Distribution of secretory component in hepatocytes and its mode of transfer into bile

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Immunoglobulin A in bile and other external secretions is mostly bound to a glycoprotein known as secretory component. This glycoprotein is not synthesized by the same cells as immunoglobulin A and is not found in blood. We now report the mechanism by which secretory component reaches the bile and describe its function in immunoglobulin A transport across the hepatocyte. Fractionation of rat liver homogenates by zonal centrifugation was followed by measurement of the amounts of secretory component in the various fractions by rocket immunoelectrophoresis. Secretory component was found in two fractions. One of these was identified as containing Golgi vesicles from its isopycnic density and appearance in the electron microscope; the other contained principally fragments of the plasma membrane of the sinusoidal face of the hepatocyte, as shown by its particle size and content of marker enzymes. Only the latter fraction bound ^{125}I -labelled immunoglobulin A added *in vitro*. At 5 min after intravenous injection of [^{14}C]fucose, the secretory component in the Golgi fraction was labelled, but not that in the plasma membrane. The secretory component in the sinusoidal plasma membrane did, however, become labelled before the first labelled secretory component appeared in bile, about 30 min after injection. We suggest that fucose is added to the newly synthesized secretory component in the Golgi apparatus. The secretory component then passes, with the other newly secreted glycoproteins, to the sinusoidal plasma membrane. There it remains bound but exposed to the blood and able to bind any polymeric immunoglobulin A present in serum. The secretory component then moves across the hepatocyte to the bile-canalicular face in association with the endocytic-shuttle vesicles which carry immunoglobulin A. Hence there is a lag before newly synthesized secretory component appears in bile.

IgA in external secretions is normally associated with a glycoprotein known as secretory component (Tomasi *et al.*, 1965). It has more recently been shown that secretory component acts as receptor to polymeric IgA on normal and neoplastic epithelial cells and is responsible for the translocation of IgA across epithelia (Brown, 1978; Crago *et al.*, 1978; Nagura *et al.*, 1979). The rapid and selective transport of polymeric IgA from blood to bile in rats has been shown to occur by the movement of endocytic vesicles across hepatocytes (Birbeck *et al.*, 1979; Mullock *et al.*, 1979). Two reports show that secretory component, which is made in rat hepatocytes (Socken *et al.*, 1979; Hinton *et al.*, 1980), is present on the surface of hepatocytes in short-term

tissue culture in a form capable of binding polymeric IgA (Orlans *et al.*, 1979; Socken *et al.*, 1979). To act as the receptor for the polymeric IgA in blood, the secretory component must be exposed on the sinusoidal surface of the hepatocytes. However, the binding of IgA to cultured hepatocytes does not prove that the secretory component is in fact on the sinusoidal surface, as it could be simply associated with the bile-canalicular plasma membrane. Indeed, large amounts of secretory component are present in bile both free and bound to IgA (Lemaître-Coelho *et al.*, 1977; Mullock *et al.*, 1978), but it has not been detected in serum (Lemaître-Coelho *et al.*, 1978).

We have therefore examined the distribution of secretory component and of IgA-binding ability in fractions separated from rat liver homogenates. We have also studied the labelling of secretory

Abbreviation used: IgA, immunoglobulin A.

component in subcellular fractions and in bile after injection of [^{14}C]fucose (Sturgess *et al.*, 1973) in an attempt to trace the pathway by which newly made secretory component reaches bile.

Materials and methods

The preparation and ^{125}I -labelling of polymeric rat IgA has been described previously (Orlans *et al.*, 1978). Rabbit antiserum specific for rat secretory component was prepared as described by Orlans *et al.* (1979). L-[1- ^{14}C]Fucose (50 $\mu\text{Ci}/\text{ml}$) was purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). Hooded rats of the University of Surrey strain were used in all experiments.

Binding of IgA to components of liver homogenates

Rat liver was homogenized in 0.25 M-sucrose/5 mM-Tris/HCl, pH 7.6, containing 2000 international units of heparin/litre, by three strokes of a Potter/Elvehjem homogenizer with a clearance of 0.33 mm between pestle and vessel. The homogenizer pestle was rotated at 900 rev./min. The volume of the homogenate was adjusted to 10 ml/g of liver. ^{125}I -labelled IgA was added to the ice-cold homogenate and, after mixing, samples were layered over a complex density gradient (Hinton *et al.*, 1970) in an A-XII zonal rotor (MSE Scientific Instruments, Crawley, Sussex, U.K.) or a linear 0.7–2 M-sucrose gradient buffered with 5 mM-Tris/HCl, pH 7.6, in the tubes of a 3 ml $\times$ 23 ml swing-out rotor. Centrifugation was for 50 min at 4000 rev./min in the A-XII zonal rotor or for 45 min at 3000 g in the 3 ml $\times$ 23 ml swing-out rotor. Further fractionation was achieved by isopycnic banding of selected fractions in a MSE B-XIV zonal rotor loaded with a linear density gradient of 0.6–2 M-sucrose buffered with 5 mM-Tris/HCl, pH 7.6. Centrifugation was for 14 h at 47000 rev./min. Preparation and fractionation of the gradients for all rotors was as described by Hinton & Dobrota (1976). Fractions collected from the zonal rotors were assayed for protein and for 5'-nucleotidase, alkaline phosphodiesterase, glucose 6-phosphatase, acid phosphatase, β -galactosidase and succinate dehydrogenase as described previously (Hinton & Norris, 1972; Mullock *et al.*, 1979). Particle-associated IgA was collected by pelleting. In brief, fractions were diluted with 0.5 vol. of 0.45 M-NaCl and centrifuged for 90 min at 115000 g. For fractions from the A-XII rotor, the pellets were washed by resuspension in 0.15 M-NaCl/5 mM-Tris/HCl, pH 7.6, and re-pelleting under the same conditions as above. Other experiments showed that it was not necessary to use such high speeds in collecting the larger particles separated in the A-XII zonal rotor; particles from fraction 11 onwards were efficiently pelleted by centrifugation for 20 min at 38000 g. The

results of experiments were processed and the average distribution after isopycnic banding was calculated as described previously (Mullock *et al.*, 1979).

Distribution of secretory component among liver cell fractions

Whole liver homogenate was fractionated by centrifugation in an A-XII zonal rotor as described above. Liver microsomal preparations ('microsomes') were fractionated by isopycnic flotation in a B-XIV zonal rotor as described by Norris *et al.* (1974). Particulate material was collected by centrifugation for 90 min at 115000 g after dilution of fractions with at least $\frac{1}{3}$ vol. of NaCl to give a final concentration of 0.15 M-NaCl. The fractions were dissolved by treatment with 1% sodium deoxycholate/0.5% Lubrol W (Cirrassol ALN-WF; I.C.I., Runcorn, Cheshire, U.K.). In some experiments insoluble material was removed by centrifugation for 30 min at 250000 g in a 3 ml $\times$ 6.5 ml swing-out rotor, but this was later found to be unnecessary. Secretory component was measured by rocket immunoelectrophoresis (Axelson *et al.*, 1973) in gels containing 0.5% Lubrol W and 0.3% antiserum specific for rat secretory component.

Electron microscopy

Pellets collected from fractions separated in the zonal rotors were fixed in 2% (w/v) osmic acid and embedded in Epon as described by El-Aaser *et al.* (1973). Grey sections were cut and examined in a JEM 100B electron microscope [Jeol (U.K.) Ltd., London N.W.9, U.K.] after counterstaining with alkaline lead citrate and uranyl acetate (Lewis & Knight, 1977).

Fucose-labelling experiments

Doses of up to 0.4 ml of [^{14}C]fucose were injected via the femoral vein of rats anaesthetized with pentobarbital (Sagatal; May and Baker, Dagenham, Essex, U.K.). When bile labelling was being examined, the bile duct was cannulated before the fucose was injected and bile collection began immediately after injection. Labelling of secretory component was examined by radioautography of the rocket-immunoelectrophoresis plates.

Results

Distributions of radiolabelled IgA bound in vitro and of secretory component

Density-gradient centrifugation was used to examine the distributions in liver homogenates of secretory component and of radiolabelled IgA bound *in vitro*. The distribution of particle-associated IgA and of marker enzymes after fractionation in an A-XII zonal rotor of a liver

homogenate to which radiolabelled IgA had been added immediately before centrifugation is shown in Fig. 1. The enzymes 5'-nucleotidase, glucose 6-phosphatase, succinate dehydrogenase and acid phosphatase were used as markers for plasma-membrane fragments, endoplasmic-reticulum fragments, mitochondria and lysosomes respectively. In other experiments (results not shown) it was shown that the distributions of alternative markers for

lysosomes and plasma-membrane fragments, namely β -galactosidase (Dobrota *et al.*, 1978) and alkaline phosphodiesterase respectively, were very similar to the distributions of acid phosphatase and 5'-nucleotidase.

Most of the ^{125}I -labelled IgA bound was found in a zone containing particles that had not significantly separated from the sample zone (centred at fraction 4 in Fig. 1). The peak of sedimentable ^{125}I -labelled

Fig. 1. Distribution of IgA-binding capacity and of protein and marker enzymes after centrifugation of a rat liver homogenate for 50 min at 4000 rev./min in an A-XII zonal rotor

^{125}I -labelled IgA was mixed with the ice-cold homogenate immediately before centrifugation. After centrifugation, 40 ml fractions were collected, their density was estimated by refractometry and samples were assayed for protein and marker enzymes. NaCl was added to a final concentration of 0.15 M to a portion of each fraction, and particulate material was collected by centrifugation. The pellets collected from fractions 3–18 were washed once with 0.15 M-NaCl. ^{125}I radioactivity was measured in the pellets. (a) O, Protein; ----, density (20°C). (b) ▲, Succinate dehydrogenase; □, glucose 6-phosphatase; △, acid β -glycerophosphatase. (c) O, 5'-nucleotidase; ●, sedimentable ^{125}I -labelled IgA. Radioactivity in fractions 3–7 is $\times 0.01$.

IgA was sharp and there was no evidence for association of IgA *in vitro* with the large vesicles with which endogenous IgA and also ^{125}I -labelled IgA injected into living rats had been associated (Mullock *et al.*, 1979). There were two small but well-defined zones containing sedimentable ^{125}I -labelled IgA centred at fractions 21 and 32. The zone centred at fraction 21 contained large sheets of plasma membrane, which electron microscopy showed were derived mainly from the bile-canalicular and contiguous faces of hepatocytes (Hinton *et al.*, 1970). The zone centred at fraction 32 has been shown by light microscopy (Hinton *et al.*, 1970) to contain unbroken cells with, in some cases, the membranes of adjacent cells still adhering.

Secretory component, measured by rocket immunoelectrophoresis after similar fractionation of liver homogenate, was found almost exclusively in the low-density region which also contained the bulk of the IgA-binding capacity (see Fig. 2). A small amount of secretory component was detected in the region containing unbroken cells.

The major peak, which contained both ^{125}I -labelled IgA and secretory component, was centred in the region containing 'microsomes'. These are derived from several types of membranes. In order to identify the type of membrane which bound the IgA, the 'microsomal' region from the A-XII rotor was further fractionated by isopycnic flotation. The results (Fig. 3) show that the IgA was bound to

particles that had a density distribution similar to those of the plasma-membrane markers 5'-nucleotidase and alkaline phosphodiesterase (results not shown) and distinct from the distribution of the endoplasmic-reticulum marker glucose 6-phosphatase.

The distribution of secretory component among microsomal subfractions (Fig. 4) showed that it was associated with two classes of fragment with mean densities 1.125 and 1.155 g/ml respectively. The first class of secretory-component-containing vesicles can be identified as fragments of the Golgi apparatus by their low density and by their appearance in the electron microscope (Plate 1a). The distribution of the higher-density fragments resembled that of bound IgA. Electron microscopy showed smooth-surfaced vesicles of indeterminate origin (Plate 1b).

Labelling of secretory component in vivo by [^{14}C]-fucose

At 5 min after an intravenous injection of [^{14}C]-fucose, labelled secretory component was detected only in the low-density microsomal material, which has been identified as fragments of the Golgi apparatus (Fig. 4e). At 20 min, label was associated with the secretory component in all of the microsomal subfractions (results not shown). In bile, labelled secretory component was first clearly seen in the samples collected between 30 and 45 min after injection (Fig. 5).

Fig. 2. Distribution of secretory component and of 5'-nucleotidase after centrifugation of a rat liver homogenate for 50 min at 4000 rev./min in an A-XII zonal rotor

Fractions were collected and examined as in Fig. 1. The secretory-component content of the particulate material was estimated by rocket immunoelectrophoresis after extraction with 1% sodium deoxycholate/0.5% Lubrol W. ----, Density (20°C); ■, protein; Δ, 5'-nucleotidase; □, secretory component.

EXPLANATION OF PLATE 1

Electron micrographs of pellets collected from fractions of rat liver microsomal material separated by isopycnic banding as described in Fig. 4

The fractions were fixed with 2% osmic acid and embedded in Epon. Sections were stained with alkaline lead citrate and uranyl acetate. (a) Materials banding at density 1.10–1.12 g/ml; (b) material banding at density 1.14–1.18 g/ml.

Fig. 3. Distribution of IgA-binding capacity after isopycnic banding of the 'microsomal' region from the A-XII rotor. Fractions were separated as in Fig. 1. Fractions 4-7 were pooled and NaCl was added to a final concentration of 0.15 M. Particulate material was collected by centrifugation. The pelleted material was resuspended in 2 M-sucrose buffered with 5 mM-Tris/HCl (pH 7.4) and loaded under a linear 0.6-2 M-sucrose gradient, which was similarly buffered. After centrifugation for 14 h at 47 000 rev./min, 20 ml fractions were collected and their density, protein and marker enzymes assayed. Pairs of adjacent fractions were pooled, diluted by the addition of $\frac{1}{2}$ vol. of NaCl to give a final concentration of 0.15 M-NaCl and centrifuged for 90 min at 115 000 g in order to separate particulate material for radioactivity counting. The results of two separate experiments were combined to give an average distribution of protein, radioactivity and enzyme activity with density. The bars at each end of the graph indicate the percentage of activity recovered at densities below 1.08 g/ml (left) or above 1.23 g/ml (right). (a) Protein; (b) glucose 6-phosphatase; (c) acid β -glycerophosphatase; (d) 5'-nucleotidase; (e) sedimentable ^{125}I -labelled IgA.

Discussion

The results presented in this paper show that both secretory component and the ability to bind radio-labelled polymeric IgA *in vitro* are chiefly associated with subcellular structures, fragments of which are recovered in the microsomal fraction of a rat liver homogenate. Subfractionation of liver microsomal preparations, however, showed that,

although IgA-binding ability was confined to a single population of vesicles with a median density of 1.147 g/ml, secretory component appeared in two distinct populations, one corresponding in density to that which bound IgA *in vitro*, and a second fraction of lower density.

The lower-density secretory-component-containing fraction had a median density of 1.125 g/ml. Beaufay *et al.* (1974) reported that the median

Fig. 4. Distribution of secretory component after isopycnic banding of the microsomal fraction of a rat liver homogenate

The homogenate was centrifuged for 15 min at 11500 *g* and the resulting pellet washed once with 0.25 M-sucrose/5 mM-Tris/HCl (pH 7.4). The supernatant was combined with the wash supernatant and centrifuged for 90 min at 115000 *g*. The sedimented microsomal fraction was resuspended in 2 M-sucrose buffered with 5 mM-Tris/HCl (pH 7.4) and loaded under a similarly buffered 0.6–2 M-linear sucrose gradient. After centrifugation for 14 h at 47000 rev./min, 20 ml fractions were collected, and their density, protein and marker enzymes assayed. Further portions were diluted with at least $\frac{1}{3}$ vol. of NaCl to give a final concentration of 0.15 M-NaCl and centrifuged for 90 min at 115000 *g*. The pellets were extracted with 1% sodium deoxycholate/0.5% Lubrol W and their secretory-component content was estimated by rocket immunoelectrophoresis. The results of four separate experiments were combined to give an average distribution of protein, secretory component and enzyme activity with density. The bars at each end of the graph indicate the percentage of activity recovered at densities below 1.08 g/ml (left) or above 1.20 g/ml (right). (a) Protein; (b) glucose 6-phosphatase; (c) 5'-nucleotidase; (d) alkaline phosphodiesterase; (e) secretory component. The line at the top of (e) shows the density range over which label could be detected in secretory component by radioautography when [14 C]fucose had been injected 5 min before the animals were killed.

density of fragments of the Golgi apparatus, as indicated by galactosyltransferase activity, was 1.127 g/ml. The appearance of this fraction in the electron microscope is very like that of the lipoprotein-containing vesicles associated with Golgi apparatus (Glaumann *et al.*, 1975). The presence exclusively in this fraction of radiolabelled secretory component 5 min after the injection of [14 C]fucose also supports the view that it contains fragments of the Golgi apparatus, the site where fucose would be added to newly synthesized protein. As there is no binding *in vitro* of IgA to these Golgi-apparatus-

derived vesicles, it is likely that the secretory component faces into the lumen of the vesicle. This indeed would be expected of a protein destined for incorporation into the outer face of the plasma membrane. Secretory component has been demonstrated in the Golgi apparatus of other cell types (Brandtzaeg, 1974; Kraehenbuhl *et al.*, 1975; Nagura *et al.*, 1979).

The higher-density secretory-component-containing fraction had a median density of 1.155 g/ml, not significantly different from the median density of 1.147 g/ml found for material that bound IgA *in*

Fig. 5. Appearance of ^{14}C -labelled secretory component in bile after intravenous injection of L-[1- ^{14}C]fucose into a rat

Bile samples were collected over 15 min intervals after injection and their secretory-component contents measured by rocket immunoelectrophoresis in gel containing 0.3% anti-(secretory component) serum. The plate was stained for protein with Coomassie Brilliant Blue R (a) and secretory component containing $[^{14}\text{C}]$ fucose was located by radioautography (b). The numbers beneath each rocket indicate, in min, the time interval over which the bile sample was collected after injection. Similar results were obtained in each of three rats.

vitro. In both cases, the distributions resembled those of marker enzymes for liver plasma membrane. We conclude that the secretory-component-containing material of higher density is derived from plasma membrane and that this material, unlike the lower-density secretory-component-containing vesicles, can bind polymeric IgA *in vitro*.

These results confirm the presence of secretory component, in a form capable of binding IgA, in plasma-membrane material from rat liver and also show that secretory component is unevenly distributed over the hepatocyte surface, since only the plasma-membrane material recovered from the microsomal fraction contained secretory component. Plasma-membrane fragments derived from the sinusoidal face of the hepatocyte are recovered largely in the microsomal fraction (Wisher & Evans,

1975; Dobrota *et al.*, 1976; Carey & Evans, 1977), whereas material from the bile-canalicular and contiguous faces is much more rapidly sedimenting and is normally recovered in the crude nuclear fraction (Hinton *et al.*, 1970; Wisher & Evans, 1975). We detected no secretory component and very little IgA-binding ability in the rapidly sedimenting plasma-membrane fragments and therefore conclude that secretory component is only displayed on the sinusoidal portion of the hepatocyte surface. This secretory component is not labelled 5 min after injection of $[^{14}\text{C}]$ fucose, but does become labelled before labelled secretory component appears in bile. Newly made secretory component does not appear in bile until 30 min after the injection of $[^{14}\text{C}]$ fucose, although labelled glycoproteins appear in serum almost immediately (Riordan *et al.*, 1974; Mullock & Hinton, 1979). A similar interval of about 30 min between intravenous injection and first detection in bile is observed for polymeric radio-labelled IgA (Orlans *et al.*, 1978; Jackson *et al.*, 1978; Fisher *et al.*, 1979).

Our results suggest the following mode of transfer of secretory component into bile. After synthesis, presumably on the rough endoplasmic reticulum, it is processed in the Golgi apparatus and packaged in secretory vesicles together with serum lipoproteins and glycoproteins. The secretory component is attached to the membrane of the Golgi-derived vesicles, which move rapidly to the sinusoidal surface of the hepatocytes. There the serum glycoproteins and lipoproteins are released, but secretory component remains associated with what has now become the extracellular surface of the plasma membrane, and is able to bind any polymeric IgA arriving in the blood. The IgA complexed with secretory component is taken up into the hepatocyte as an endocytic vesicle (Mullock *et al.*, 1979) and transported across the hepatocyte to the bile-canalicular face, with which it fuses. There the complex is released, possibly by the detergent action of bile salts (Coleman *et al.*, 1977).

Clearly other proteins could be supplied to the biliary surface of hepatocytes by these 'shuttle vesicles', which move across the cell without fusing with lysosomes. Similar processes probably occur in other epithelia. Immunocytochemical studies by Nagura *et al.* (1979) have shown that IgA crosses cultured neoplastic colon cells in the same way as we now conclude IgA is transported from blood to bile across hepatocytes.

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