Steroid Metabolism in the Rabbit

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

By W. TAYLOR AND T. SCRATCHERD

Department of Physiology, The Medical School, University of Newcastle upon Tyne

(Received 26 October 1966)

1. [4-¹⁴C]Testosterone was administered to anaesthetized male and female New Zealand White rabbits as a single injection or as a 45–60 min. infusion. 2. After a single dose a total of approx. 56–80% of the radioactivity was excreted in bile and urine. After infusion total recovery of radioactivity was approx. 63–75%. 3. The mean ratio of metabolites in urine to those in bile was 0.77 ± 0.41 (range 0.3–1.5). 4. Bile and urine samples were hydrolysed successively by β -glucuronidase, cold acid and hot acid. In both bile and urine neutral metabolites extracted by ethyl acetate-ether were found mainly after β -glucuronidase hydrolysis, but a considerable proportion of the dose was converted into substances not extractable from alkaline aqueous solution after all forms of hydrolysis used.

Anaesthetized cats with a cannula in the common bile duct and with the urinary bladder intubated excrete metabolites of intravenously administered progesterone, corticosterone and testosterone almost completely in the bile. Only a small fraction of the dose, at most 5% and usually only about 1%, appears as metabolites in the urine. Intact cats excrete administered steroid hormones almost exclusively in the faeces, so if enterohepatic circulation of steroid metabolites occurs in cats the reabsorbed metabolites are not excreted in the urine (for references see Taylor, 1965; Taylor & Scratcherd, 1965). The anaesthetized rabbit, on the other hand, excretes metabolites of intravenously administered [4-¹⁴C]progesterone in both bile and urine (Taylor & Scratcherd, 1965). Since there are differences in the rate of excretion and degree of conjugation of progesterone and testosterone metabolites in cats (Taylor & Scratcherd, 1961; Archer, Scratcherd & Taylor, 1965) the excretion of testosterone metabolites by male and female rabbits has now been investigated. A preliminary account of part of this work has been published (Taylor & Scratcherd, 1966).

MATERIALS AND METHODS

[4-¹⁴C]Testosterone (29.2 mc/m-mole) was obtained from The Radiochemical Centre, Amersham, Bucks. Paper chromatography in two solvent systems showed that at least 97% of the radioactivity had the same R_f as testosterone even after storage of the labelled steroid in ethanol at -15° for 12 weeks. The hormone was given as a single rapid intravenous injection or by intravenous infusion in 25 ml. of

0.15 M-NaCl over 45–60 min. New Zealand White rabbits, starved overnight but allowed access to water, were anaesthetized with urethane. All injections were made through a cannula in the external jugular vein. The common bile duct was cannulated and the cystic duct tied off, and the urinary bladder intubated. Because of the low production of urine by most animals a small polythene cannula was incorporated in the glass tube in the bladder; when necessary the urinary bladder was washed out with 2 ml. of 0.15 M-NaCl. Samples of bile (1 hr. intervals) and of urine (2 hr. intervals) were collected for a total period of 6 hr. after administration of the labelled testosterone. Full details of the method of preparation of the animals and of treatment of bile and urine samples were as described by Taylor & Scratcherd (1961, 1963, 1965) except that ethyl acetate-ether (2:1, v/v) was used for extraction of metabolites from bile and urine.

Assay of radioactivity. This was carried out on duralumin planchets described by Taylor & Scratcherd (1961). The planchets (1 in. diam.) were mounted on spacing rings to permit insertion into the bakelite holders of the automatic sample-changer. Counting of samples was carried out with a thin end-window flow counter operating in the proportional range at 2300 v. Argon+methane (90:10) was obtained from the British Oxygen Co. (Wembley, Middx.). The counting apparatus incorporating a preamplifier, multi-matic scaler and auto-computer with print-out was the SCS-5 assembly of Tracerlab (G.B.) Ltd. (Weybridge, Surrey). Counting efficiency for ¹⁴C under the conditions used was approx. 35%.

RESULTS

The excretion of radioactive metabolites of testosterone in the bile and urine of male and female rabbits is shown in Fig. 1 (single dose) and Fig. 2 (continuous infusion over 45–60 min.). In the

animals given the single dose of steroid the largest percentage excretion of metabolites occurred in the first hour, but when the steroid was given by

Fig. 1. Cumulative excretion of radioactivity in bile (●) and urine (▲) of rabbits after a single intravenous dose of [4-¹⁴C]testosterone. The values, means and ranges (indicated by the vertical lines), were obtained from four male and four female animals. The dose of steroid was 31.7–46.2 μ g., and the weight of the animals was 1.8–2.9 kg. For other details see the text.

Fig. 2. Cumulative excretion of radioactivity in bile (●) and urine (▲) of two male and two female rabbits after administration of [4-¹⁴C]testosterone as a continuous infusion in 25 ml. of 0.15 M-NaCl over 45–60 min. The dose of steroid was 46.2–44.8 μ g., and the weight of the animals 1.2–2.2 kg. Means and ranges are shown.

infusion a considerable proportion of the dose was excreted in the second 1 hr. bile sample. In one animal given the steroid by infusion there was a greater proportion of the dose excreted in the first 1 hr. bile sample. Because of the low spontaneous urine flow in many of the animals it was necessary to rinse out the bladder with 0.15 M-sodium chloride as described in the Materials and Methods section. However, the low production of urine did not appear to have any marked effect on the amount of steroid metabolites excreted by the kidney. For instance, one animal excreted 14.9% of the dose in the urine although low urine production necessitated rinsing of the bladder with 0.15 M-sodium chloride, and another animal produced 11.5 ml. of urine and this contained only 14.0% of the dose. Similarly, another animal produced 13.1 ml. of urine containing 41.8% of the dose, and another animal excreted only 25.1% of the dose in 47.7 ml. of urine. In all but one instance the greatest proportion of metabolites in urine was excreted in the first 2 hr. collection period, indicating that there is a rapid excretion of metabolites in both bile and urine irrespective of whether the steroid is administered as a single dose or as an infusion.

In spite of the wide range of excretion of metabolites in both bile and urine it can be seen from Fig. 1 that when the steroid was given as a single dose a lower proportion of the dose was excreted in urine than in bile; of the eight animals treated in this way, only one excreted more radioactivity in bile than in urine. When the steroid was given by infusion, however, three of the four animals excreted more radioactivity in urine than in bile. Fig. 2 shows that, after 6 hr., the mean values of excretion of radioactivity in bile and urine were equal (see also Table 1). The mean ratio of metabolites in urine to those in bile was 0.77 ± 0.41 (range 0.3–1.5); the corresponding values for excretion of progesterone metabolites by rabbits were 0.62 ± 0.14 (range 0.44–0.89) (Taylor & Scratcherd, 1965).

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

The steroid was given to rabbits 1–8 as a single dose and to rabbits 9–12 as a continuous infusion over 45–60 min. Collections of bile and urine were made for 6 hr. Results are given as means with the ranges in parentheses.

		Distribution of radioactivity (% of dose in sample)				
		Neutral ethyl acetate-ether-soluble radioactivity after hydrolysis by:				
Animals		Total	β -Glucuronidase	Cold acid	Hot acid	Unaccounted for
1–8	Bile	40.8 (22.5–59.5)	22.2 (10.1–30.5)	3.7 (2.9–5.6)	2.3 (1.2–4.6)	12.1 (5.2–22.4)
	Urine	23.5 (14.0–41.8)	12.0 (5.5–25.3)	2.4 (1.4–4.7)	1.9 (0.3–3.0)	7.5 (4.1–11.2)
9–12	Bile	33.5 (24.8–45.5)	18.4 (13.5–28.0)	2.5 (1.2–3.5)	2.0 (1.9–2.1)	11.7 (7.1–13.5)
	Urine	33.5 (24.4–39.7)	16.9 (12.5–21.2)	2.2 (1.2–3.6)	2.2 (1.6–2.7)	11.8 (9.1–16.0)

The mean biliary excretion of radioactivity was $35 \pm 10.1\%$ (S.D.) (variance 136) for males and $45.2 \pm 8.6\%$ (variance 99) for females. By using Bessel's correction for the small sample number and Student's *t* test, the ratio of the difference of the means to the standard error of the difference was $0.2 < P < 0.3$. Therefore there is no significant difference in total dose of steroid excreted in the bile of male and female rabbits. No sex difference was observed in the percentage of the dose excreted in the urine or in the total percentage dose excreted in bile plus urine.

Table 1 shows the distribution of neutral ethyl acetate-ether-soluble metabolites after successive hydrolysis of the bile and urine samples by limpet-powder β -glucuronidase, cold acid solvolysis and hot acid and the amount of radioactivity not extractable from the alkaline aqueous solution after the hydrolyses and extractions. The results are recorded as the means and ranges of the percentages of the dose of radioactivity present in each fraction.

DISCUSSION

The investigation of the metabolism of steroid hormones by rabbits *in vivo* appears to have been neglected (Dorfman & Ungar, 1965) except for studies with progesterone and certain of its derivatives and analogues (Crépy, Judas & Jayle, 1964; Crépy, Judas, Pasqualini, Nunez & Jayle, 1966; Taylor, 1965; Taylor & Scratcherd, 1965).

The results shown in Figs. 1 and 2 indicate that, in most of the animals investigated, there was a greater proportion of the dose of testosterone excreted in the bile than in the urine of male and female rabbits when the steroid was given as a single dose. When the steroid was given as an infusion three of the four animals excreted more of the dose in the urine than in the bile; after the 6 hr. period of the experiment the total excretion of radioactivity

in the bile and urine was equal. It is not possible to state whether this was due to the lower rate of administration of the steroid or because of the administration of 20–25 ml. of 0.15 M-sodium chloride with the steroid. However, it may be significant that urine production was no greater in the infused animals than in the animals given the steroid as a single dose. The degree of hydration of the animals was not measured, but it was observed that those animals that had a full bladder when operated upon usually had a better spontaneous urine flow than those animals with empty bladders. All urine was removed from the bladder before that organ was intubated. The same phenomenon was also observed in similar experiments in which progesterone was administered to rabbits (Taylor & Scratcherd, 1965). The apparent independence of the proportion of the dose of administered steroid excreted by the liver and kidney and the relative volumes of bile and urine produced by cats and rabbits has already been discussed (Taylor, 1965; Taylor & Scratcherd, 1965). However, since no enterohepatic circulation of metabolites could have occurred under the present experimental conditions it is possible that the proportions of testosterone metabolites excreted in the bile (and hence faeces) and urine of intact rabbits might be different from those reported here. With progesterone metabolites it seems that enterohepatic circulation does not occur, or does not influence the proportion of the dose appearing as metabolites in urine. Cooke, Rogers & Thomas (1963) recovered 14.7–37.1% of labelled metabolites in the urine after subcutaneous administration of labelled progesterone to rabbits. The anaesthetized bile-duct-cannulated rabbit excretes about the same proportion (11–23.9%) of intravenously administered progesterone as metabolites in urine (Taylor & Scratcherd, 1965). However, it has been stressed that different species may excrete the same steroid by different routes and

Table 2. Comparison of excretion of metabolites of $[4-^{14}\text{C}]$ progesterone and $[4-^{14}\text{C}]$ testosterone in bile and urine of rabbits and cats

Percentage of dose recovered											
Rabbit				Cat							
Bile			Urine	Total	Bile			Urine	Total		
Progesterone*				Progesterone†							
Single dose			35.3-40.2	21.8-23.9	59.2-62.0	65.6-67.1			0.5	66.1-67.1	
Infusion			22.5-30.7	11.2-20.0	36.8-48.8	26.2-35.2			0.4-0.6	26.6-35.8	
Testosterone‡				Testosterone§							
Single dose			22.5-59.5	14.0-41.8	55.8-80.1	68.8-79.1			3.3-5.5	74.3-82.4	
Infusion			24.8-45.5	24.4-39.7	62.9-74.9	70.0-77.3			2.9-3.3	73.3-80.2	

* From Taylor & Scratcherd (1965).

‡ Present investigation.

† From Taylor & Scratcherd (1961).

§ From Archer *et al.* (1965).

that the same species may excrete different steroids by different routes (for full discussion see Taylor, 1965), and the excretion of metabolites of testosterone by intact rabbits requires investigation.

As shown in Table 1 the radioactivity extractable after β -glucuronidase hydrolysis constitutes the major fraction of the dose. The 'glucuronides' account for about half of the radioactivity in the bile and urine samples. Metabolites hydrolysed by cold acid and hot acid form only a small part of the 'conjugated' radioactivity. However, a considerable proportion of the dose cannot be extracted from neutral aqueous solution after the successive hydrolytic procedures. These metabolites, the nature of which is unknown, have been observed in similar experiments with other species and steroids (for references see Taylor, 1965). They occur in both bile and urine to an extent usually greatly exceeding the sum of the cold-acid- and hot-acid-hydrolysed metabolites.

Table 2 summarizes the results of experiments in which labelled progesterone and testosterone have been administered to cats and rabbits prepared as described in the Materials and Methods section. Although in the cat virtually all of the excreted progesterone metabolites appear in the bile the total excretion of metabolites is about the same as that observed with rabbits. With testosterone, on the other hand, the cat excretes a larger proportion of the dose, mainly in the bile, particularly when the steroid is given as a single dose. Both rabbits and cats excrete a higher total percentage of a dose of

testosterone than of progesterone over the 6 hr. of the experimental period. This difference between excretion of the two steroids is most clearly marked when the hormones are administered as an infusion over 45–60 min. The results in Table 2 clearly show how different species excrete steroid metabolites by different routes and also how the same species may excrete metabolites of different steroids to a greater or smaller extent in bile and urine under the experimental conditions employed.

This work was financed by a grant to W.T. from the Medical Research Council. The technical assistance of Miss Yvonne Turner is gratefully acknowledged.

REFERENCES

- Archer, S. E. H., Scratcherd, T. & Taylor, W. (1965). *Biochem. J.* **94**, 778.
 Cooke, A. M., Rogers, A. W. & Thomas, G. H. (1963). *J. Endocrin.* **27**, 299.
 Crépy, O., Judas, O. & Jayle, M. F. (1964). *Bull. Soc. Chim. biol., Paris*, **46**, 905.
 Crépy, O., Judas, O., Pasqualini, J. R., Nunez, E. A. & Jayle, M. F. (1966). *Europ. J. Steroids*, **1**, 171.
 Dorfman, R. I. & Ungar, F. (1965). *Metabolism of Steroid Hormones*. New York and London: Academic Press Inc.
 Taylor, W. (1965). *Proc. N.A.T.O. Advanced Study Inst., Newcastle upon Tyne: The Biliary System*, p. 399. Ed. by Taylor, W. Oxford: Blackwells Scientific Publications.
 Taylor, W. & Scratcherd, T. (1961). *Biochem. J.* **81**, 398.
 Taylor, W. & Scratcherd, T. (1963). *Biochem. J.* **86**, 114.
 Taylor, W. & Scratcherd, T. (1965). *Biochem. J.* **97**, 89.
 Taylor, W. & Scratcherd, T. (1966). In *Excerpta Med. no. 101: Androgens*, p. 142. Ed. by Vermeulen, A.