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COMMUNICATIONS

Transfer of oestrone glucuronide to the medium in an isolated rat liver perfusion system

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Oestrogens are excreted predominantly via the biliary route in the rat and undergo an extensive enterohepatic circulation (Sandberg, Kirdani & others, 1967). While investigating the mechanism of oestrone glucuronide transfer to bile in an isolated liver perfusion system, it became apparent that there was considerable transfer of the steroid in the direction of the perfusing medium. Recent studies have suggested that various organic compounds may be absorbed into the systemic circulation from the rat biliary tree (Clark, Hirom & others, 1971). The present study was undertaken to determine whether a similar absorptive process for the oestrogen could account for its observed transfer to the medium.

The perfusion system utilized has been previously described (Watanabe, 1977). The livers from female Wistar rats, 170–200 g, were placed in a humidified perfusion chamber maintained at 37° and perfused with an oxygenated medium consisting of Krebs-Ringer bicarbonate buffer, pH 7·4, containing 5% bovine serum albumin, 11 mM glucose, 11·5 mM sodium lactate and 20% heparinized whole rat blood. The pressure on the portal vein was maintained at 14 cm of water. Retrograde intrabiliary injections were made with a Hamilton syringe over a period of 15 s and the needle was removed from the bile duct cannula at 30 s. The recycling of the medium was interrupted at the start of the injection and the total effluent collected fractionally over 4 min. The flow rate of the perfusate was 14 ml min^{-1}. Bile collections were started immediately following removal of the syringe. A mixture of [6,7-^{3}H]oestrone glucuronide, 12·3 μCi mg^{-1}, at a concentration of 8 mg ml^{-1} saline and [U-^{14}C]erythritol, 18·9 μCi mg^{-1}, at a concentration of 2 mg ml^{-1} were administered retrogradely in volumes of 18, 35 or 70 μl. The lowest volume used was expected to be below that of the distended biliary tree capacity since the liver weights averaged 7·7 g (6–9·8 g). Fujimoto (1975) has estimated the distended capacity for a 10 g liver to be 37 μl.

Bile, medium and liver samples were combusted to ^3H_2O and $^{14}CO_2$ (Parkard Oxidizer, Model 305, Packard Instruments Co., Downers Grove, Ill.) which were collected in separate vials and assayed for radioactivity by liquid scintillation spectrometry. The counting effeciencies were constant at 28% for ^{3}H and 65% for ^{14}C.

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For an injection volume of 18 μl, the recovery of oestrone glucuronide in the bile over the 4 min collection period was similar to that for erythritol (Table 1). Peterson & Fujimoto (1973) recovered similar proportions of an erythritol dose with an approximately equivalent injection volume of 25 μl 10 g^{-1} liver. It is apparent that even where distended capacity is not exceeded, a significant proportion of erythritol was absorbed into the circulation. The amount of oestrone glucuronide collected in the medium over 4 min was, however, much lower. In Fig. 1, typical curves for the appearance of radioactivity in the medium are shown. Of particular interest were the ^{3}H to ^{14}C ratios found in the 0 to 0·5 min medium samples. The measured ratio, in terms of counts min^{-1}, for the injected material averaged 1·02 ± 0·02 (standard error). In the 18 μl group, the initial ratio (0 to 0·5 min sample) was much lower, averaging 0·37 ± 0·06 in 4 experiments. From an analysis of liver samples obtained at the end of the experiment (Table 1), it appears likely that the relatively lesser absorption of oestrone glucuronide into the circulation results, at least partly, from its greater retention by the liver. The radioactivity found in the livers must arise solely from biliary absorption since the perfusing medium was not recirculated through the liver in these experiments. This suggests that absorption from the biliary tree, at least for the steroid, proceeds through the hepatic cells. There is some evidence for hepatic protein binding of oestrone glucuronide (Ketterer, Tipping & others, 1976). The involvement of

Table 1. *Recoveries of [6,7-^{3}H]oestrone glucuronide and [U-^{14}C]erythritol following retrograde intrabiliary injection (RII).* Values are reported as % of dose administered ± standard error. The bile and medium values represent the quantities accumulated over a period of 4 min. Liver samples were obtained at the end of the 4 min. The figures in parentheses represent the number of experiments.

RII Volume	Bile ^{3}H	Bile ^{14}C	Medium ^{3}H	Medium ^{14}C	Liver ^{3}H	Liver ^{14}C
18 µl	80·5 ± 5·9(4)	80·6 ± 5·1(4)	2·9 ± 0·8(4)	12·4 ± 2·1(4)	2·5 ± 0·5(2)	0·2 ± 0·1(2)
35 µl	65·4 ± 4·3(5)	51·6 ± 6·2(5)	9·3 ± 2·6(5)	40·9 ± 6·0(5)	9·6 ± 2·2(3)	0·9 ± 0·4(3
70 µl	37·9 ± 4·7(5)	24·2 ± 4·3(5)	30·0 ± 5·8(5)	70·9 ± 5·9(5)	19·9 ± 1·7(	4·6

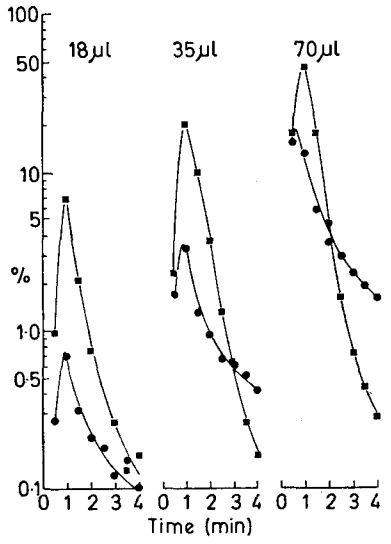

FIG. 1. Absorption into the medium of [6,7-^{3}H]oestrone glucuronide (●——●) and [U-^{14}C]erythritol (■——■) following retrograde intrabiliary injection. The figure above each curve represents the volume of injection. Ordinate = % of dose.

intracellular binding may explain the observed greater hepatic concentration of oestrone glucuronide relative to that of erythritol.

The shape of the descending portion of the curves in Fig. 1 differ for the two compounds. Whereas the post-peak concentration of erythritol appeared to decay in a mono-exponential manner, the decay curve for oestrone glucuronide was bi-exponential. The latter is suggestive of a two compartment pharmacokinetic model (Barr, 1968) indicating a modification due to distribution or distribution and binding. Since there is some evidence for hepatocellular binding, it appears possible that the terminal decay rate in the oestrone glucuronide curve is a consequence of slower release from such binding sites.

With the 35 μl dose, a volume which according to Fujimoto's (1975) estimate would slightly exceed the distended capacity, the initial medium ratio was 0·70 ± 0·17. With the largest injection volume, in spite of considerable amounts of oestrone glucuronide ultimately retained by the liver, the initial ^{3}H/^{14}C ratio of 1·05 ± 0·16 did not differ from that of the injected mixture. The unchanged ratio suggests a transient unrestricted movement of the injected material into the circulation. It is of interest that, whereas the bile volume during the 4 min following the 18 μl injection exceeded the pre-injection flow by 17·8 ± 1·34 μl, with the larger injections, the excess bile flow did not adequately account for the volumes introduced into the system. With the 35 μl injections the excess bile volume was 27·6 ± 0·99 μl or 79% of the administered volume. The corresponding figures for the 70 μl injections were 34·6 ± 3·8 μl or 49·5%. Although the initial ratio suggests transfer to the medium of oestrone glucuronide which is unrestricted by hepatocellular binding, that there is also an increased absorption into the hepatocytes with the larger volumes is apparent from the greater quantities of the steroid recovered in the liver. It is suggested that, where the injection volume exceeds the distended capacity, a transient general stretching of the membranes occurs which leads to, not only an increased absorption into the hepatocytes, but also transfer at other site(s) which are impermeable under non-distended conditions. The transient nature of the perturbations is indicated by the rapid decrease in the ^{3}H/^{14}C ratios. A shift of the oestrone glucuronide peak, in Fig. 1, to the left of that for erythritol, especially apparent in the 70 μl group, would appear to suggest a graded recovery such that the larger molecule, oestrone glucuronide (mol. wt = 446·5), is excluded earlier than erythritol (mol. wt = 122). This interpretation would be consistent with the finding of lower recoveries of erythritol in the bile than of the oestrogen with the larger volumes of injections (Table 1).

It can be concluded that, even where the distended capacity is not exceeded, there is a measurable absorption of both erythritol and oestrone glucuronide from the biliary tree. Recoveries in bile show that the extent of absorption is similar for both compounds. In the case of the oestrogen, however, hepatocellular binding appears to hinder its transfer to the circulation. That portion which is retained in the liver is presumably more susceptible to transfer back to the bile, thus limiting net absorption from the biliary tree.

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