Steroid Metabolism in the Cat

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

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1. [4-¹⁴C]Testosterone was administered intravenously to anaesthetized male cats as a single injection or as a 45–60 min. infusion. 2. Most of the administered radioactivity was excreted in the bile (70–80%); only 2.9–5.5% of the dose was excreted in the urine. 3. Bile and urine samples were hydrolysed successively to yield glucuronide, 'cold-acid-hydrolysed' and 'hot-acid-hydrolysed' fractions. 4. The proportion of glucuronides in bile decreased in successive samples, but cold- and hot-acid-hydrolysed metabolites showed no consistent change. 5. After hydrolysis most of the radioactivity in both bile and urine could not be extracted by ether from neutral aqueous solution.

Using conventional methods based on the Zimmermann reaction, Borrell (1963) could not detect any 17-oxo steroids in the urine of normal cats even after the administration of testosterone propionate or adrenocorticotrophic hormone. However, the cat may excrete metabolites of testosterone without a 17-oxo group in the urine, or the metabolites may be excreted in the bile and faeces. Taylor & Scratcherd (1961, 1962, 1963) have shown that male cats excrete metabolites of progesterone and corticosterone almost entirely in the bile or faeces. The biliary and urinary excretion of metabolites of intravenously administered [4-¹⁴C]testosterone by anaesthetized male cats has therefore been studied.

MATERIALS AND METHODS

[4-¹⁴C]Testosterone (17 β -hydroxyandrost-4-en-3-one) (21.5 mc/m-mole) was obtained from The Radiochemical Centre, Amersham, Bucks. Paper chromatography in hexane-methanol-water (10:9:1, by vol.) showed that 95–99% of the radioactivity had the same R_F as authentic testosterone. The labelled steroid was not purified further, but was stored in ethanol at -15° ; no detectable change in purity occurred after 8 weeks' storage. The steroid was administered intravenously either as a single dose (0.4 ml. of stock ethanol solution in 0.6 ml. of 0.15 M-NaCl) or by continuous infusion over 45–60 min. (0.4 ml. of stock solution in 20–25 ml. of 0.15 M-NaCl). Male cats were anaesthetized with chloralose-urethane, and hepatic bile and urine were collected. The operative procedures and all other materials, and methods of hydrolysis, extraction and radioactive assay, were as described by Taylor & Scratcherd (1961, 1963). Preliminary analyses of bile and urine showed that less than 1%, usually less than 0.5%, of the dose of steroid

was excreted as metabolites extractable before hydrolysis, and so the determination of this 'free steroid' fraction was omitted.

RESULTS

Table 1 shows the excretion of radioactivity in the bile of male cats after a single rapid injection (cats 1, 2 and 3) and after 45–60 min. infusion (cats 4 and 5) of [4-¹⁴C]testosterone. Also included in Table 1 are the volumes of bile collected and the total solids to show the degree of biliary function maintained during the experiments. Except for cat 1, the variations in the volumes of bile (1.0–3.2 ml./hr.) and percentage solids (1.9–2.9 g./100 ml.) are comparable with those obtained in other experiments (1.3–3.5 ml./hr. and 0.6–2.8 g./100 ml.) in which liver bile was collected by tying off the cystic duct and cannulating the common bile duct (Taylor & Scratcherd, 1963). Cat 1 excreted relatively larger volumes of bile, with a concentration of total solids greater than normally found. It was observed that this more concentrated bile was green gall-bladder bile and not the usual golden-brown hepatic bile. Post-mortem examination revealed that the ligature around the cystic duct had become untied and gall-bladder bile had contaminated the liver bile. However, this did not materially affect the excretion of steroid metabolites.

Table 1 shows that excretion of radioactivity after the administration of the steroid is rapid, 68.8–80.1% of the dose being excreted after a single dose and 70.0–77.3% after infusion. In the single-dose experiments the first bile samples

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

A single dose was given to cats 1, 2 and 3, and cats 4 and 5 were dosed for 45-60 min. Experimental details are given in the text.

Cat no.	Wt. (kg.)	Dose of steroid		Collection period (hr.)	Vol. of bile (ml.)	Bile		Distribution of radioactivity (% of total in sample)				Total recovery after all hydrolyses
		(μg.)	(10 ⁻⁶ x counts/min.)			Total solids		Radio-activity (% of dose)	Neutral ether-soluble radioactivity			
						(mg.)	(g./100ml.)		Glucuronide	Cold-acid-hydrolysed	Hot-acid-hydrolysed	
1	3.0	45	3.59	1	2.2	50	2.3	30.6	23.0	14.5	12.0	49.5
				1	2.1	62	2.9	23.0	8.2	6.0	9.5	23.7
				1	3.2	251	7.8	10.9	8.1	8.0	14.0	30.1
				1	3.1	244	7.9	6.1	7.8	10.5	13.5	31.8
				1	3.1	221	7.1	4.3	6.8	12.5	15.5	34.8
				1	1.2	141	11.7	4.2	6.4	10.0	11.5	27.9
						Total 79.1						
2	3.1	65	5.66	1	1.3	37	2.9	23.6	7.8	9.2	15.2	32.8
				1	1.7	38	2.3	20.2	3.9	4.5	15.2	24.0
				1	1.3	33	2.5	14.0	2.5	3.1	14.0	19.8
				1	1.3	31	2.5	10.0	2.4	3.3	13.8	19.8
				1	1.2	28	2.4	7.1	1.7	3.9	6.4	12.0
				1	1.0	23	2.4	5.2	2.4	3.4	7.5	13.3
						Total 80.1						
3	3.4	66	5.74	1	2.7	59	2.2	25.8	21.8	8.9	15.0	45.7
				1	2.9	56	2.0	20.0	15.7	4.5	10.2	30.4
				1	2.4	47	1.9	9.3	10.6	5.7	11.2	27.5
				1	2.5	48	2.0	6.6	6.4	3.6	7.4	17.4
				1	2.4	52	2.2	3.9	7.2	8.0	10.1	25.3
				1	2.4	46	1.9	3.2	5.7	8.8	12.5	27.2
						Total 68.8						
4	3.5	62	5.37	1	1.5	36	2.4	13.0	6.3	9.2	13.8	29.3
				1	1.3	31	2.4	20.0	8.0	8.0	18.8	31.4
				1	1.3	30	2.4	15.4	5.2	10.7	13.7	29.6
				1	1.3	32	2.8	10.6	5.0	5.0	6.6	17.5
				1	1.3	29	2.5	6.7	6.7	6.3	4.6	17.6
				1	1.0	27	2.7	4.3	4.8	4.8	9.1	18.7
						Total 70.0						
5	3.3	66	5.71	1	2.5	65	2.6	14.6	9.6	10.8	10.7	31.1
				1	2.5	53	2.1	21.1	9.2	9.0	15.0	33.2
				1	2.4	60	2.5	16.1	4.9	13.0	13.5	31.4
				1	2.4	66	2.8	13.1	4.6	12.2	8.5	25.3
				1	2.1	51	2.4	8.2	2.3	5.5	7.2	15.0
				1	2.0	45	2.1	4.2	4.3	7.3	8.3	19.9
						Total 77.3						

Table 2. *Excretion of radioactivity in urine of male cats after the intravenous injection of [4-¹⁴C]testosterone*

Experimental details are given in the text.

Cat no.	Collection period (hr.)	Vol. of urine (ml.)	Distribution of radioactivity (% of total in sample)							
			Urine			Neutral ether-soluble radioactivity				Total recovery after all hydrolyses
			Total solids		Radioactivity (% of dose)	Glucuro-nide	Cold-acid-hydrolysed	Hot-acid-hydrolysed		
			(g.)	(g./100 ml.)						
1	6	20.3	2.0	9.8	3.3	2.8	7.8	2.6	13.2	
2	6	6.0	1.6	26.7	3.5	3.1	1.6	4.5	9.2	
3	6	19.5	2.4	12.3	5.5	2.9	1.3	Sample lost	—	
4	6	21.3	1.9	8.9	3.3	2.8	2.2		4.6	9.6
5	6	8.3	1.9	23.6	2.9	1.6	1.6	4.4	7.6	

contained a greater amount of radioactivity than subsequent samples, whereas when the steroid was given over the longer period maximal excretion occurred in the second hour. Table 1 also shows the distribution of excreted neutral metabolites after consecutive hydrolysis with β -glucuronidase, 2N-sulphuric acid-ether (cold acid) and heating the aqueous solution at a pH of less than 1 (hot acid). Determination of radioactivity remaining in the aqueous residue after all forms of hydrolysis is a tedious and relatively inaccurate procedure and was not done in these experiments. Table 1 shows the total percentage recovery of radioactivity after all hydrolysis of bile samples, and the last column shows the total amount of radioactivity recovered. Taylor & Scratcherd (1961, 1963) have shown that this unaccounted-for radioactivity represents water-soluble metabolites, which may be unhydrolysed conjugates, or acid, phenolic or other substances not extractable from alkaline aqueous solution by ether.

Table 2 shows the corresponding results for the urine samples obtained from the same cats. Because of the small amount of radioactivity excreted in the urine the samples taken at 6 hr. intervals from each animal were combined for analysis, hydrolysis and extraction. The period over which the steroid was administered does not appear to affect to any great extent the total radioactivity or proportions of the different types of conjugate in the urine.

DISCUSSION

Many animal species, including man, convert testosterone to a greater or lesser extent into 17-oxo steroids: these metabolites have been found in the urine of dogs, rats, rabbits and other animals (for references see Borrell, 1963). However, Borrell (1963) was not able to detect any 17-oxo

steroids by the Zimmermann reaction in the urine of male or female cats, even after the intramuscular administration of up to 20 i.u. of adrenocorticotrophic hormone or 5 mg. of testosterone propionate.

It is now established that different animal species may excrete different proportions of metabolites of the same steroid in the urine and bile or faeces, and that the same species may excrete different proportions of different steroids by the hepatic and renal routes. For example, rats excrete metabolites of progesterone (Shen, Elliott, Doisy & Doisy, 1954), testosterone (Barry, Eidinoff, Dobriner & Gallagher, 1952) and cortisol (Hyde & Williams, 1957) predominantly in the bile or faeces, whereas in man urine is the principal route of excretion of the metabolites of these steroids (Sandberg & Slaunwhite, 1958; Fukushima, Bradlow, Dobriner & Gallagher, 1954; Hellman *et al.* 1954). When different steroids are administered to the same animal, however, different proportions of metabolites appear in bile and urine; e.g. rats excrete about 25% of administered progesterone in the urine (Shen *et al.* 1954), the corresponding values for other steroids being 10% for testosterone (Barry *et al.* 1952) and 35% for cortisol (Hyde & Williams, 1957). A number of theories has been put forward to explain what determines the route of excretion of steroid metabolites. These theories have been fully discussed by Taylor & Scratcherd (1961, 1963).

Sandberg & Slaunwhite (1956) studied the metabolism of [4-¹⁴C]testosterone in adult male and female human subjects. They found that normal subjects excreted the radioactivity almost completely in the urine; 50% of the dose was excreted as metabolites in the urine in the first 4 hr. after intravenous administration of the steroid. Patients with biliary fistulae excreted 12–14% of

Table 3. *Total excretion of radioactivity in bile and urine of male cats after the intravenous administration of progesterone, corticosterone and testosterone by a single rapid injection or by infusion over 45–60 min.*

Experimental details for the experiment with testosterone were as given in the text.

Steroid	Excretion (% of dose)			
	Bile		Urine	
	Single dose	Infusion	Single dose	Infusion
Progesterone*	67.1	35.2	0.5	0.6
	65.6	26.2	0.5	0.4
Corticosterone†	85.8	91.7	1.1	0.6
	90.9	83.8	0.9	0.4
Testosterone	80.1	70.0	3.5	3.3
	68.8	77.3	5.5	2.9
	79.1		3.3	

* Taylor & Scratcherd (1961).

† Taylor & Scratcherd (1963).

the dose in the bile, whereas intact subjects excreted only 6% in the faeces. This finding indicates that some reabsorption and enterohepatic circulation of testosterone metabolites occurs in man, and that part of the reabsorbed metabolites are subsequently excreted in the urine. Work with progesterone and corticosterone in cats (Taylor & Scratcherd, 1961, 1962, 1963) shows that enterohepatic circulation and subsequent urinary excretion of reabsorbed metabolites of those two steroids do not occur to any significant extent. No information is yet available about the role of the enterohepatic circulation in the urinary excretion of testosterone metabolites in cats.

The present investigation has shown that the rate of biliary excretion of testosterone metabolites more closely resembles that of corticosterone than of progesterone, especially when the steroid is administered over 45–60 min. (Table 3). Testosterone and progesterone are firmly bound to albumin, whereas corticosteroids are only weakly bound. Sandberg & Slaunwhite (1956) have suggested that there is a relationship between the binding of a steroid to serum albumin and the extent of its biliary excretion. If this were so one would expect testosterone to be excreted in a way more closely resembling that of progesterone, whereas it resembles that of corticosterone. However, it is known that progesterone and some of its metabolites are stored in adipose tissue (Davis & Plotz, 1957), and this may explain the relatively slow excretion of administered progesterone, particularly when the steroid is administered over 60 min. The rapidity of the biliary excretion of testosterone suggests that no appreciable storage of testosterone in adipose tissue occurs in the cat.

Another peculiarity of the cat is that a greater proportion of the administered testosterone appears

as metabolites in the urine than is found when progesterone and corticosterone are administered. This is a low value compared with urinary excretion in other species, but is significantly higher than the amounts of metabolites of progesterone and corticosterone found in cat urine under the same conditions.

In previous work with progesterone (Taylor & Scratcherd, 1961) and corticosterone (Taylor & Scratcherd, 1963) it was observed that the proportions of different conjugates appearing in bile samples varied with the time after the administration of the steroids. The most consistent change was a decrease in the proportion of glucuronides and an increase in water-soluble metabolites in successive samples. Table 1 shows that the same phenomenon occurs with testosterone metabolites, particularly when the steroid is given as a single dose. The proportions of cold- and hot-acid-hydrolysed metabolites show no such consistent or well-marked variation, although there appears to be a tendency for the later bile samples to contain smaller proportions of these conjugates than the early samples.

The failure of Borrell (1963) to detect 17-oxo steroids in cat urine is not surprising in view of the fact that only some 3% of the administered radioactivity was found in the urine. Of the 3% only one-tenth could be obtained in the ether-soluble neutral fraction after hydrolysis (Table 2). The present investigation therefore supports the view that, in the anaesthetized and the normal cat, the major route of excretion of steroid hormones is via the bile and faeces.

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