Steroid Metabolism in the Cat

BILIARY AND URINARY EXCRETION OF METABOLITES OF [4-¹⁴C]OESTRADIOL

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1. [4-¹⁴C]Oestradiol was administered to seven male, seven female and two castrated male cats as a single intravenous injection. Bile and urine were collected for 6 h. 2. The radioactivity was excreted mainly in the bile of all animals (53–60%); only approx. 1% of the dose appeared in the urine. 3. Bile and urine samples were hydrolysed successively by β -glucuronidase, cold acid and hot acid. There were significant differences ($P < 0.005$) between the percentage of the dose present in the bile fractions hydrolysed by β -glucuronidase (male, $9.0 \pm 1.7\%$; female, $18.6 \pm 1.45\%$) and by cold acid (male, $18.9 \pm 1.44\%$; female $12.1 \pm 1.02\%$). The excretion of radioactivity in these fractions by the castrated male cats was closer to that of female cats. 4. Approx. 20–27% of the dose could not be extracted from aqueous solution (pH 10.5) by ethyl acetate–ether after hydrolysis.

Metabolites of the neutral steroids progesterone, testosterone, corticosterone and cortisone are excreted predominantly in the urine of man and other animals (Taylor, 1965). However, when these steroids are administered intravenously to anaesthetized cats metabolites of the hormones are excreted almost completely in the bile; only about 1% of the dose appears as metabolites in the urine (Taylor, 1969; Taylor & Scratcherd, 1961, 1963; Archer, Scratcherd & Taylor, 1965). Intact cats excrete neutral steroid hormone metabolites almost exclusively in the faeces (Taylor & Scratcherd, 1962).

By contrast, metabolites of the phenolic steroids (oestradiol, oestrone and oestriol) are excreted to a large extent in the bile of man, rodents and other animals (Sandberg & Slaunwhite, 1957, 1965; Adlercreutz, 1962; Pearson & Martin, 1966; Adlercreutz & Luukkainen, 1967).

The excretion of oestradiol by male, female and castrated male cats has now been investigated.

MATERIALS AND METHODS

[4-¹⁴C]Oestradiol (51.4 mCi/mmol), from The Radiochemical Centre, Amersham, Bucks., U. K., was found by paper chromatography and t.l.c. to be at least 98% pure and was used without further purification. The steroid was stored in ethanol at -15°C ; after 9 weeks some decomposition occurred, amounting to only 1% of the radioactivity. The steroid was administered intravenously as a single dose (0.4 ml of ethanolic stock solution plus 1.6 ml of 0.15 M-NaCl). The cats (1.5–3 kg) were of unknown age, and the two castrated males had been operated on at

an unknown time before purchase. The animals were anaesthetized with chloralose–urethane after induction with ether. The common bile duct was cannulated and the urinary bladder intubated. Full details of the operative procedure have been described (Taylor & Scratcherd, 1961, 1967). Each animal received approx. 40 μg (6 μCi) of oestradiol. Bile was collected in 1 h samples and urine in 2 h samples.

Samples of bile and urine were hydrolysed and extracted by a modification of the method of Taylor (1969). The sample was adjusted to approx. pH 4.5 by addition of 2 M-acetic acid. An equal volume of 0.5 M-sodium acetate buffer, pH 4.5 (containing 0.25 M-KH₂PO₄ to inhibit steroid sulphatases), containing 10000 Fishman units of β -glucuronidase was added, and the mixture was incubated at 45°C for 24 h. Without adjustment of pH, the solution was extracted three times with 5 vol. of ethyl acetate–ether (3:1, v/v). The combined extracts were washed twice with 0.1 vol. of saturated NaHCO₃ and twice with 0.1 vol. of water. The washings were added to the original aqueous sample. The extracts were dried over anhydrous Na₂SO₄, filtered, evaporated to dryness and the residue was dissolved in 2.0 ml of ethanol. The aqueous sample was made 2 M with respect to H₂SO₄, an equal volume of ether was added and the mixture was kept at room temperature for 72 h with occasional shaking. The ether was removed and the aqueous phase was extracted three times with 5 vol. of ethyl acetate–ether. The combined ether and ethyl acetate–ether extracts were washed three times with 0.1 vol. of saturated NaHCO₃ and twice with 0.1 vol. of water. The washings were added to the original aqueous solution, and the extracts treated as described above. After ether had been removed from the aqueous solutions by a current of air, the pH was approx. 1. The solutions were heated in a boiling-water bath for 1 h, and then extracted as before. The combined extracts were washed

three times with 0.1 vol. of saturated NaHCO_3 and twice with 0.1 vol. of water. Three washings of the extracts were required after the cold-acid and hot-acid hydrolysis to ensure that the pH of the final NaHCO_3 -washed solution was 10.5.

Student's *t* test was used to assess the significance of differences.

RESULTS

The excretion of radioactivity associated with metabolites of oestradiol in bile and urine of seven male, seven female and two castrated male cats is shown in Table 1. The radioactivity was excreted mainly in the bile and only about 1% of the dose appeared in the urine. There was no significant difference ($0.05 < P < 0.1$) between the total excretion of radioactivity in bile by male and female cats, and the amount excreted in bile of the castrated males was within the range of excretion by male and female animals. Table 1 also shows the percentage recovery of radioactivity in the phenolic fraction after hydrolysis by β -glucuronidase, cold acid and hot acid, and also the percentage of radioactivity that could not be extracted from aqueous solution at pH 10.5 by ethyl acetate-ether. There was a highly significant difference ($P < 0.005$) between male and female animals in the percentage of the dose appearing in the fractions hydrolysed by β -glucuronidase. In bile from the male cats this fraction, presumably glucuronides, contained half as much radioactivity as that from females. Male cats excreted significantly greater ($P < 0.005$) amounts of cold-acid-hydrolysable radioactivity, presumably sulphates, than did female animals. There was no significant difference ($0.05 < P < 0.1$) between male and female animals in the amount of

radioactivity that could be extracted after hot-acid hydrolysis or in the amount that could not be extracted after hydrolysis. With the two male castrated cats the values for radioactivity in the fractions hydrolysed by β -glucuronidase and by cold acid in the bile were closer to the values for female than for male cats.

No significant differences were observed between any of the fractions excreted in the urine of male and female animals.

DISCUSSION

Metabolites of neutral steroid hormones are excreted in bile and urine, but there are major differences in the pattern of excretion of one steroid by different species and of different steroids by the same species (Taylor, 1965). The cat is the only common laboratory animal that excretes metabolites of neutral steroids exclusively in the bile; only about 1% of an intravenously administered dose appears in the urine (Taylor, 1969). If an enterohepatic circulation of neutral steroid-hormone metabolites occurs in the cat, the reabsorbed metabolites are not subsequently excreted in the urine (Taylor & Scratcherd, 1962). Such reabsorption and subsequent urinary excretion of metabolites of neutral steroids occurs in most other species, including man (Taylor, 1965).

Oestrogen metabolites are excreted to a major extent in the bile of most species, including man; the degree of enterohepatic circulation of these metabolites and their subsequent excretion in urine varies from species to species, and also depends on the particular oestrogen administered (Adlercreutz, 1962; Sandberg & Slaunwhite, 1957; Adlercreutz &

Table 1. *Excretion of radioactivity in bile and urine of male and female cats after intravenous injections of $[4\text{-}^{14}\text{C}]\text{oestradiol}$*

The steroid was given as a single dose. Bile and urine were collected for 6 h. Results for male and female animals are expressed as means $\pm$ s.e.m. Significance of differences was assessed by Student's *t* test. The results for the two castrated males are expressed as means, with ranges in parentheses.

No. of animals		Sex	Distribution of radioactivity (percentage of dose in sample)					Not extracted after hydrolysis
			Total	Phenolic ethyl acetate-ether-soluble radioactivity after hydrolysis by:				
				β -Glucuronidase	Cold acid	Hot acid		
7	Male	Bile	60.4 $\pm$ 3.34	*9.0 $\pm$ 1.7	†18.9 $\pm$ 1.44	5.1 $\pm$ 1.17	27.3 $\pm$ 4.41	
		Urine	1.2 $\pm$ 0.22	0.2 $\pm$ 0.02	0.2 $\pm$ 0.03	0.2 $\pm$ 0.05	0.7 $\pm$ 0.12	
7	Female	Bile	53.3 $\pm$ 3.52	*18.6 $\pm$ 1.45	†12.1 $\pm$ 1.02	3.8 $\pm$ 0.65	20.7 $\pm$ 2.87	
		Urine	0.9 $\pm$ 0.11	0.1 $\pm$ 0.001	0.1 $\pm$ 0.02	0.1 $\pm$ 0.02	0.5 $\pm$ 0.09	
2	Male, castrated	Bile	53.1 (50.4–55.9)	24.3 (17.2–31.4)	10.2 (8.2–12.3)	3.5 (2.2–4.4)	15.3 (14.4–16.5)	
		Urine	0.8 (0.78–0.86)	0.1 (0.04–0.14)	0.08 (0.04–0.13)	0.05 (0.04–0.06)	0.6 (0.45–0.62)	
				* $P < 0.005$	† $P < 0.005$			

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Luukkainen, 1967; Sandberg, Kirdani, Back, Wyeman & Slaunwhite, 1967). For instance, Sandberg *et al.* (1967) found that in animals with a biliary fistula the percentage of a dose of labelled oestrone excreted in 4 h in bile and urine respectively were: male rats, 58.1 and 4.2%; male guinea pigs, 58.7 and 20.2%; female rabbits, 17.0 and 36.1; man, 72 and 15%. When oestrone was administered to intact animals the percentages of the dose excreted in 3 days in faeces and urine respectively were: rats, 10.8 and 7.4%; guinea pigs, 7.5 and 63.9%; rabbits, 16.2 and 68.8%; man, 7 and 85%. The excretion of oestrogens by intact cats has not been studied.

In the present work it was shown that cats excrete 53–60% of an intravenous dose of oestradiol as metabolites in the bile in 6 h; only about 1% of the dose appears in the urine. Also, there is a highly significant difference between the percentage of the dose appearing in the glucuronide fraction of bile from male (9%) and female (18.6%) cats. A significant sex difference was also observed in the metabolites appearing in the cold-acid-hydrolysed fractions, the values for the male cat being 18.9% and for the female 12.1% (Table 1).

There are at least two possible explanations for these sex differences. In man, oestrone is excreted mainly as its sulphate (Hobkirk, Nilsen & Blahey, 1969), whereas oestradiol and oestriol are excreted as glucuronides or as compounds doubly conjugated with both glucuronic acid and sulphuric acid (Adlercreutz & Luukkainen, 1967). Species and sex differences in metabolism and excretion of a wide range of steroid hormones and their metabolites are well established (Rao & Taylor, 1965a; Sandberg *et al.* 1967). Sex difference in steroid metabolism has been attributed to the influence of sex hormones on steroid-metabolizing systems. For example, liver of the female rat is more active than that of the male in reducing the A ring of Δ^4 -3-oxosteroids. This activity is decreased if the female animals are treated with testosterone, and the activity of the liver from male animals approaches that from female animals if they are castrated or treated with oestrogens (Yates, Herbst & Urquhart, 1958; Leybold & Staudinger, 1959). Such sex difference might also apply to the metabolism of oestrogens. Male cats may convert the administered oestradiol into oestrone and other metabolites that are more readily conjugated with sulphuric acid than with glucuronic acid, whereas female cats produce metabolites that are more likely to form glucuronides.

Another explanation for the sex differences observed in the present work is that male and female cats convert oestradiol into the same metabolites, but the sexes may differ in their ability to form conjugates of these. The liver of the female rat has a greater ability to convert dehydroepiandrosterone

into its sulphate than has that of the male (Roy, 1956). Microsomes of the liver of male rats are about four times as active as those of the female in forming the glucuronide of *o*-aminophenol; this activity is reversed by administering testosterone to female rats and oestrogens to male rats (Inscoc & Axelrod, 1960). Isselbacher & Axelrod (1955) reported that the UDP glucuronyltransferase system(s) of rat liver show a sex difference towards steroid aglycones. Rao & Taylor (1965a) observed marked sex and species differences in the ability of liver homogenate to convert metabolites of progesterone into glucuronides. Liver homogenate of the female cat *in vitro* converts progesterone into metabolites conjugated mainly with glucuronic acid (Rao & Taylor, 1965b).

Other explanations of the observed phenomena are possible, but the two proposed seem to be the most probable on the basis of information so far available. Whatever the cause of this sex difference in glucuronide and sulphate formation, the results obtained with the two castrated male cats indicate that the phenomena are probably sex-hormone-dependent. The amounts of glucuronides and sulphates formed by these two animals were much closer to the values obtained with the female than with the male animals (Table 1).

Another steroid diconjugate, oestradiol 3-glucuronide 17 α -*N*-acetylglucosaminide, has been isolated from rabbit urine after administration of oestrone benzoate and [16-¹⁴C]oestrone (Layne, Sheth & Kirdani, 1964). β -Glucuronidase of mammalian tissues is accompanied by β -*N*-acetylglucosaminidase (Levy & Conchie, 1966), but the existence of the latter enzyme in the molluscan β -glucuronidase used in the present work has not been studied and it is possible that part of the radioactivity released by β -glucuronidase was originally present as a single or double conjugate of *N*-acetylglucosamine.

Kuss (1969) incubated oestradiol with rat liver and isolated the water-soluble conjugates, *S*-(2,3,17 β -trihydroxy-1,3,5(10)-oestratrien-1-yl)glutathione and its 4-yl isomer. These compounds are thioethers and, if formed by cats *in vivo*, might contribute to the radioactivity which could not be extracted after hydrolysis, because of the presence of the free carboxyl group in the glutathione moiety. Extreme conditions, involving treatment with Raney nickel, were used by Kuss (1968) to separate the glutathione from the steroid.

Taylor (1965, 1969, 1970) observed no sex differences in the biliary and urinary excretion of metabolites of progesterone, testosterone and cortisone in the rabbit. In all previous investigations in this laboratory on steroid metabolism in cats only male animals were used.

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