Biliary Excretion of Mestranol in the Female Rat: Effect of Neomycin Pretreatment on Enterohepatic Recirculation

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Mestranol (17α-ethynylestradiol 3-methyl ether) is widely used in contraceptive formulations of the combination type. The metabolism and route of excretion of this compound has been well studied (Bolt & Remmer, 1972a,b; Fotherby, 1974). The effectiveness of mestranol as an orally active oestrogen is dependent on its o-demethylation by the microsomal mixed-function oxidase system of the liver to ethynylestradiol and the subsequent availability of this latter compound (Bolt & Remmer, 1973). Ethynylestradiol undergoes extensive enterohepatic recirculation after the hydrolysis of biliary conjugates in the gut, which accounts for the relatively long half-lives of such synthetic steroids in animals (Smith, 1974). Clearly, factors which affect the hydrolysis and reabsorption of the biliary conjugates of mestranol and its metabolites may be important in determining the effectiveness of these compounds as oestrogens; this aspect seems to have received little attention. The gut microfloral population is a major source of hydrolytic enzymes in the intestine (Scheline, 1973; Drasar & Hill, 1974) and may play an important role in the recirculation of mestranol and its metabolites.

Fig. 1. Biliary excretion of radioactivity after administration of [4-14C]mestranol (0.25 μCi) to female rats

Results are expressed as a percentage of the administered dose. ○, Intraduodenal administration; □, intraperitoneal administration; points represent means±S.E.M. of three (intraperitoneal route) or four animals (intraduodenal route). Mean bile flow rates, ±S.E.M.: intraduodenal administration, 738±62μl/h; intraperitoneal administration, 593±185μl/h.
Results are expressed as a percentage of the administered dose. ■, Control rats; ○, neomycin-pretreated rats; points represent means ± S.E.M. of three control and four neomycin-pretreated animals. Mean bile flow rates ± S.E.M.: 672 ± 54 μl/h (controls); 553 ± 44 μl/h (neomycin treated); ○, one animal which received 250 mg of saccharolactone in 0.5 ml of 0.9% NaCl immediately before the administration of labelled bile (bile flow in this animal was 684 μl/h).

The present work was designed to (i) establish the rate of biliary excretion of [4-14C] mestranol after intraperitoneal and intraduodenal administration and (ii) study biliary excretion after intraduodenal administration of labelled mestranol conjugates in neomycin-treated rats.

[4-14C]Mestranol (specific radioactivity 59.8 mCi/mmol) was obtained from New England Nuclear Chemicals (Dreieichenhain, W. Germany). Female Wistar albino rats (220–240 g) were used in all experiments. Anaesthesia was achieved with Nembutal. After surgery, cannulae were inserted into the bile duct of all animals and also into the duodenum of animals receiving intraduodenal administration of mestranol or its metabolic conjugates. All cannulae were exteriorized, and after suturing, bile collection commenced. Bile was collected for 6–8 h as half-hourly samples after dosing the animals with [4-14C]mestranol (0.25 μCi, 5.3 μg/kg) in 1 ml of 0.9% NaCl.

Samples (50 μl) of all bile samples were dissolved in 0.5 ml of water and 4 ml of a Synperonic NXP/toluene/2,5-diphenyloxazole scintillant by the method of Wood et al. (1975). Radioactivity was determined in an LKB 1210 liquid-scintillation counter and the excretion of radioactivity in the bile calculated as a percentage of the dose given.

The 30–90 min bile samples collected from animals given labelled mestranol by intraperitoneal injection were used in the experiments to determine the biliary excretion of radioactivity after intraduodenal administration to neomycin-treated and control
rats. Ether extraction of such samples at pH 5.0 showed them to contain at least 98% of the radioactivity in a conjugated form. Bile containing the labelled conjugates was diluted with 0.9% NaCl such that ~1000 d.p.s. was administered to each animal. Biliary excretion of radioactivity was again expressed as a percentage of this dose. Experimental rats received neomycin sulphate orally (100mg in 0.2ml of water) twice daily for 4 days and once on the fifth day 1–2 h before the experiment.

Fig. 1 shows the observed rates of biliary excretion of mestranol after intraperitoneal and intraduodenal administration. Biliary excretion is rapid in both cases, but far more so for intraduodenal administration. Reference to the mean bile-flow rates given in Fig. 1 shows that after 3 h 35–39% of the intraperitoneal and 72–75% of an intraduodenal mestranol dose had been excreted in the bile. The results suggest that an active transport mechanism may be involved in the absorption of mestranol across the wall of the intestine.

The biliary excretion of radioactivity after intraduodenal administration of metabolic conjugates of mestranol into normal and neomycin-treated rats is shown in Fig. 2. In addition the rate of excretion after administration of conjugated bile plus the β-glucuronidase inhibitor saccharolactone is shown. After 4 h a significant decrease in the rate of excretion of radioactivity was observed in both cases compared with controls. This lower excretion rate can be interpreted as reflecting a decrease in the rate of deconjugation, since the absorption of the free steroids and their subsequent biliary excretion is rapid (see curve for intraduodenal administration, Fig. 1).

It seems on this evidence that the gut microflora are responsible (in part) for the hydrolysis of conjugated mestranol and its metabolites.

The fact that recirculation is not totally prevented in neomycin-treated animals is not surprising, since neomycin does not suppress all groups of gastro-intestinal microorganisms (e.g. Bacteriodes; Finegold et al., 1965) and there is likely to be a contribution from hydrolytic enzymes of tissue origin. Preliminary incubations in vitro have shown that rat caecal micro-organisms are capable of extensive deconjugation of biliary conjugates of mestranol.


Aromatization of Shikimic Acid in the Rat and the Role of Gastrointestinal Micro-Organisms

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Shikimic acid [(-)-3α,4α,5β-trihydroxycyclohexene-1-carboxylic acid] is a compound of widespread occurrence in plants and certain fruits (Bohm, 1965). After investigations into the toxic principle(s) of the bracken fern (Pteridium aquilinum), shikimic acid has been reported as being carcinogenic in mice (Evans & Osman, 1974). The present report describes an introductory examination into the fate of this compound in the rat, together with details of the metabolism of the compound by rat caecal micro-organisms.