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The Effects of Triton WR1339 and Asialo-fetuin on the Hepatic Uptake of Circulating Native and Asialo-carcinoembryonic Antigen

By PETER THOMAS and MERVYN JONES
Institute of Cancer Research, The Haddow Laboratories, Clifton Avenue, Sutton, Surrey SM2 5PX, U.K., and The Chester Beatty Research Institute, Fulham Road, London SW3 6JB, U.K.

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Uptake of carcinoembryonic antigen from the circulation of the mouse is inhibited by treatment of the animal with Triton WR1339, but not by asialo-fetuin. Uptake of asialo-carcinoembryonic antigen is inhibited by asialo-fetuin, but not by Triton WR1339. These results reflect the different mechanisms of uptake of the glycoproteins. The presence or absence of sialic acid does not seem to be important in governing Kupffer-cell uptake, but when terminal galactoses are exposed on a glycoprotein, uptake by hepatocytes is preferred. Kupffer-cell uptake of carcinoembryonic antigen is not due to the formation of high-molecular-weight complexes in the blood stream. The effect of asialo-fetuin and Triton WR1339 on the biliary excretion of carcinoembryonic antigen and asialo-carcinoembryonic antigen is discussed.

Carcinoembryonic antigen is a sialoglycoprotein of mol.wt. about 180000. As with many other sialoglycoproteins, removal of sialic acid results in an increased uptake of the circulating glycoprotein by the liver (Thomas & Hems, 1975). The native glycoprotein, however, has a surprisingly short half-life in the circulation of both rat and mouse and again the liver is the major site of accumulation (Thomas & Hems, 1975; Thomas *et al.*, 1977). Two hepatic receptors for glycoproteins have been recognized, that for galactose (Ashwell & Morell, 1974) and that for *N*-acetylglucosamine (2-acetamido-2-deoxy-D-glucose) (Stockert *et al.*, 1976). Neither of these receptors appear to be involved in the hepatic uptake of circulating native carcinoembryonic antigen (Thomas *et al.*, 1976). Carcinoembryonic antigen is initially removed from the circulation of rats and mice by the liver Kupffer cells and this is followed by a transfer to the parenchymal cells. Asialo-carcinoembryonic antigen, however, is removed from the circulation directly by the parenchymal cells and this presumably involves binding to the galactose receptor (Thomas *et al.*, 1977).

This study is concerned with the effect of asialo-fetuin as a galactose-receptor blocker (Ashwell & Morell, 1974) and of Triton WR1339 as an inhibitor of Kupffer-cell function (Cotmore & Carter, 1973) on the hepatic uptake of circulating native and asialo-carcinoembryonic antigen.

Materials and Methods

Isolation of carcinoembryonic antigen was from liver metastases of colorectal carcinomas by the method of Krupey *et al.* (1972). Asialo-carcinoembryonic antigen was prepared by treatment of carcinoembryonic antigen with neuraminidase (Westwood *et al.*, 1974). Fetuin was purchased from Sigma Chemical Company, Kingston upon Thames, Surrey, U.K., and asialo-fetuin prepared as described for asialo-carcinoembryonic antigen. Triton WR1339 was obtained from Winthrop Laboratories, Newcastle-upon-Tyne, U.K. The sialic acid content of the glycoproteins was determined by using the thiobarbituric acid method (Warren, 1959).

Female mice of the Balb C-strain were used throughout this study and were supplied with food and water *ad libitum*. Intravenous injections of 25μg of the glycoproteins (0.2ml in iso-osmotic saline) were given via the tail vein after warming the animals for a few minutes. Blood samples were taken by piercing a tail vein other than that used for injection and withdrawing 20μl with a heparinized pipette. Blood samples were added to 0.5ml of 0.05M-phosphate-buffered saline solution, pH7.4, containing 0.1% human serum albumin and heparin as an anticoagulant. Erythrocytes were removed by centrifugation and the carcinoembryonic-antigen content of the supernatants determined on a 0.2ml sample by radioimmunoassay (Laurence *et al.*, 1972). Triton WR1339 (0.25ml) was injected intraperitoneally as a 10% (w/v) solution in iso-osmotic saline. The mice were divided into four groups. Group 1 received no Triton. Group 2 received a single dose. Group 3 received two doses at 24h intervals. Group 4 received three doses at 24h intervals. Uptake of carcinoembryonic antigen was determined in these animals at 24 and 48h after their last Triton injection.

Asialo-fetuin with either carcinoembryonic antigen or asialo-carcinoembryonic antigen was dissolved

together in the ratios of 1:1, 2.5:1, 10:1 or 20:1 by weight and injected intravenously for the rate of carcinoembryonic antigen and asialo-carcinoembryonic antigen uptake to be determined.

Gel filtration was carried out on a calibrated column (100cm×1.6cm) of Bio-Gel A 1.5m, with 0.1M-phosphate buffer, pH7.4, containing 0.9% NaCl as the eluting solvent. Samples of carcinoembryonic antigen or asialo-carcinoembryonic antigen were injected into mice via the tail vein and the mice killed 1min after injection. Blood was collected, erythrocytes removed by centrifugation and the plasma samples (0.5ml) applied to the Bio-Gel column. The elution position of the glycoprotein was determined by radioimmunoassay (Laurence *et al.*, 1972) of the fractions.

Results and Discussion

Fig. 1 shows the removal of both carcinoembryonic antigen and asialo-carcinoembryonic antigen from the circulation of the mouse. Carcinoembryonic antigen has a half-life of 4min, whereas asialo-carcinoembryonic antigen has a half-life of 1.5min. In the presence of asialo-fetuin little or no effect is observed on the half-life of native carcinoembryonic antigen, with an increase in half-life at the highest concentration of asialo-fetuin to 6min. In the presence of the two highest concentrations of asialo-fetuin, however, the half-life of asialo-carcinoembryonic antigen is increased from 1.5min to 6min, a similar half-life to that found with native carcinoembryonic antigen under these conditions (Fig. 2). When mice were treated with Triton, however, there was only a marginal effect on the uptake of asialo-carcinoembryonic antigen, whereas the effect on carcinoembryonic-antigen uptake was marked. Table 1 shows the effect of the various amounts of Triton on the half-lives of both carcinoembryonic antigen and asialo-carcinoembryonic antigen. Although Triton does cause some damage to liver parenchymal cells (Cotmore & Carter, 1973), the effect on the galactose-receptor function was minimal and asialo-glycoprotein uptake was normal, though it is possible that subsequent inhibition of lysosomal digestion of the glycoproteins may have occurred. It can be seen from the above data that removal of sialic acid from carcinoembryonic antigen in itself does not affect Kupffer-cell uptake, but when terminal galactose residues are exposed the uptake by hepatocytes becomes important and in general the majority of the circulating asialo-glycoprotein is now removed by this route and may be observed by radioautography of the ^{125}I-labelled molecule (Thomas *et al.*, 1977), or by immunoperoxidase staining (Heyderman & Neville, 1977; E. Heyderman & P. Thomas, unpublished work). Thus when the liver-cell galactose receptors are blocked by an excess of asialo-fetuin then the uptake of asialo-carcinoembryonic antigen is mainly brought about by a mechanism similar to that of the native glycoprotein. The presence of an

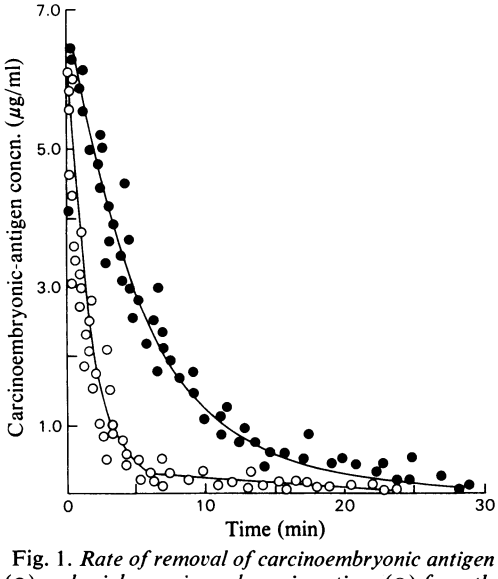

Fig. 1. *Rate of removal of carcinoembryonic antigen* (●) *and asialo-carcinoembryonic antigen* (○) *from the circulation of the mouse after intravenous injection*
For details see the text.

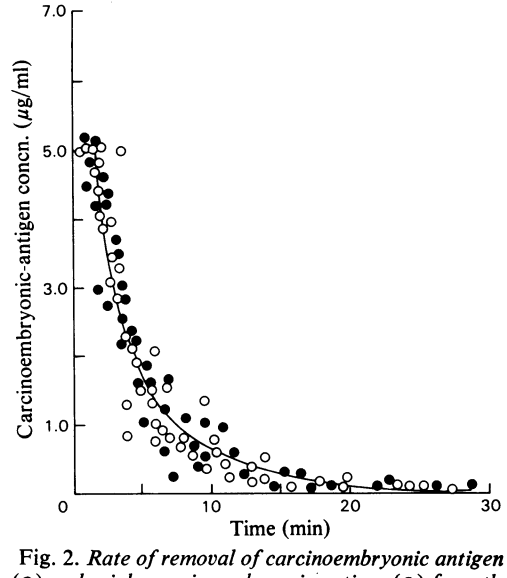

Fig. 2. *Rate of removal of carcinoembryonic antigen* (●) *and asialo-carcinoembryonic antigen* (○) *from the circulation of the mouse in the presence of* 0.25*mg of asialo-fetuin* (*ratio* 10:1 *by weight*)
For details see the text.

Table 1. *The effect of Triton WR1339 on the uptake of circulating native and asialo-carcinoembryonic antigen*
For experimental details see the text. Values and errors are calculated to the nearest 0.5 min.

Triton dose [0.25 ml of 10% (w/v) in saline]	Native carcinoembryonic-antigen half-life (min)		Asialo-carcinoembryonic-antigen half-life (min)	
Time after injection (h) ...	24	48	24	48
Single injection	5.0±0.5	6.5±1.0	2.0±0.5	2.0±0.5
Two injections at 24h intervals	7.0±1.0	11.0±1.5	2.0±0.5	2.5±0.5
Three injections at 24h intervals	12.5±2.0	12.5±3.0	2.0±0.5	2.5±1.0
No Triton (control values)	4.0±0.5		1.5±0.5	

excess of asialo-fetuin does not seem to influence greatly the removal of carcinoembryonic antigen or asialo-carcinoembryonic antigen by the Kupffer cells, the half-life of both asialo and native carcinoembryonic antigen in this case becoming identical. The mechanism of Kupffer-cell uptake is not certain, though the presence or absence of sialic acid does not seem to be important. However, it is possible that the initial Kupffer-cell uptake of native carcinoembryonic antigen is due to large aggregates of the glycoprotein being formed in the circulation, perhaps by binding to other plasma proteins. However, gel filtration on Bio-Gel A 1.5m showed no evidence of any change in molecular weight of either carcinoembryonic antigen or asialo-carcinoembryonic antigen after injection into the mice.

These experiments do not fully clarify the reason why native carcinoembryonic antigen should be specifically taken out of the circulation by liver Kupffer cells. However, it does appear that when terminal galactoses are exposed on carcinoembryonic antigen and binding to the galactose receptors occurs, then uptake by liver parenchymal cells takes precedence over Kupffer-cell uptake. The asialo-carcinoembryonic-antigen molecule will still retain its ability to be removed from the circulation by Kupffer cells as, in the presence of an excess of another asialo-glycoprotein, removal of this asialo-glycoprotein by Kupffer cells becomes the predominant pathway. The effect of both asialo-fetuin and of Triton WR1339 has also been examined with regard to the biliary excretion of these glycoproteins in the rat (P. Thomas & J. W. Summers, unpublished work). No obvious effect on the rate of excretion or quantity of glycoprotein excreted via the bile was observed. This would give further support to the suggestion that biliary excretion occurs via a different mechanism from direct Kupffer-cell or parenchymal-cell uptake (Thomas & Summers, 1978).

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