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THE BILIARY EXCRETION OF CIRCULATING ASIALOGLYCOPROTEINS IN THE RAT

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SUMMARY

Following intravenous injection into the rat a small proportion (0.5 - 3.0%) of asialo α_1-acid glycoprotein, asialo fetuin, asialo CEA* and native CEA are excreted in an apparently unchanged form in the bile. The maximum excretion rate occurs one hour after injection in all cases. The possibility of a novel pathway for glycoprotein uptake by the liver is discussed.

The role of the liver in the removal and catabolism of circulating asialoglycoproteins has been well documented (1). During *in vitro* studies to investigate the metabolism of native and asialocarcinoembryonic antigen (CEA) by the perfused rat liver, it was observed that a considerable amount of the glycoprotein (10%) was excreted in the bile (2) in what appeared to be an unchanged state (3). We have now extended this investigation and report on the biliary excretion by the rat *in vivo* of a number of asialoglycoproteins following their intravenous administration.

MATERIALS AND METHODS

Both carcinoembryonic antigen and its asialo derivative were prepared as described previously (4). Human α_1-acid glycoprotein was obtained from plasma (5) and fetuin was purchased from the Sigma Chemical Co. Ltd. The asialo derivatives of both α_1-acid glycoprotein and fetuin were prepared by treatment of the native glycoproteins with neuraminidase (*v. cholerae*) as described previously for asialocarcinoembryonic antigen (4).

Iodinations were carried out by the chloramine T method (6) for CEA and asialo-CEA and by the solid supported lactoperoxidase method (7) for asialo α_1-acid glycoprotein and fetuin.

Male August rats (approximately 250 gm) were anaesthetised with 'Sagatal' (sodium pentabarbitone) administered intraperitonealy. Anaesthesia was maintained for the duration of the experiments and then the animals were killed by cervical dislocation. Bile ducts were cannulated using 0.5 mm bore

* CEA = Carcinoembryonic Antigen

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teflon tubing and bile collected sequentially for either fifteen or thirty minutes. The iodine labelled glycoproteins were injected intravenously via the femoral vein thirty minutes after the commencement of bile collection. Bile samples were examined for their protein content (8), CEA content by radioimmunoassay (9) and for total ^{125}I content. Some bile samples were also examined by gel-filtration on a Sephadex G-200 column (100 cm x 1.6 cm) equilibrated with 0.1 M phosphate buffered saline.

RESULTS AND DISCUSSION

The pattern of the biliary excretion of asialo-CEA, asialo-fetuin and asialo α_1-acid glycoprotein along with that for native CEA is shown in Figure 1. There is a rapid excretion into the bile of all the glycoproteins tested with a miximum occurring about one hour after the initial injection and falling to virtually background levels after two hours. The pattern of biliary excretion of CEA and asialo-CEA as determined by radioimmunoassay is shown in Figure 2.

Gel-filtration of bile obtained from the above experiments showed no apparent molecular weight change in the biliary excreted glycoproteins. Dilution curves of the biliary excreted CEA and asialo-CEA by radioimmunoassay were parallel to that obtained with the standard pre-injection samples of these glycoproteins. Specific activities of the ^{125}I-labelled glycoproteins were similar after excretion in the bile to the samples injected. It is likely, therefore, that little breakdown of the protein chain has occurred during the passage of these glycoproteins from the circulation to the bile. This conclusion is particularly apt for CEA and asialo-CEA where minor alterations to the protein chain (i.e. limited hydrolysis by acid and proteolytic enzymes or breakage of disulphide bridges) have previously been found to cause considerable loss in its ability to bind to antibody in the radioimmunoassay (4, 10). Significant degradation of the carbohydrate side chains must also be negligible as no detectable alterations in molecular weights could be observed.

This pathway for the biliary excretion of circulating glycoproteins appears to be of only minor quantitative significance. Only 0.5 - 3.0% (these figures refer an injection of 100 μg of glycoprotein) of the injected

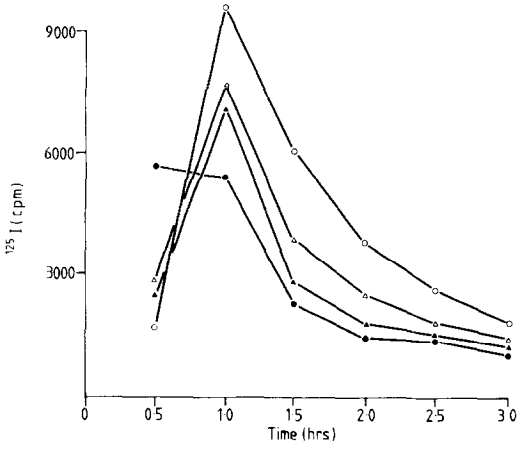

Figure 1 - Biliary excretion of ^{125}I-labelled glycoproteins in the rat. ● asialofetuin, ○ asialo α_1-acid glycoprotein, △ asialo CEA, ▲ native CEA. Glycoproteins (100 μg) were injected via the femoral vein.

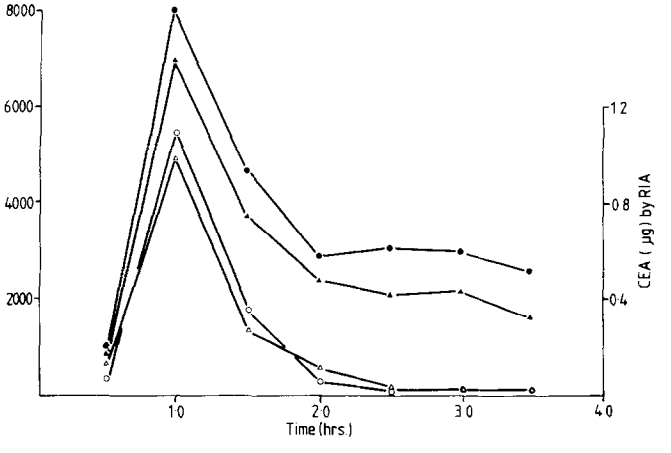

Figure 2 - Biliary excretion of native CEA and asialo CEA in the rat. ● CEA (^{125}I), ▲ asialo CEA (^{125}I), ○ CEA by radioimmunoassay, ○ asialo CEA by radioimmunoassay.

materials appear in the bile, though in the case of CEA and asialo-CEA the liver retains immunoassayable material for a number of hours after biliary excretion has ceased (Figure 3). This pathway, however, may be physiologically important as a recent report (11) has shown considerable amounts of secretory

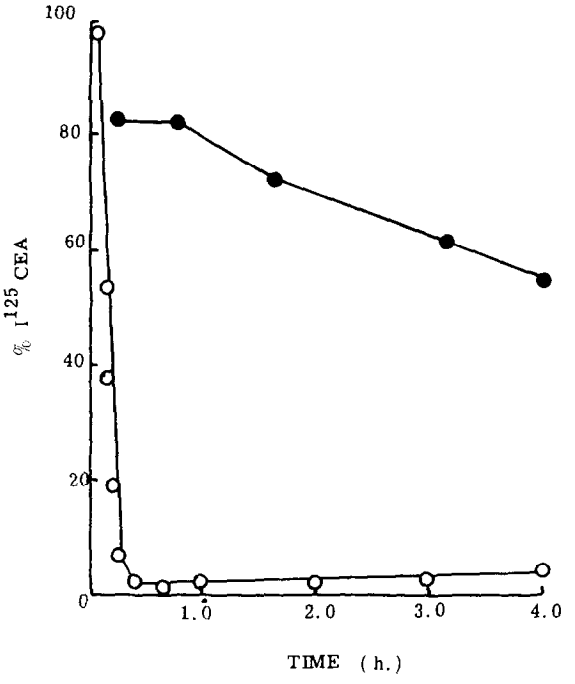

Figure 3 - Removal of ^{125}I-CEA from the circulation of the rat and its appearance in the liver. ○ circulating levels, ● liver levels.

IgA and free secretory component to be present in rat bile. It has been suggested that these materials enter the bile from the circulation and not by direct liver synthesis (11). Also materials with immunological cross reactivity with CEA have also been found in relatively large quantities in human bile (2, 12).

As in the case of other asialoglycoproteins, asialo-CEA is largely removed from the circulation by the liver parenchymal cells. Native CEA, however, is removed exclusively by the Kupffer cells with a subsequent transfer to the hepatocytes (13). This transfer is relatively slow (one to three hours) although it can be seen that the biliary excretion of CEA is quite rapid and short lived (Figure 2). It is possible, therefore, that the small fraction of circulating glycoprotein molecules which are subsequently excreted in the bile are removed from the blood-stream by a mechanism different from that involving the remainder of such molecules.

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