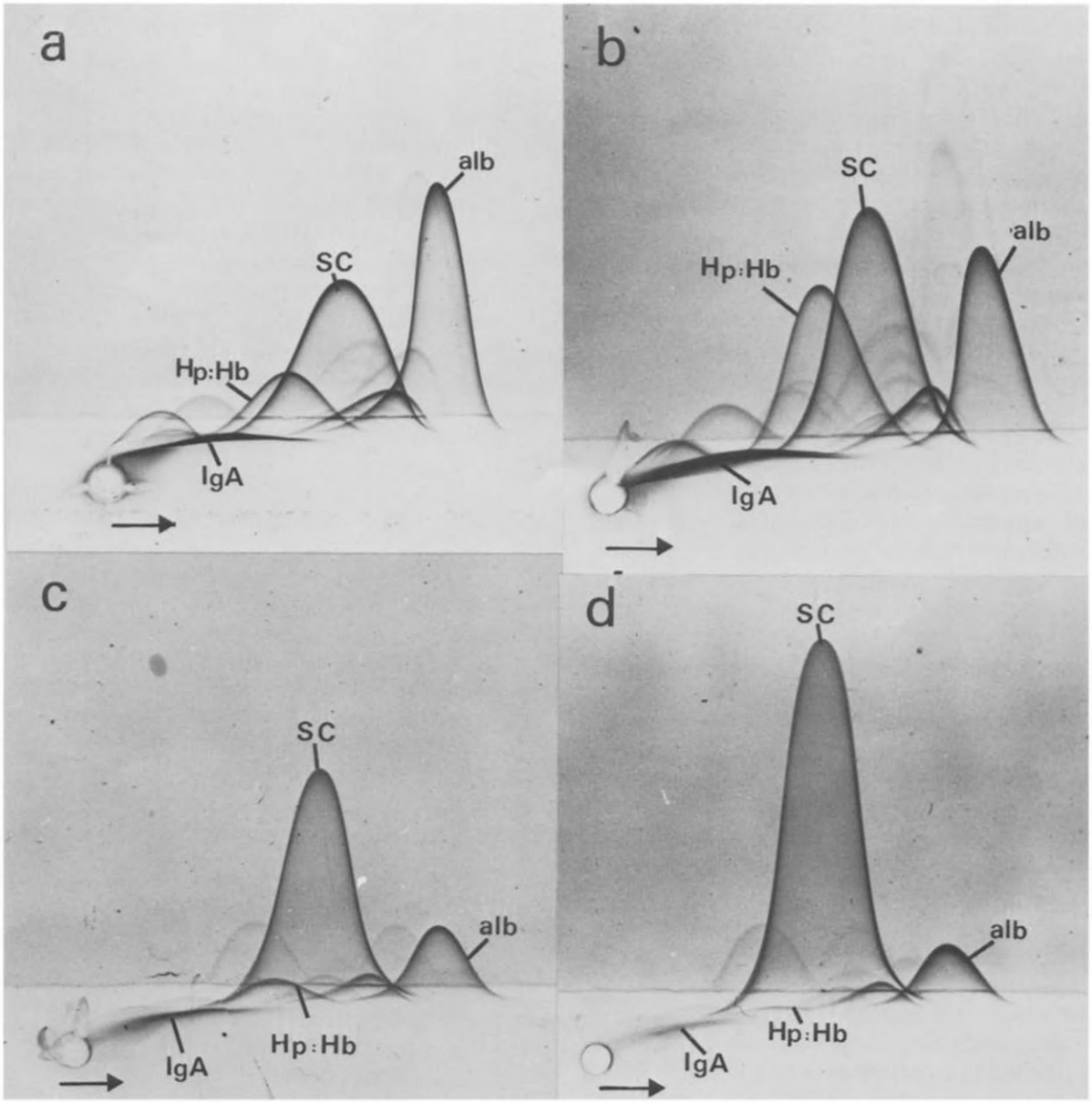

Fig.2. Patterns obtained after crossed immunoelectrophoresis of 7 μl aliquots of bile collected at various times after cannulation of the bile duct. Electrophoresis was against an anti-(rat bile) antiserum [9]. Plates were stained for protein with Coomassie brilliant blue R. Arrows show the direction of electrophoresis in the first dimension. The peaks are labelled as follows: alb, albumin; SC, secretory component; Hp : Hb, haptoglobin : haemoglobin complex (called bile protein 8 in earlier publications); IgA, immunoglobin A. (a) Bile collected whilst liver remained in rat. (b) Bile collected during first 40 min perfusion. (c) Bile collected between 110 and 125 min after beginning of perfusion. (d) Bile collected between 155 and 170 min after beginning of perfusion.

procedures preceding removal of the liver. However, later bile samples (figs.2, c and d) showed progressively decreasing levels of all the biliary proteins with the sole exception of secretory component. In the specimen illustrated in fig.2d, collected between 155 and 170 min after the natural blood supply to the liver had been replaced, only traces of IgA and haptoglobin : haemoglobin complex are visible, but the level of secretory component has increased. These high levels were maintained until sampling stopped 4.5–5 h after perfusion first began.

4. Discussion

The isolated perfused rat liver appeared to behave normally with regard to glycoprotein synthesis during the first few hours of perfusion as shown by the amounts of acid-insoluble [^{14}C]fucose. However, the protein composition of the bile changed markedly. Rat serum proteins are no longer bathing the sinusoidal face of the hepatocytes except insofar as they are synthesised de novo by the liver and diluted into the perfusion medium. IgA, which is not synthesised by the rat liver, is not supplied at all. It is therefore to be expected that bile levels of all the proteins common to serum and bile should fall, whether they normally reach the bile by means of endocytic vesicles or whether, as has been suggested in man and dog for albumin and those other serum proteins present in bile at concentrations less than 1% of their concentration in serum, they diffuse across the tight junctions between hepatocytes [10,11]. In fact, small, but significant amounts of those serum proteins which are synthesised by the hepatocytes continue to appear in bile even after lengthy perfusion of the liver. This observation does not, however, rule out diffusion as the mechanism for transfer of major serum proteins into bile. Diffusion will take place from the space of Disse and, in view of the very rapid discharge of proteins from hepatocytes, the protein composition of the fluid in the space of Disse will not necessarily be the same as that of the serum.

The amount of free secretory component being secreted into bile after a lengthy perfusion is, however, greater than the amount which was being secreted in the bile originally collected from the intact animal (fig.2). The ratio of newly synthesised biliary glycoprotein/newly synthesised serum glycoprotein is of the same order of magnitude in the perfused liver as in the intact animal even though amounts of most glycoproteins are greatly reduced in bile as discussed above and haptoglobin, in the absence of rat haemoglobin with which to complex, has almost vanished from bile. There appears to be no normal leakage of newly synthesised glycoprotein to the bile in the perfused liver since acid-insoluble [^{14}C]fucose levels in the bile showed at least as long a lag phase as in intact animals. We conclude that free secretory component is being synthesised and transported to the bile long after any IgA is available to bind. Hence the movement of the endocytic vesicles from the sinusoidal face of the hepatocyte to the bile canalicular face does not have to be initiated by binding of IgA to secretory component. The haptoglobin : haemoglobin complex which travels in association with the same or similar endocytic vesicles cannot be responsible for initiation of vesicle movement since rat haemoglobin is no longer available and the level of the complex in bile becomes very low. Hence it appears that the endocytic shuttle vesicles which normally transfer IgA, haptoglobin : haemoglobin and free secretory component to bile do not require binding of IgA or haptoglobin : haemoglobin to move from the sinusoidal face of hepatocytes to the bile canalicular face. Other workers [12] have found that the uptake of membrane proteins into hepatocytes is not inhibited by cytochalasin B and hence microfilaments do not appear to be involved.

It would seem that the formation of endocytic vesicles from the sinusoidal surface of hepatocytes is not dependent on the binding of serum proteins such as IgA. Hence the primary function of the vesicles appears to be the supplying of plasma membrane to the bile canalicular face of the cell where the membrane is being continuously eroded by the bile [13].

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References

[1] Birbeck, M. S. C., Cartwright, P., Hall, J. G., Orlans, E. and Peppard, J. (1979) Immunology 37, 477–484.

[2] Mullock, B. M., Hinton, R. H., Dobrota, M., Peppard, J. and Orlans, E. (1979) Biochim. Biophys. Acta 587, 381–391.
[3] Hinton, R. H., Mullock, B. M., Peppard, J. and Orlans, E. (1980) Biochem. Soc. Trans. 8, 114.
[4] Hinton, R. H., Dobrota, M. and Mullock, B. M. (1980) FEBS Lett (in press).
[5] Socken, D. J., Jeejeebhoy, K. N., Bazin, H. and Underdown, B. J. (1979) J. Exp. Med. 150, 1538–1548.
[6] Orlans, E., Peppard, J., Fry, J. R., Hinton, R. H. and Mullock, B. M. (1979) J. Exp. Med. 150, 1577–1581.
[7] Lemaître-Coelho, I., Jackson, G. D. F. and Vaerman, J.-P. (1977) Eur. J. Immunol. 7, 588–590.
[8] Brewster, D., Jones, R. S. and Parke, D. V. (1977) Xenobiotica 7, 601–609.
[9] Mullock, B. M. and Hinton, R. H. (1979) FEBS Lett. 106, 121–124.
[10] Dive, Ch. and Heremans, J. F. (1974) Eur. J. Clin. Invest. 4, 235–239.
[11] Dive, Ch., Nadalini, R. A., Vaerman, J. P. and Heremans, J. R. (1974) Eur. J. Clin. Invest. 4, 241–246.
[12] Stanley, K. K., Edwards, M. R. and Luzio, J. P. (1979) Biochem. J. 186, 56–69.
[13] Coleman, R., Holdsworth, G. and Vyvoda, O. S. (1977) in: Membrane Alterations as a Basis for Liver Injury (Popper, H. et al. eds) pp. 143–156, MTP, Lancaster.