Effect of β -Glucuronidase upon the Hydrolysis¹ of Corticoids in Cat Urine

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Robinson & Williams (1958) have raised the question of the formation of glucuronides by cat liver. During an investigation of the influence on elimination of urinary corticoid by cats of block of the reticulo-endothelial system by injection of trypan blue (Arteta & Borrell, 1958), it was observed that incubation of cat urine with β -glucuronidase had little or no effect on the amount of Porter-Silber-reacting corticoids extractable from cat urine. This observation has been further studied and results indicate that only small amounts of corticoid glucuronides are excreted by the cat.

METHODS

Corticoid determinations, by a modification of the method of Silber & Porter (1954), were carried out on extracts of portions of cat urine incubated at pH 6.5 for 22 hr. with and without added β -glucuronidase, or extracted immediately after adjustment of urinary pH to pH 7.0 or pH 1.5. The activity of the β -glucuronidase preparation was checked almost daily in routine analyses of human urine.

When incubation with enzyme was studied, the freshly collected 24 hr. specimen was filtered and adjusted to pH 6.5. Two samples, from 10 to 25 ml. depending on the volume available, were made up to 25 ml. each with water and 10 ml. of 0.1M-phosphate buffer (prepared from KH_2PO_4 and Na_2HPO_4), pH 6.5, 100 Sigma units of β -glucuronidase and 10 000 units of penicillin (Merck and Co. Ltd., U.S.A.) were added. Incubation was carried out at 37° for 22 hr. The reaction mixtures were extracted with 100 ml. of freshly redistilled chloroform and shaken for 90 sec. If an emulsion formed the samples were centrifuged. The chloroform extracts were concentrated *in vacuo* in a water bath below 42° to approx. 20 ml. The extracts were washed by shaking them for 30 sec. in plastic-capped tubes with 2 ml. of 0.1N-NaOH and centrifuged. The volume of chloroform was adjusted to 25 ml. and 20 ml. portions were shaken for 45 sec., in clean tubes, with 2 ml. of phenylhydrazine-sulphuric acid reagent. After centrifuging, the phenylhydrazine-sulphuric acid layer was placed in a water bath at 60° for 30 min. and the absorption determined with a Hilger Spekker absorptiometer (H760) with a no. 601 filter and appropriate blanks. Control experiments showed that the presence of penicillin did not influence the results.

RESULTS

Recovery from 5–10 μg . of cortisone (Merck and Co. Ltd., U.S.A.), added to four urines containing

1.5–2.8 μg . of endogenous Porter-Silber-reacting material, indicated that the extraction procedure gave 97–104 % recovery of free cortisone. The maximum variation between triplicate determinations was 8 % at low levels of corticoid content and 4 % at higher levels. Incubation with or without β -glucuronidase made no difference in the recovery of cortisone added to urines (Table 1).

Table 2 presents the results of extractions of pooled and individual cat urines, the corticoid values after incubation with β -glucuronidase being compared with those obtained by extraction at pH 7.0 or at pH 1.5. There appears to be a tendency toward slightly higher values after incubation with enzyme, but the differences are not significant for the numbers analysed. In some individual cases, the value after incubation with glucuronidase was lower than that obtained by simple extraction, confirming the opinion that the increases seen after incubation with β -glucuronidase are not significant.

Cortisone or adrenocorticotrophic hormone was administered to other cats to see if the material hydrolysable by glucuronidase remained low at higher levels of corticoid excretion. The results in Table 3 show that there was no change in the ratio of glucuronide to free corticoid under these conditions.

Table 1. *Effect of incubation at pH 6.5 with and without β -glucuronidase on corticoids of cat urine*

For experimental details see text.

Vol. of urine (ml.)	β -Glucuronidase (Sigma units)	Cortisone added (μg .)	Corticoids/sample (as μg . of cortisone)
20	300	0	3.24
20	100	0	3.00
20	0	0	2.90
15	100	0	2.52
15	100	10	12.47
15	0	10	11.95
25	100	0	2.86
25	0	0	2.76
25	100	5	8.00
25	0	5	8.17
0	0	5	4.99
20	100	0	1.47
20	0	0	1.47
20	100	5	6.28
20	0	5	6.33
0	0	5	5.04

Table 2. *Corticoids extracted from cat urine at pH 7.0, pH 1.5 or after incubation with β -glucuronidase*Results are given as means $\pm$ s.e. with ranges in parentheses.

Source of urine	No. of experiments	Corticoids (as μ g. of cortisone/24 hr.)		
		β -Glucuronidase	pH 7.0	pH 1.5
Pool (3 cats)	6	9.2 $\pm$ 1.1 (4.1-10.9)	8.1 $\pm$ 0.9 (3.6-12.6)	—
Individuals	4	9.1 $\pm$ 1.3 (6.0-12.2)	7.6 $\pm$ 0.8 (5.6-9.2)	—
Pool (4 cats)	2	4.4 (4.1, 4.6)	4.6 (3.3, 5.9)	3.8 (3.0, 7.5)
Pool (3 cats)	3	6.4 $\pm$ 0.7 (5.7-7.9)	—	7.6 $\pm$ 0.1 (7.4-7.8)
Individuals	2	—	5.7 (4.8, 6.6)	5.8 (5.4, 6.2)

Table 3. *Corticoids extracted from urine at pH 7.0 or after incubation with β -glucuronidase, after administration of cortisone or adrenocorticotrophic hormone to cats*Results are given as means $\pm$ s.e.

Compound administered	No. of cats	Corticoids (as μ g. of cortisone/24 hr.)	
		β -Glucuronidase	pH 7
Adrenocorticotrophic hormone*	4	17.5 $\pm$ 3.1	14.7 $\pm$ 1.9
Cortisone†	2	34.5 $\pm$ 5.2	34.0 $\pm$ 5.3

* 25 i.u. (Adrecortan) was injected into each cat.

† Two cats (D and E): three urine collections each. Cat D received 1 mg. of cortisone (Altesona)/kg. on day 1, 2.5 mg./kg. on days 2 and 3. Cat E received 3 mg./kg. daily for 3 days.

DISCUSSION

Results presented are in contrast with those published for human urine (e.g. Mason, 1954), where at least 80 % of the corticoids excreted are excreted as glucuronides. Peron & Dorfman (1958) have demonstrated that the bulk of the steroids isolated from guinea-pig urine are unconjugated. It is impossible to state with certainty from the data presented whether there are no steroid glucuronides in cat urine or whether there is an inhibitor for β -glucuronidase. In view of the fact that trebling the amount of enzyme had no effect and in view of the work of Robinson & Williams (1958) it appears probable that glucuronide formation is deficient in the cat.

SUMMARY

1. Corticoids have been determined in the urines of cats with and without incubation with β -

glucuronidase. After incubation with glucuronidase there does not appear to be a statistically significant increase.

2. Results are considered to support the finding of Robinson & Williams (1958) of a lack of glucuronide formation by cats.

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