Steroid Metabolism in the Cat

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

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(Received 8 January 1969)

1. [4-¹⁴C]Cortisone was administered to anaesthetized male cats as a single injection or as a 45–60 min. infusion. 2. After the single dose a total of 69.6–89.6% of the radioactivity was excreted in bile, and 0.5–7.1% in urine. After infusion total recovery in bile was 73.4–92.1%, and 1.2–1.7% in urine. 3. When bile and urine samples were hydrolysed successively by β -glucuronidase, cold acid and hot acid, most of the radioactivity was converted into substances not extractable from neutral aqueous solution by ethyl acetate–ether. 4. In bile, metabolites hydrolysable by β -glucuronidase were found in only small proportions (3–4%) of the dose.

Anaesthetized cats with a cannula in the common bile duct and the urinary bladder intubated excrete metabolites of intravenously administered labelled progesterone, corticosterone and testosterone almost completely in the bile; only about 1% of the dose appears as metabolites in the urine (Taylor, 1965; Taylor & Scratcherd, 1965, 1967). Intact cats excrete administered steroid hormones almost entirely in the faeces so, if enterohepatic circulation of steroid metabolites occurs, the reabsorbed metabolites are not subsequently excreted in the urine (Taylor & Scratcherd, 1962). The excretion of cortisone metabolites by anaesthetized male cats has now been investigated.

MATERIALS AND METHODS

[4-¹⁴C]Cortisone (17 α ,21-dihydroxypregn-4-ene-3,11,10-trione) (27.5 mc/m-mole), obtained from The Radiochemical Centre, Amersham, Bucks., was purified once by paper chromatography and twice by t.l.c. before use. The steroid was stored as an ethanol solution at –15°; after 5 weeks some decomposition occurred, amounting to only 1% of the total radioactivity. The steroid was administered intravenously either as a single dose (0.4 ml. of ethanol stock solution plus 0.6 ml. of 0.15 M-NaCl) or by continuous infusion over 45–60 min. (0.4 ml. of ethanol stock solution plus 25 ml. of 0.15 M-NaCl). The cats were anaesthetized with chloralose–urethane after induction with ether.

The procedure used by Taylor & Scratcherd (1967) for collection of rabbit urine was used with a small polythene cannula incorporated into the glass tube inserted into the urinary bladder, which allowed the bladder to be washed out with 2 ml. of 0.15 M-NaCl passed gently up the cannula from a syringe. The saline washings were added to the particular urine sample being collected at that time. Samples of bile were collected every 1 hr. and of urine every 2 hr. for a total period of 6 hr. after administration of the steroid. Each

animal received approx. 66 μ g. (5 μ C) of cortisone; the weights of the animals ranged from 2.1 to 4.0 kg. Full details of the operative procedure and methods of hydrolysis of bile and urine samples have been described by Taylor & Scratcherd (1961, 1963, 1967).

Ethyl acetate–ether (3:1, v/v) was used for extraction of metabolites from aqueous media. This mixture produced less emulsification than ethyl acetate alone, and was effective in extracting metabolites quantitatively.

Assay of radioactivity. This was carried out on Duralumin planchets as described by Taylor & Scratcherd (1961). Counting of samples was done with an ultra-thin end-window counter operating in the Geiger range at 1750 v. Helium+butane (98.3:1.7, v/v) was obtained from Air Products Ltd. (Stoke-on-Trent, Staffs.). The counting apparatus was the SCS-5 assembly of Tracerlab (G.B.) Ltd. (Weybridge, Surrey). At infinite thinness counting efficiency was approx. 35% for ¹⁴C.

RESULTS

The excretion of radioactive metabolites of cortisone in bile and urine of six male cats is shown in Table 1. When the steroid was given as a single dose the largest proportion of the dose (34.5–51%) in any single sample was in the bile collected over the first 1 hr.; when the steroid was given as an infusion the largest proportion of the dose (45.4–51.8%) was excreted in the second 1 hr. bile sample, but 18.2–26.6% was present in the first bile sample. The total excretion of radioactive metabolites in bile over the 6 hr. period was not affected by the mode of administration of the cortisone. Only a very small proportion of the dose was excreted in the urine except in one animal (7.1%); in this experiment the first 2 hr. urine sample was contaminated with blood and it alone contained 5.1% of the dose.

Table 1. *Excretion of radioactivity in bile and urine of male cats after intravenous injection of [4-¹⁴C]cortisone*

The steroid was given to cats 1-3 as a single dose, and to cats 4-6 as a continuous infusion over 45-60 min. Bile and urine were collected for 6 hr.

		Distribution of radioactivity (% of dose in sample)				
Animal nos.		Neutral ether-ethyl acetate-soluble, after hydrolysis				Unaccounted for
		Total	By β -glucuronidase	By cold acid	By hot acid	
1-3	Bile	82.5 (69.6-89.6)	3.2 (2.6-4.1)	3.7 (2.0-4.8)	7.1 (5.0-10.0)	68.5 (57.2-74.6)
	Urine	3.0 (0.5-7.1)	0.33 (0.09-0.75)	0.11 (0.02-0.27)	0.30 (0.01-1.06)	2.3 (0.38-5.53)
		85.5				
4-6	Bile	83.9 (73.4-92.1)	4.1 (3.8-4.3)	2.3 (1.8-3.1)	5.2 (3.5-6.0)	72.4 (61.8-78.7)
	Urine	1.4 (1.2-1.7)	0.30 (0.15-0.44)	0.35 (0.24-0.40)	0.06 (0.04-0.08)	0.83 (0.61-1.07)
		85.3				

Successive hydrolyses by β -glucuronidase, by cold-acid solvolysis and finally by hot acid released only a small amount of radioactive metabolites that could then be extracted from neutral, aqueous solution by ethyl acetate-ether. This applied to the radioactivity from both bile and urine (Table 1).

DISCUSSION

Borrell (1958, 1963a,b) studied the excretion of endogenous steroid hormone metabolites in cat urine, but was unable to detect the presence of corticosteroid metabolites by the Porter-Silber reaction or with Blue Tetrazolium. Apart from this work little is known about endogenous steroid hormone metabolism in the cat. However, the present work and that of Taylor & Scratcherd (1961, 1963) and Archer, Scratcherd & Taylor (1965) firmly establishes that in the cat steroid hormone metabolites are excreted almost completely in bile under the experimental conditions employed. Experiments with intact cats (Taylor & Scratcherd, 1962) have shown that little, if any, enterohepatic recirculation of steroid hormone metabolites occurs in cats. Metabolites of cortisone are excreted almost completely over the 6 hr. of the experiment (Table 1), as were those of corticosterone (Taylor & Scratcherd, 1963); those of testosterone are excreted to a slightly less extent than the corticosteroids (Archer *et al.* 1965), but there is no marked difference in the amount excreted when the steroids are administered as a single dose or by infusion. By contrast, progesterone metabolites are not excreted completely in 6 hr. after a single dose of the steroid is given, and there is marked retention of metabolites when the progesterone is given by infusion. This is probably due to the passage of progesterone or its metabolites into fat depots (Davis & Plotz, 1957).

In human subjects cortisone metabolites are excreted almost entirely in the urine. Peterson,

Pierce, Wyngaarden, Bunim & Brodie (1957) recovered 90% of a dose of labelled cortisone from the urine of intact patients; no radioactivity was found in the faeces. When the steroid was given to patients with T-tube bile drainage only 4% of the dose appeared as metabolites in bile, whereas 90% of the dose was recovered as metabolites in the urine, i.e. the reverse of the pattern of excretion exhibited by cats.

The biliary excretion of foreign compounds of different structure and molecular weight has been extensively studied by Abou-El-Makarem, Millburn, Smith & Williams (1967a,b), Abou-El-Makarem, Millburn & Smith (1967c) and Millburn, Smith & Williams (1967a,b). These authors have suggested that one of the major factors in determining that a compound will be appreciably or extensively excreted by the biliary route in rats is that the compound or its metabolite(s) should have a minimal molecular weight of 325 ± 50 and contain a strongly polar anionic group. Rodents are known to excrete metabolites of many steroid hormones appreciably or almost completely in bile (Taylor, 1965), and these steroid metabolites would meet the criteria laid down by Millburn *et al.* (1967a,b). Abou-El-Makarem *et al.* (1967b) studied the biliary excretion of four compounds of molecular weight ranging from 93 to 495 in eight species of animals, including the cat. From these experiments they found a clear species difference in the extent of biliary excretion of the compounds tested. They found that the rat, dog and hen were 'good excretors', the rabbit, guinea pig and rhesus monkey 'poor excretors' and the cat and sheep were in an intermediary position. However, in all the species tested there was a definite tendency for an increase in biliary excretion of compounds or their metabolites with increase in molecular weight. Cortisone has a molecular weight of 360 and it is reasonable to suppose that at least some of

its metabolic products in the cat would have molecular weights greater than this. The almost complete excretion of cortisone metabolites by the biliary route conflicts with the finding by Abou-El-Makarem *et al.* (1967b) that the cat does not excrete succinylsulphathiazole (7% of an intravenous dose) or phenolphthalein glucuronide (34% of dose) to the same extent as stilboestrol glucuronide (77% of dose). This suggests that in the cat the steroids are more readily taken up in the pathway(s) leading to biliary excretion than succinylsulphathiazole or phenolphthalein glucuronide, although other explanations may be found for this.

The results of Taylor & Scratcherd (1961, 1963) and Archer *et al.* (1965) show that there is a definite decrease in the order progesterone, testosterone, corticosterone and cortisone in the proportion of metabolites excreted as glucuronides and as metabolites hydrolysable by cold acid; but there is no apparent pattern in the proportions of metabolites hydrolysed by hot acid, and there is a corresponding increase in the amount of metabolites that cannot be accounted for after hydrolysis and extraction. The differences in the proportions of glucuronides and cold-acid-hydrolysable metabolites might be due to lower activity of the glucuronide-forming system for cortisone metabolites than for those of progesterone. It has been shown that species differences exist in the ability of animal liver homogenates to form glucuronides of progesterone metabolites (Rao & Taylor, 1965). W. Taylor (unpublished work) has found that rat and rabbit liver homogenates convert testosterone and cortisone into metabolites hydrolysable by β -glucuronidase less readily than progesterone.

Approximately 70% of the dose of cortisone could not be extracted from neutral aqueous solution after the successive hydrolytic procedures used (Table 1). The corresponding values for other steroids are progesterone (38%), testosterone (48%) and corticosterone (55%) (Taylor & Scratcherd, 1961, 1963; Archer *et al.* 1965). The possible nature

of this unaccounted-for radioactivity has been discussed in those earlier papers. It is not known whether these substances are conjugates that have not been hydrolysed by the methods used, or whether they are acidic or phenolic substances formed as a result of rupture of the steroid ring. These metabolites have been found in investigations both *in vivo* and *in vitro*, and their identity is as yet unknown (Taylor, 1965).

The technical assistance of Miss Yvonne Turner and a grant from the Medical Research Council are gratefully acknowledged.

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