J. steroid Biochem. Vol. 22, No. 6, pp. 781–788, 1985
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0022-4731/85 $3.00 + 0.00
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INTESTINAL ABSORPTION OF OESTRONE, OESTRONE GLUCURONIDE AND OESTRONE SULPHATE IN THE RAT *IN SITU*—I. IMPORTANCE OF HYDROLYTIC ENZYMES ON CONJUGATE ABSORPTION

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(*Received* 29 *March* 1984)

Summary—The biliary excretion of steroid after administration of [^{3}H]oestrone ([^{3}H]E_1), [^{3}H]oestrone glucuronide ([^{3}H]E_1G) and [^{3}H]oestrone sulphate ([^{3}H]E_1S) into the hepatic portal vein of anaesthetized rats was very rapid with more than 70% of E_1S and greater than 80% of E_1 and E_1G excreted in the first 30 min. There was a lag period in the biliary excretion of E_1S, this was less apparent with E_1 and absent with E_1G. Biliary excretion accurately reflects the amount of steroid in the portal circulation and was therefore used as an assessment of absorption from the gastrointestinal (GI) tract.

Absorption (as judged by excretion in bile) was least after administration of each steroid into the stomach. The extent of absorption correlated well with the lipophilicity of the steroids as shown by their relative partition coefficients between *n*-octanol and pH 6.5 phosphate-buffered saline ($E_1 \geqslant E_1S \geqslant E_1G$). There was no significant difference in excretion profile when the steroids were given into the caecum (at 5 h, E_1, 46.3 ± 9.1%; E_1G, 42.2 ± 14.5%; E_1S, 39.9 ± 7.1%). The similarity, despite marked differences in physicochemical properties, suggested conjugate hydrolysis to the parent steroid. In contrast, after administration into the small intestine, excretion of E_1 was very rapid and was maximal at 1 h (72.5 ± 8.0%); E_1G showed a near-linear excretion rate (1 h, 14.4 ± 3.0%; 5 h, 80.0 ± 11.7%), whereas in comparison E_1S excretion was low (1 h, 12.1 ± 2.4%; 5 h, 36.9 ± 2.7%). The involvement of hydrolytic enzymes in conjugate absorption was assessed. Ampicillin pretreatment (200 mg/kg/day for 2 days) reduced the absorption of E_1G from both the proximal and distal small intestine (by approximately 50%) but had no effect on the absorption of E_1S. There was, therefore, evidence that quantitative absorption of E_1G requires prior hydrolysis (by mammalian and/or microbial enzymes) but intact absorption of E_1S from this region of the tract was implicated. Ampicillin pretreatment reduced the absorption of both conjugates (greater with E_1S) from the caecum; hydrolysis clearly precedes absorption from the caecum.

The above findings were supported by an *in vitro* study which showed that ampicillin pretreatment abolished the hydrolysis of E_1S by caecal contents but only partially reduced the hydrolysis of E_1G. The presence of mammalian glucuronidase enzyme may account for this difference.

INTRODUCTION

Studies on the absorption and enterohepatic circulation (EHC) of steroid sulphates in both animals and man have indicated that in addition to the hydrolysis of the conjugates by the gut microflora [1–3], intact absorption may also be important [4–6].

In a previous study in germfree rats [7], we found unequivocal evidence of intact absorption of oestrone sulphate (E_1S) from the small intestine of the rat. This was in agreement with the work of Schwenk *et al.* [6] who studied the absorption of E_1S (^{3}H and ^{35}S) from rat intestinal loops with intact arterial blood supply and found that 86% of the steroid appearing in portal blood was E_1S.

There is less information available on the extent to which intact absorption of glucuronides has a role in EHC. Bock and Winne [8] using closed jejunal loops with venous collection in rats, demonstrated that naphthol glucuronide was transported from the intestinal lumen into the blood apparently without hydrolysis by the gut flora. They suggested that the absorption of unchanged glucuronide may contribute to a considerable extent to the EHC of drugs in addition to the hydrolysis by the gut flora and subsequent absorption of the aglycone. Lisboa *et al.* [9] have also suggested that oestrone glucuronide (E_1G) is actively transported from the mucosal to the serosal side of rat everted gut sacs.

The present work was designed to directly compare the absorption of E_1, E_1S and E_1G at various levels of the gastrointestinal tract and the involvement of hydrolytic enzymes in the absorption of the conjugates.

EXPERIMENTAL

Chemicals

All chemicals and reagents were obtained from Sigma Chemical Co., London, BDH Chemicals Ltd, Poole, or Koch-Light Laboratories Ltd, Colnbrook, unless otherwise specified. They were of AnalaR or AristaR grade, or the equivalent.

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Radioactive materials

[6,7-^{3}H(*N*)]Oestrone ([^{3}H]E_1; in 9:1, benzene–ethanol, 50.0 Ci/mmol) and [6,7-^{3}H(*N*)]oestrone 3-sulphate ([^{3}H]E_1S; ammonium salt, in ethanol, 59.0 Ci/mmol) were obtained from New England Nuclear (NEN), Dreieichenhain, West Germany. [6,9-^{3}H(*N*)]Oestrone 3-β-D-glucuronide ([^{3}H]E_1G; in 2% ethanol, 2.4 Ci/mmol) was supplied by Amersham International plc, Amersham.

The purity of the radioactive materials was checked by thin-layer chromatography (TLC) in the appropriate solvent systems: (a) [^{3}H]E_1 was chromatographed in dichloromethane–ethylacetate, 3:2, v/v (silica gel, Merck, W. Germany), (b) [^{3}H]E_1G in ethanol–glacial acetic acid–water, 20:1:4, by vol (cellulose, Merck), and (c) [^{3}H]E_1S in methanol or ethyl acetate–methanol–ammonium hydroxide, 75:25:2, by vol (silica gel, Merck). They were found to be at least 97% pure.

Animals

Mature female rats (170–250 g) of the Wistar strain were housed in groups of about 10 in cages in well-ventilated rooms at a temperature of approx 24°C. The animals were allowed free access to food and water until the evening prior to the study when food, but not water, was withdrawn. During the time of fasting, they were kept in cages with a wide mesh wire bottom to prevent coprophagy as far as possible.

In some experiments, the animals were dosed with the antibiotic, ampicillin sodium (Penbritin®, Beecham Research Laboratories, Brentford) solution by oral intubation. A total daily dose of 200 mg/kg b.wt was given for 2 days just prior to the experimental study. The last dose was usually given about 16 h before the experiment. The control animals received water by the same dosing regimen.

Experimental procedures

Study 1—portal vein study. Rats were anaesthetized with urethane (14%, w/v, in 0.9% NaCl; 1 ml/100 g b.wt, i.p.). A midline abdominal incision was made to expose the viscera and the bile duct was cannulated with a piece of polyethylene tubing (PE25, Portex Ltd, Hythe). The dosing solution, containing [^{3}H]E_1, (10 μCi/ml, 3.7 μM in ethanol–saline, 1:9, v/v), [^{3}H]E_1G (9 μCi/ml, 3.7 μM in saline) or [^{3}H]E_1S (10 μCi/ml, 3.7 μM in saline), 0.1 ml/100 g.b.wt, was administered over a period of about 25 s into the portal vein via a catheter (PE25 tubing) tipped with a broken 25G needle. The preparation was kept moist and warm with a cotton swab soaked with warm saline and a lamp.

Bile was collected into preweighed vials at 5 min intervals for 30 min and thereafter at 10 min intervals to 240 min (4 h). The vials containing the bile samples were weighed again in order to estimate the volume of bile excreted in each time period. The total radioactive content of each bile sample was measured.

Study 2—in situ *closed loop study.* Rats were anaesthetized with urethane, the abdomen was opened and the bile duct cannulated (PE25 or PE50 tubing). The appropriate parts of the GI tract were isolated by means of ligatures placed at the following places: (a) *stomach*—the pyloroduodenal junction; (b) *small intestine*—the pyloroduodenal and the ileocaecal junctions; and (c) *caecum*—the ileocaecal and the caecocolonic junctions. In some experiments, the small intestine was further subdivided into proximal and distal segments. The division between the proximal and distal small intestine (PSI and DSI, respectively) was arbitrarily set at approx 30 cm distal to the ligament of Treitz (length, mean $\pm$ SD, 33.6 $\pm$ 5.8 cm, $n = 25$, PSI; 48.0 $\pm$ 8.7 cm, $n = 28$, DSI). The ligatures were placed underneath the mesenteric blood vessels so as not to compromise any blood flow to and from the isolated loop.

[^{3}H]E_1, [^{3}H]E_1G or [^{3}H]E_1S (concentrations as above) in either phosphate-buffered saline (PBS; pH 6.5) or 1:9 (v/v) ethanol–PBS was instilled into the isolated loop through a 26G needle over a period of about 20 s. The syringe and the needle stayed in position throughout the experiment. The preparation was kept moist and warm all the time. Bile samples were collected at 15 and 30 min, and thereafter at half-hourly intervals to 5 h. Duplicate aliquots (50–100 μl) of each bile sample were removed and the radioactive content determined.

In some experiments where the effect of ampicillin pretreatment on the absorption of the steroids was studied, the two conjugates of oestrone (in PBS alone) were instilled into the isolated PSI, DSI or caecum of untreated and ampicillin-pretreated animals. The collection of bile and the subsequent treatment of the bile samples were as described above.

Study 3—in vitro *incubation study.* Rats were killed by a blow on the head. The whole caecal contents of the untreated and ampicillin-pretreated rats were rapidly emptied into the sterile McConkey bottles (25 ml capacity), each containing 10 ml autoclaved thioglycolate broth (0.60 g bacto-yeast extract, 0.60 g peptone, 0.60 g D-glucose, 0.05 g DL-cysteine hydrochloride and 0.1 mg resazurin dye in 100 ml 0.1 M phosphate buffer pH 6.5). After thorough mixing of the inoculated broth, aliquots of this caecal suspension (0.5 ml) were then dispensed into sterile Bijoux bottles (5 ml capacity), which contained 1.5 ml sterile thioglycolate broth. Control samples containing only 2.0 ml sterile broth, but *no* caecal contents, were also included. At zero-time, 0.5 ml [^{3}H]E_1, [^{3}H]E_1G or [^{3}H]E_1S (0.2 μCi/ml; 3.7 μM) was added to the appropriate bottles (including the controls) and stoppered. All the control and the inoculated samples were incubated with the steroids at 37°C in a water bath (without shaking). The thioglycolate broth used in this study maintained an anaerobic environment inside the bottle throughout the incubation period, and this was monitored by any change in the colour of the indicator present in the broth.

At the end of $\frac{1}{2}$, 2, 4, 6 and 24 h, the appropriate bottles were removed. Duplicate 0.4 ml aliquots of each incubate were vortexed in stoppered tubes with 3 ml diethyl ether for 1 min and then centrifuged (2 min at 1,000 *g*) to separate the ether and the aqueous phases. The aqueous layer was frozen by immersion in a methanol–dry ice (CO_2) mixture and the ether extract decanted into a clean scintillation vial insert. The ether fraction was evaporated to dryness under vacuum and the aqueous fraction (frozen) allowed to thaw at room temperature. The radioactive content of the dried ether fraction and of duplicate 100 μl aliquots of the aqueous fraction were determined.

Estimation of radioactivity

The radioactivity in the dosing solutions, the bile samples and the incubates was estimated by liquid scintillation spectrometry using 4 ml of a commercially prepared micellar scintillant (NE260, Nuclear Enterprise, Edinburgh, or 299TM, Packard, Reading).

Statistical analysis

Unless otherwise specified, the data are expressed as the mean $\pm$ SD of n experiments. The statistical significance of the difference between two means was calculated using Student's t-test for unpaired samples.

RESULTS

Study 1—Biliary excretion of steroid after administration of oestrone and its conjugates into the hepatic portal vein

The cumulative biliary excretion of steroid after administration of [^{3}H]E_1, [^{3}H]E_1G or [^{3}H]E_1S into the hepatic portal vein is shown in Fig. 1(a, b and c). The biliary excretion of the oestrogens was very rapid; more than 70% of E_1S and more than 80% of E_1 and E_1G was excreted in the first 30 min. The cumulative percentage excretion of E_1 and the conjugates in the bile reached near-maximal values by 1 h (E_1, 85.7 $\pm$ 4.0%; E_1G, 92.1 $\pm$ 4.7%: E_1S, 85.0 $\pm$ 3.3%) and very little increase occurred thereafter (at 4 h, E_1, 88.1 $\pm$ 3.6%; E_1G, 95.6 $\pm$ 2.8%; E_1S, 91.0 $\pm$ 2.7%).

The insets show clearly that the maximal rate of biliary excretion occurred at a different time for each steroid studied: E_1G (fastest), within 5 min; E_1, between 5 and 10 min; and E_1S (slowest), between 10 and 15 min. There was also an apparent lag period in the biliary excretion of E_1S; this was less noticeable with E_1 and apparently absent with E_1G.

Study 2—Biliary excretion of steroid after administration of oestrone and its conjugates at various levels of the GI tract: Effect of ampicillin pretreatment

Absorption at various levels of GI tract. Figure 2(a, b and c) shows the biliary excretion of steroid after administration of [^{3}H]E_1, [^{3}H]E_1G or [^{3}H]E_1S into the stomach, the small intestine and the caecum. The results obtained after administration of the three steroids into the PSI and the DSI are given in Fig. 3(a, b and c).

The biliary recovery of steroid after administration into the stomach was clearly the lowest for each steroid studied (at 5 h, E_1, 22.4 $\pm$ 5.6%; E_1G, 6.0 $\pm$ 4.0%; E_1S, 10.7 $\pm$ 4.0%) when compared with the corresponding biliary recovery after administration into the small intestine (at 5 h, E_1, 77.4 $\pm$ 6.7%; E_1G, 80.0 $\pm$ 11.7%; E_1S, 36.9 $\pm$ 2.7%) or the caecum (at 5 h, E_1, 46.3 $\pm$ 9.1%; E_1G, 42.2 $\pm$ 14.5%; E_1S, 39.9 $\pm$ 7.1%).

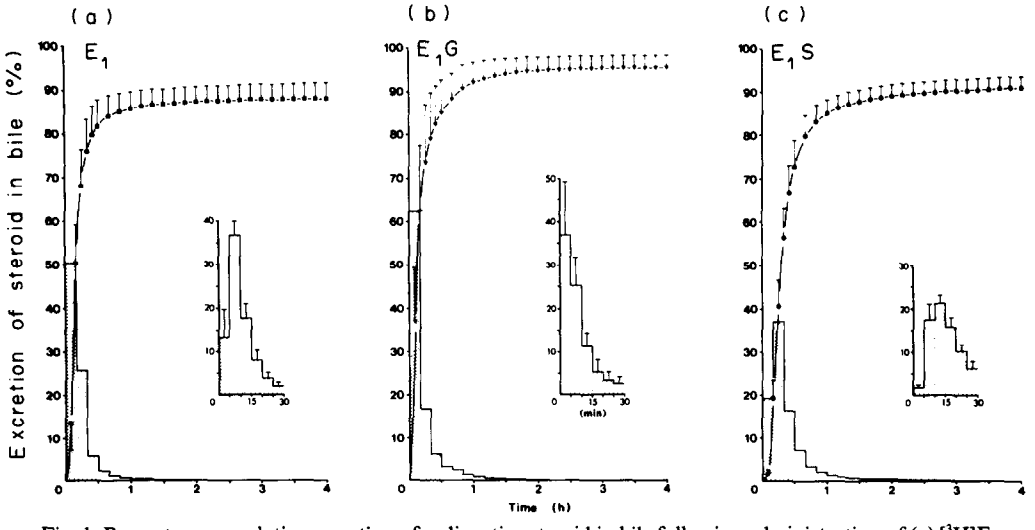

Fig. 1. Percentage cumulative excretion of radioactive steroid in bile following administration of (a) [^{3}H]E_1 (■——■), (b) [^{3}H]E_1G (▼——▼) and (c) [^{3}H]E_1S (●——●) into the hepatic portal vein of the rat. Percentage excretion of radioactive steroid in each time period (5 or 10 min) is represented by the histograms. The insets are excerpts from the histograms and show the percentage of steroid excreted in each 5 min period over the first 30 min. Each point is the mean $\pm$ SD of 4 experiments.

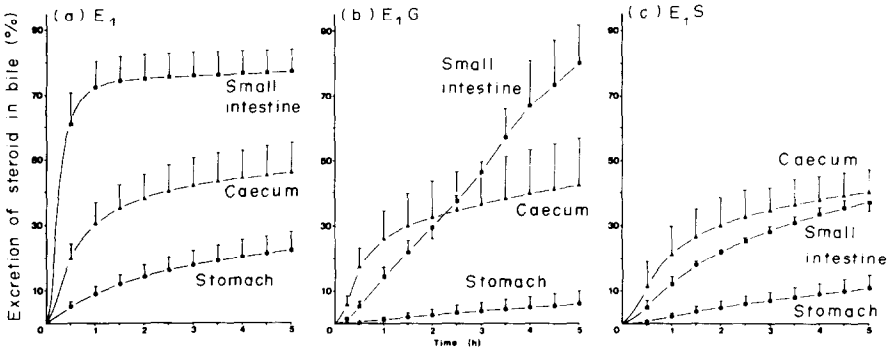

Fig. 2. Percentage cumulative excretion of radioactive steroid in bile following administration of (a) [^{3}H]E_1, (b) [^{3}H]E_1G and (c) [^{3}H]E_1S into the stomach (●——●), small intestine (■——■) and caecum (▲——▲) of the rat. Each point is the mean ± SD of 3–5 experiments.

There was no significant differences in the biliary excretion profile when E_1 and the conjugates were given into the caecum. Excretion was initially relatively rapid (at 1 h, E_1, 30.6 ± 6.2%; E_1G, 26.2 ± 8.3%; E_1S, 21.4 ± 8.6%), and this quickly declined to a low near-constant rate of about 2% per h.

In contrast, after administration into the small intestine, excretion of the absorbed E_1 was very rapid and was maximal at 1 h (72.5 ± 8.0%); E_1G showed a near-linear excretion rate of about 15% per h (1 h, 14.4 ± 3.0%; 3 h, 46.4 ± 3.2%; 5 h, 80.0 ± 11.7%) whereas in comparison, excretion of the absorbed E_1S was low (1 h, 12.1 ± 2.4%; 3 h, 28.1 ± 1.5%; 5 h, 36.9 ± 2.7%). When the small intestine was further subdivided into PSI and DSI to study the absorption of E_1 and the conjugates from these regions, excretion of the E_1 absorbed from the DSI was found to be significantly slower ($P \leqslant 0.005$) than that absorbed from the PSI during the first 30 min but thereafter, there was no significant difference (Fig. 3a). The mean excretion of E_1G absorbed from the DSI although higher than that absorbed from the PSI at all time points was not significantly different (Fig. 3b). In contrast, the biliary excretion profiles for E_1S absorbed from the PSI and the DSI were significantly different at all time points except 0.5 h (Fig. 3c). Unlike the hyperbolic shape obtained for the excretion of E_1S after administration into the PSI (as is the case with the whole small intestine, see Fig. 2c), the biliary excretion of E_1S was at a near-constant rate of about 5% per h following administration into the DSI (1 h, 5.4 ± 1.5%; 3 h, 15.7 ± 4.6%; 5 h, 26.2 ± 7.4%).

Ampicillin pretreatment. The effect of ampicillin pretreatment on the biliary excretion of steroid after administration of [^{3}H]E_1G or [^{3}H]E_1S into the PSI and the DSI is shown in Fig. 4(a and b) and Fig. 5(a and b) respectively, and that after administration of [^{3}H]E_1, [^{3}H]E_1G or [^{3}H]E_1S into the caecum is shown in Fig. 6(a, b and c). Ampicillin pretreatment reduced the amount of E_1G recovered in bile following administration into the PSI, the DSI or the caecum (by ~50% in the three regions). However, pretreatment with ampicillin only caused a reduction in the biliary recovery of E_1S absorbed from the caecum (by ~85%, see Fig. 6c), and not from the PSI or the DSI. The biliary recovery of E_1 after administration in the caecum was not significantly different

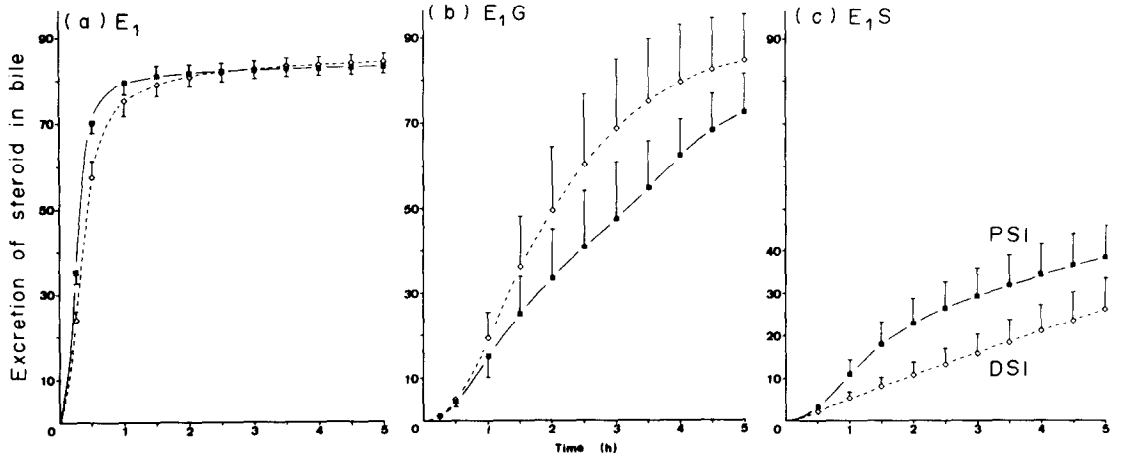

Fig. 3. Percentage cumulative excretion of radioactive steroid in the bile following administration of (a) [^{3}H]E_1, (b) [^{3}H]E_1G and (c) [^{3}H]E_1S into the proximal small intestine (PSI, ■——■) and distal small intestine (DSI, ◇——◇) of the rat. Each point is the mean ± SD of 3–7 experiments.

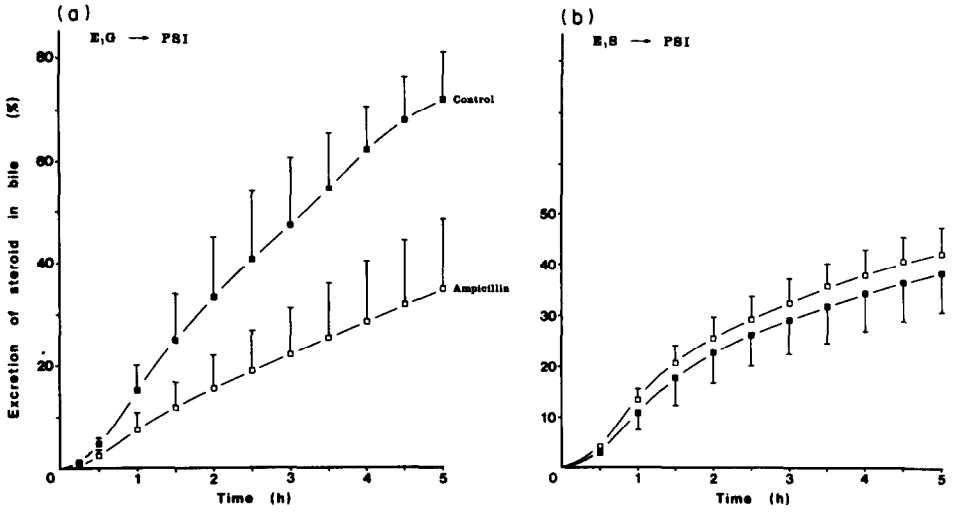

Fig. 4. Percentage cumulative excretion of radioactive steroid in bile following administration of (a) [^{3}H]E_1G or (b) [^{3}H]E_1S into the PSI of control (■——■) or ampicillin-pretreated (□——□) rats. Each point is the mean ± SD of 4 or 5 experiments.

in the ampicillin pretreatment group, although there was a trend towards an increase (Fig. 6a). Bile flow rates between the control and the ampicillin-pretreated animals were not significantly different.

Study 3—In vitro *hydrolysis of the conjugates by the caecal/GI contents: Effect of ampicillin pretreatment*

The extent of hydrolysis of the conjugates by the caecal contents of control and ampicillin-pretreated rats, as indicated by an increase in the ether-extractable fraction of radioactive steroid, is shown in Fig. 7(a and b). The percentage of E_1, E_1G and E_1S in the control samples, i.e. *not* inoculated with caecal contents, which was ether-extractable after 6 or 24 h incubation was 95.9 ± 2.3%, 1.4 ± 0.3% and 0.8 ± 0.1% respectively ($n = 7$ for each). Incubation of E_1 with the inoculated samples did not significantly alter the efficiency of ether extraction at any time point; this was true for the caecal contents of control rats (96.4 ± 1.9%; $n = 20$) and also of ampicillin-pretreated rats (96.7 ± 1.1%; $n = 13$). The hydrolysis of E_1G by the caecal contents of control rats was already maximal (97.0 ± 0.9%) after ½ h incubation at 37°C and was maintained for 24 h (Fig. 7a). Ampicillin pretreatment markedly reduced, but did not abolish, the hydrolytic activity of the caecal contents towards E_1G. Hydrolysis of E_1G by the caecal contents of ampicillin-pretreated rats at ½ h incubation was only 23% of the control value, but this gradually increased with incubation time until it reached a near-maximal value (89.3 ± 1.6%) by the end of the 24 h incubation. In contrast, the hydrolysis of the sulphate conjugate by the control caecal contents did not reach the steady state or maximal value

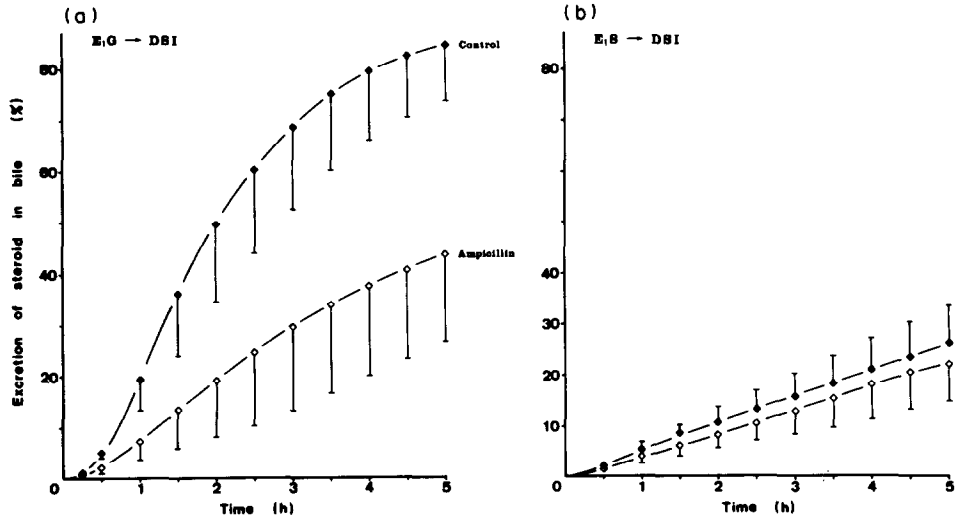

Fig. 5. Percentage cumulative excretion of radioactive steroid in bile following administration of (a) [^{3}H]E_1G or (b) [^{3}H]E_1S into the DSI of control (◆——◆) or ampicillin-pretreated (◇——◇) rats. Each point is the mean ± SD of 4–6 experiments.

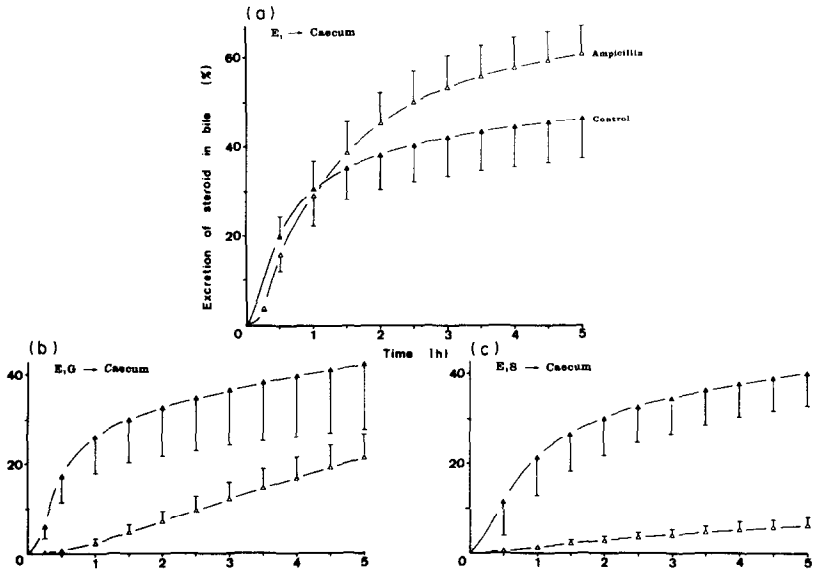

Fig. 6. Percentage cumulative excretion of radioactive steroid in bile following administration of (a) [^{3}H]E_1, (b) [^{3}H]E_1G or (c) [^{3}H]E_1S into the caecum of control (▲——▲) or ampicillin-pretreated (△——△) rats. Each point is the mean ± SD of 3 or 4 experiments.

(85.8 ± 13.7%) until after 6 h incubation (Fig. 7b). Ampicillin pretreatment abolished the caecal oestrone sulphatase activity completely (less than 2% in 24 h).

DISCUSSION

Biliary excretion has been used by many workers as an indirect measure of absorption [10–13]. This method is based on the assumption that the recovery of the drug (biotransformed or not) in the bile quantitatively reflects the amount present in the hepatic portal vein system. In the text we have used the term biliary recovery of E_1, E_1G or E_1S to indicate excretion of radioactivity in bile following administration of the individual steroids; no metabolite identification studies were performed.

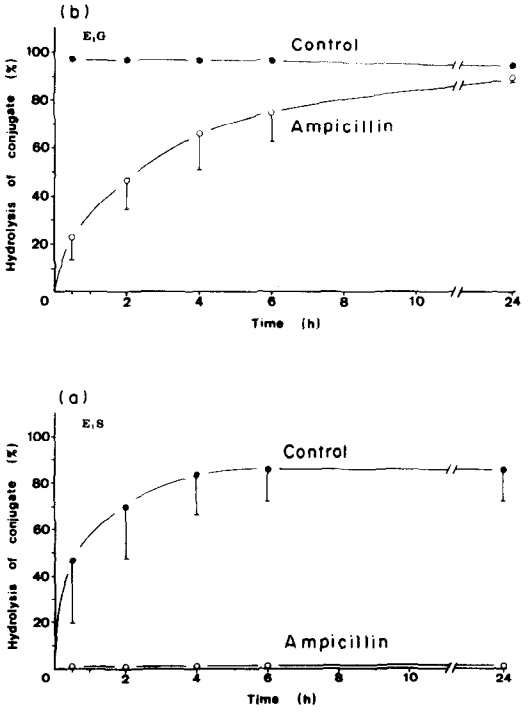

Fig. 7. Time course for the *in vitro* hydrolysis of [^{3}H]E_1G and [^{3}H]E_1S by the caecal contents of control (●——●) and ampicillin-pretreated (○——○) rats. Each point is the mean ± SD of 5 (control) or 3 ampicillin experiments.

The results of study 1 have clearly confirmed the usefulness of biliary recovery as an indirect measurement of absorption of E_1 and the two conjugates. Between 70 and 85% of the steroid administered into the portal circulation was recovered in the bile in $\frac{1}{2}$ h and almost complete biliary recovery was obtained after 1 h. It is interesting to note the differences in the time-to-peak in biliary excretion of the three steroids studied ($E_1G \leqslant E \leqslant E_1S$). This finding is in agreement with the presupposition that the glucuronide conjugate can be excreted in bile unchanged and therefore no delay occurred as would otherwise be caused by metabolism. It also adds further evidence to the findings of other workers [13–15] that E_1 and E_1S are further metabolized in the liver before excretion in the bile, mainly as the glucuronide conjugate.

Absorption of the three oestrogens was least from the stomach. There was a positive correlation between the fraction of dose absorbed and the partition coefficient of the steroid (determined as a partitioning between *n*-octanol and pH 6.5 phosphate buffer; $E_1 \geqslant E_1S \geqslant E_1G$). Since the latter is an estimate of lipophilicity it tended to suggest that the absorption of these three compounds from the stomach was by a non-ionic diffusion (passive) mechanism. However, since the pH of the stomach in the rat is generally between 4 and 5 [16] and the oestrogens were administered in pH 6.5 buffer, it is unlikely that non-specific acid hydrolysis of the two conjugates could have

occurred to any major extent in the stomach. Hydrolysis of the conjugates by the specific enzyme(s) produced by the small number of microorganisms which may inhabit the stomach (temporarily or otherwise) cannot be totally ruled out, although arylsulphatase-producing bacteria are known to be strict anaerobes [17] and therefore normally populate the lower GI tract.

There were quite distinct differences in the absorption of the three oestrogens from the small intestine (also PSI and DSI). As with the stomach, E_1, was absorbed from the small intestine (also PSI and DSI) at a rate much faster than the corresponding conjugates, E_1G and E_1S. But unlike in the stomach, the relative "rate" and extent of absorption of the three oestrogens from the small intestine (Fig. 2), including the PSI and DSI (Fig. 3), do not mirror their relative lipophilicity in that E_1S, which is more lipophilic than E_1G, was much less well absorbed than E_1G. This agrees with the results of Schwenk *et al.* [6], who have previously reported an approx 4-fold increment in the intestinal absorption of E_1 over E_1S. Similar rate differences between free and sulphated androgens [18], and free and sulphated EE_2 [19] have also been reported. Possible causes for this unexpectedly faster and more extensive absorption of E_1G, when compared with E_1S, are the presence of β-glucuronidase, but not sulphatase, or the presence of much more β-glucuronidase than sulphatase in the small intestinal contents. The subsequent release of the more lipophilic aglycone on hydrolysis would enhance the absorption of the conjugate.

In order to assess the contribution of hydrolysis to the overall absorption of conjugates, animals pretreated with ampicillin were included in the study since gut microflora is the major source of hydrolytic enzymes in the GI tract. The importance of intestinal microbial β-glucuronidase in the absorption of E_1G from the PSI, the DSI and the caecum of the rat was demonstrated by a significant reduction ($P \leqslant 0.005$, $\leqslant 0.01$ and $\leqslant 0.05$, respectively), in the absorption of the glucuronide conjugate after ampicillin pretreatment. It is uncertain from this study, however, whether the remaining fraction of E_1G absorbed after ampicillin pretreatment was due to intact absorption of the conjugate, to mammalian β-glucuronidase activity, to the residual β-glucuronidase activity released from the dead microbes or a combination of the above factors. A survey of the literature reveals very few reports on the intact absorption of glucuronide conjugates [8, 20–22]. The presence of mammalian β-glucuronidase in the intestinal contents, although in a much smaller quantity, is well established [23, 24]; the optimum pH for the mammalian enzyme ($\sim$4.5) being lower than that for the microbial enzyme ($\sim$6.5).

In contrast to E_1G, E_1S absorption from the small intestine (PSI, DSI) was not affected by ampicillin pretreatment. The effectiveness of ampicillin in suppressing anaerobic as well as aerobic bacteria has previously been demonstrated [25]. Furthermore, there has been no report of mammalian sulphatase being sequestered into the lumen of the GI tract. Therefore, the results obtained in this study suggested that E_1S was absorbed intact from the small intestine (PSI and DSI). In contrast, the near-complete elimination of E_1S absorption from the caecum of ampicillin-pretreated rat indicated that hydrolysis of the sulphatase conjugate normally precedes its absorption. This is in agreement with previous work [7, 13].

Ampicillin pretreatment did not significantly alter the absorption of E_1 from the caecum. This indicated that the physiological changes which usually accompany antibiotic pretreatment (noticeably an enlargement of the caecum and an increase in the fluidity of the caecal contents) did not in themselves significantly alter the absorption parameters of E_1 from the caecum. This may not be true for other more polar oestrogens.

The absorption of both E_1 and E_1S from the DSI was slower than from the PSI, particularly during the initial experimental period. The absorption of a compound as lipophilic as E_1 is most likely to occur by passive diffusion across the epithelial cells into the portal circulation. The reduction in the epithelial surface area and/or the reduction in GI motility and/or the reduction of blood supply to the DSI region, when compared with the PSI, may account for the delay observed. Interaction with compounds (e.g. bile salts or mucin) which may be present to different extents in these two regions of the small intestine may also be responsible for the reduced absorption of E_1 from the DSI. Furthermore, there may be a change in the intestinal metabolic activity towards E_1 from the duodenal down to the ileal region. Similar reasons may explain the slight delay observed with E_1S from the DSI.

The results obtained for the effects of ampicillin pretreatment on the *in situ* absorption of the two conjugates from the caecum were supported by the *in vitro* study which showed that the pretreatment abolished the hydrolysis of E_1S by the caecal contents but only partially reduced the hydrolysis of E_1G. As explained for the *in situ* study, the presence of mammalian β-glucuronidase enzymes may account for this difference on the effect of ampicillin on the hydrolysis of the two conjugates.

Acknowledgements—S.M.S. was supported by the Mersey Regional Health Authority and was in receipt of an ORS award.

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