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Steroid Metabolism in the Rabbit

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

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1. [4-^{14}C]Cortisone was administered to anaesthetized male and female New Zealand White rabbits as a single injection or as a 45–60 min infusion. 2. The method of administration of the steroid did not significantly affect the total excretion of radioactivity in bile and urine [83.8 ± 10.8% (S.D.)]. 3. The mean ratio of metabolites in urine to those in bile was 0.97 ± 0.23% (range 0.64–1.3). 4. When bile and urine samples were hydrolysed successively by β-glucuronidase, cold acid and hot acid, neutral metabolites extracted by ethyl acetate–ether were found mainly after hydrolysis by β-glucuronidase. 5. An approximately equal proportion of the dose was converted into substances not extractable from alkaline aqueous solution after hydrolysis.

Anaesthetized rabbits with a cannula in the common bile duct and with the urinary bladder intubated excrete metabolites of intravenously administered progesterone and testosterone in bile and urine (Taylor & Scratcherd, 1965, 1967). The excretion of radioactivity associated with cortisone metabolites by rabbits has now been investigated.

MATERIALS AND METHODS

[4-^{14}C]Cortisone (27.5 mCi/mmol) obtained from The Radiochemical Centre, Amersham, Bucks, U.K., was the sample described by Taylor (1969). The steroid was administered as a single rapid injection (0.4 ml of ethanolic stock solution plus 0.6 ml of 0.15 M-NaCl) or by continuous infusion over 45–60 min (0.4 ml of ethanolic stock solution plus 25 ml of 0.15 M-NaCl). New Zealand White rabbits (1.9–2.4 kg) were anaesthetized with urethane after induction with ether. Each animal received approx. 66 μg (5 μCi) of cortisone. Details of the operative procedure and methods of hydrolysis of bile and urine have been described by Taylor & Scratcherd (1961, 1965, 1967). Samples of bile were collected every hour and of urine every 2 h for a total period of 6 h after administration of the steroid. Ethyl acetate–ether (3:1, v/v) was used to extract metabolites from aqueous media. Radioactivity was assayed on Duralumin planchets as described by Taylor (1969).

Student's *t* test, with Bessel's correction for small sample numbers, was used to assess significance of differences.

RESULTS

The excretion of radioactivity in bile and urine of the four male and four female rabbits is shown in Table 1. Animals 1–4 were given the cortisone as a single rapid injection, and animals 5–8 received the steroid as an infusion over 45–60 min. The results are expressed as means ± S.E.M. No significant difference ($P > 0.3$) was observed in the total dose of steroid excreted in bile and urine of animals 1–4 and 5–8, and there was no significant difference in excretion of radioactivity by male and female animals. The percentage of the dose appearing in the various fractions after hydrolysis of bile and urine was not affected significantly ($P > 0.3$) by the method of administering the steroid or the sex of the animal. Indeed, when the values for the eight animals are combined (Table 1), there is a remarkable similarity between the percentage of the total radioactivity excreted in bile and urine. This similarity is also seen in the percentage of radioactivity recovered in the fractions of bile and urine respectively hydrolysed by β-glucuronidase, cold acid and hot acid, and in the radioactivity that could not be extracted after hydrolysis.

DISCUSSION

The metabolism of adrenocortical hormones in rabbits *in vivo* has not been studied (Dorfman & Ungar, 1965). Bush (1951, 1953) identified cortisol and corticosterone in rabbit blood, and the latter steroid and 11-dehydrocorticosterone were isolated from the same source by Reif & Longwell (1958).

The results (Table 1) indicate that, under the experimental conditions employed, rabbits excrete metabolites of intravenously administered cortisone in equal proportions in bile and urine. The mean

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

The steroid was given to rabbits 1–4 (two males, two females) as a single dose and to rabbits 5–8 (two males, two females) as a continuous infusion over 45–60 min. Bile and urine were collected for 6 h. Results are expressed as means ± S.E.M.

		Distribution of radioactivity (percentage of dose in sample)				
			Neutral ethyl acetate–ether-soluble radioactivity after hydrolysis by:			
Animals		Total	β-Glucuronidase	Cold acid	Hot acid	Not extracted after hydrolysis
1–4	Bile	42.4 ± 3.96	17.2 ± 2.20	4.1 ± 0.78	2.9 ± 1.14	18.1 ± 2.00
	Urine	41.7 ± 5.31	19.0 ± 3.31	3.8 ± 1.12	3.2 ± 1.23	15.6 ± 5.06
5–8	Bile	42.4 ± 1.82	20.5 ± 0.81	2.9 ± 0.56	2.7 ± 0.67	16.3 ± 1.66
	Urine	40.9 ± 3.77	16.9 ± 1.03	2.7 ± 0.84	2.1 ± 0.37	19.2 ± 3.52
1–8, Totals	Bile	42.5 ± 2.00	18.9 ± 1.21	3.5 ± 0.50	2.8 ± 0.61	17.3 ± 1.25
	Urine	41.3 ± 3.00	18.0 ± 1.65	3.3 ± 0.68	2.7 ± 0.63	17.4 ± 2.93

ratio of radioactivity in urine to that in bile was 0.97 ± 0.23(S.D.); the corresponding values for metabolites of progesterone (Taylor & Scratcherd, 1965) and testosterone (Taylor & Scratcherd, 1967) were 0.62 (±0.14) and 0.77 (±0.41) respectively. This finding is in keeping with the concept that in man and other animals a greater proportion of metabolites of adrenocortical hormones than of less polar steroids is excreted in urine than in bile (Taylor, 1965).

The method of administering the cortisone to rabbits did not significantly affect the total excretion of radioactivity, the amount of radioactivity excreted in bile and urine or the radioactivity present in the various fractions after hydrolysis. This is in contrast with the results obtained with other steroids. When progesterone was administered to two rabbits as a single dose 35.3 and 40.2% of the dose was excreted in the bile and 23.9 and 21.8% in the urine. However, when the steroid was given by infusion to four rabbits lower recoveries of radioactivity were obtained from bile (range 22.5–30.7%) and from urine (range 11.2–20.2%) (Taylor & Scratcherd, 1965). When testosterone was administered by single injection into two rabbits 40.8 ± 6.4% (S.D.) was recovered from bile but only 23.5 ± 8.4% (S.D.) from urine. When the steroid was given by infusion to four rabbits 33.5 ± 6.9% (S.D.) was recovered from bile and 33.5 ± 5.4% (S.D.) from urine (Taylor & Scratcherd, 1967).

The excretion of steroid metabolites by rabbits is markedly different from that in cats. Taylor (1969) administered [4-^{14}C]cortisone to anaesthetized male cats and found that approx. 83% of the dose was excreted in the bile and only approx. 1% in the urine. The present results and those of Taylor (1969) extend and confirm earlier work with rabbits (Taylor & Scratcherd, 1965, 1967) and with cats (Taylor & Scratcherd, 1961, 1963; Archer, Scratcherd & Taylor, 1965). There is a definite increase in the order progesterone, testosterone and cortisone in the total amount of administered steroid excreted in bile of cats and in bile and urine of rabbits.

There are also marked differences, between cats and rabbits, in the proportions of the dose present in the various fractions after hydrolysis. This is marked in the β-glucuronidase-hydrolysed fraction. Cats excrete only about 3–4% of the dose in the bile in this fraction (Taylor, 1969), whereas about 18% of the dose is excreted in this fraction in both bile and urine of rabbits (Table 1). This greater ability of the rabbit to convert a labelled steroid into radioactive metabolites hydrolysable by β-glucuronidase is probably due to the higher activity of the UDP-glucuronyltransferase system(s) in rabbit than in cat liver (Dutton & Greig, 1957).

Taylor & Scratcherd (1961, 1963), Archer *et al.* (1965) and Taylor (1969) found a definite decrease in the order progesterone, testosterone, corticosterone and cortisone in the proportion of radioactivity excreted as glucuronides when these steroids were administered to cats. This decrease in the order progesterone (Taylor & Scratcherd, 1965), testosterone (Taylor & Scratcherd, 1967) and cortisone with respect to glucuronide formation is also exhibited by rabbits when allowance is made for the total amount of radioactivity excreted in the 6 h of the experiment. Rao & Taylor (1965) showed that species differences exist in the ability of animal liver homogenate to form glucuronides of progesterone metabolites, and the ability of rabbits to form different proportions of steroid glucuronides

might be due to differences in activity of the glucuronide-forming systems towards different steroids *in vivo*. The absence of a sex difference in the amount of glucuronides of cortisone metabolites formed by rabbits *in vivo* is also in agreement with the studies made *in vitro* by Rao & Taylor (1965): they found no sex difference in the ability of rabbit liver homogenate to convert progesterone metabolites into glucuronides.

The other major difference between the metabolism of cortisone by cats and rabbits is in the amount of radioactivity in bile and urine that could not be extracted after hydrolysis: this accounts for about 70% of the dose in the bile of cats (Taylor, 1969), but only about 17% in both bile and urine of rabbits (Table 1). The possible nature of this unaccounted-for radioactivity has been discussed by Taylor (1965). Allen, Cooke & Thomas (1968) studied the metabolites of progesterone in rabbit urine after administration of labelled steroid. They found that about 62% of the radioactivity present in the unaccounted-for fraction was extractable from ether into sodium hydrogen carbonate solution. At least some of these acidic metabolites contained a carboxylic acid moiety at C-21, and it is reasonable to suppose that the terminal hydroxymethyl group of the cortisone side chain might also be converted into C-21 carboxylic acids by cats and rabbits. The identity of the cortisone metabolites present in cat bile and rabbit bile and urine is not known.

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