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Urinary Steroids of Cats

1. ABSENCE OF 17-OXO STEROIDS

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Not much information is available on steroid metabolism in the cat. Borrell (1958) reported that cats do not excrete corticoid glucosiduronates via the urine, though small amounts of Porter-Silber chromogens were present as the free compounds. Taylor & Scratcherd (1960, 1962) have shown that metabolites of administered [4-¹⁴C]progesterone and [4-¹⁴C]corticosterone are excreted mainly in the bile, with only a small amount of radioactivity appearing in the urine. Setekleiv, Skang & Kaada (1961) showed an increase in the plasma 17-hydroxy corticosteroids after cerebral-cortical and amigdaloid stimulation.

To the best of the author's knowledge, the presence of 17-oxo steroids has not been reported in the urine of cats, although such compounds have been studied in the urines of dogs (Glenn & Heftmann, 1951; Tewell & Freeman, 1954), rats (Richter 1962; Munday & Blane, 1960; Paroli & Melchiorri, 1961), rabbits (Davis, Slater & Krichesky, 1949), cows (Klyne & Wright, 1959), goats (Klyne & Wright, 1957), guinea pigs (Brooks, Clayton & Hammant, 1960) and pigs (Huis in't Veld, Louwerens & Reilingh, 1961).

The study of steroid metabolism in the cat is desirable for more than the obtaining of knowledge of comparative endocrinology. Since the cat appears to be deficient in its ability to conjugate materials with glucuronic acid (Borrell, 1958; Hartiala, 1955; Dutton & Greig, 1957; Dutton, 1959; Robinson & Williams, 1958; Stevenson & Dutton, 1962), it is possible that a study of steroid metabolism in the cat may reveal other pathways for the conjugation of steroids.

In the present paper the results of studies of the urinary excretion of 17-oxo steroids by the cat are reported.

METHODS

Animals. Adult male and female cats weighing 2.5–3 kg. were used. To obtain complete collections of urine the bladders were placed between the skin and the ventral musculature by using the operative procedure of Arteta & Borrell (1958). The animals were not used until at least 2 weeks after the operation.

Treatment of urine. No preservatives were added to the urine. Hydrolysis and extraction were usually performed on the day of collection, but in a few experiments urines were kept at 4° for 2 days.

Urine samples were hydrolysed and extracted by one of the four procedures following:

Method A. The urinary steroids were acid-hydrolysed and extracted with ether, and the acidic components removed by washing the ether with 2N-sodium hydroxide, as described by Pincus (1945). The washed extracts were dried on a steam bath.

Method B. After acidification, the urine was extracted with ether continuously for 48 hr. The ether extracts were treated as in method A.

Method C. The urine was acid-hydrolysed and extracted continuously with carbon tetrachloride according to the procedure of Dorfman & Shipley (1956). The final carbon tetrachloride extracts were evaporated to dryness under reduced pressure. The residues were dissolved in ether and the ether extracts were treated as in method A.

Method D. The sample of urine was brought to boiling under reflux. Then 0.1 vol. of conc. hydrochloric acid was added and the boiling was continued for 10 min. After cooling under running water, 0.33 vol. of carbon tetrachloride was added and the mixture was refluxed again for 10 min. This first extract was removed, and a second portion

of solvent was added to the urine and refluxed for 10 min. The combined carbon tetrachloride extracts were washed with sodium hydroxide and water. The washed extracts were evaporated to dryness under reduced pressure.

Girard reaction. Ketonic fractions were separated from the total neutral fractions obtained by method D by using Girard T reagent (Girard & Sandulesco, 1936).

Colour reaction. Convenient portions from the different preparations were assayed for Zimmermann chromogens, by using either aqueous (Pincus, 1945) or ethanolic potassium hydroxide (Medical Research Council Committee on Clinical Endocrinology, 1951). The colour intensities and absorption spectra were determined in a Unicam spectrophotometer (SP. 600) having a range 350–1000 m μ . Dehydroepiandrosterone was used as the reference standard.

RESULTS

Four differing procedures for hydrolysis and extraction were used for various reasons. Method A, which is widely used for the extraction of 17-oxo steroids from human urine, was unsatisfactory for cat urines because it yielded troublesome ether–urine emulsions. Method B can cause destruction of steroids and artifact formation during the prolonged exposure to acid and heat. Method C, with carbon tetrachloride, is also widely used for obtaining 17-oxo steroids from human urine. It does not lead to emulsion formation. However, it also involves prolonged exposure to acid and heat. Method D was therefore devised. In method D, the exposure to heat and acid was minimal.

'Zimmermann chromogen' values were determined in 33 total neutral extracts from pooled urines and individual urines, made by the four procedures. In all cases an atypical yellow colour was obtained.

Fig. 1. Absorption spectra for Zimmermann chromogens of 20 μ g. of dehydroepiandrosterone (Δ) and of the ketonic ($\bullet$) and non-ketonic ($\circ$) fractions of extracts of cat urine. For each 24 hr. urine sample, ketonic extracts were dissolved in 5 ml. of ethanol and non-ketonic extracts in 50 ml. of ethanol. Samples of 0.1 ml. of each fraction were used for the colorimetric determination.

Dehydroepiandrosterone standards included in each run gave a normal colour.

Because the colour obtained was atypical, the total neutral-lipid extracts made by method D from five individual cats were separated into ketonic and non-ketonic fractions. Of the total neutral-lipid chromogen, only 10 % of the chromogenic material was recovered in the ketonic fraction, and 90 % of the chromogenic material was in the non-ketonic fraction. The Zimmermann chromogen was an atypical yellow for both non-ketonic and ketonic extracts. As shown in Fig. 1, neither fraction had an absorption maximum at 520 m μ , the absorption maximum for dehydroepiandrosterone. Results did not differ when ethanolic potassium hydroxide was used instead of aqueous potassium hydroxide. Also, for full colour development and development of the entire spectra shown, both potassium hydroxide and *m*-dinitrobenzene were needed: 20 μ g. of dehydroepiandrosterone added to total neutral-lipid extracts and to urinary ketonic fractions reacted with *m*-dinitrobenzene and potassium hydroxide to give the typical Zimmermann chromogen, and there was no diminution of its colour.

A single intramuscular injection of 20 i.u. of adrenocorticotrophic hormone (ACTH) was given to each of five cats, and 12 i.u. to each of another group of four cats. Urines were collected for the next three 24 hr. periods after the administration of ACTH. No significant changes were observed in the absorption at 520 m μ of the atypical chromogenic material in either the total neutral extracts or the ketonic or non-ketonic fractions.

No difference could be found between the urines of male and female cats. Therefore 5 mg. of testosterone propionate was administered to each of two cats by intramuscular injection. There was no increase in the atypical chromogen and no appearance of material with maximum absorption at 520 m μ .

DISCUSSION

From the above results it appears that cats do not excrete 17-oxo steroids in the urine. Cat urinary extracts purified with Girard's reagent do not give the typical Zimmermann reaction for 17-oxo steroids. The absorption maximum is at 370 m μ , not at 520 m μ , and the extinction (corrected for dilution) of the non-ketonic fraction at 520 m μ is greater than that of the ketonic fraction. The absorption spectra obtained with total neutral, ketonic and non-ketonic extracts of cat urine were similar to those reported by Glenn & Heftmann (1951) for extracts from urines of dogs.

Not only was no sex difference in the excretion of chromogenic material found, but the administration of testosterone propionate did not lead to detectable excretion of 17-oxo steroids.

After the administration of ACTH no change in the 17-oxo steroid could be demonstrated, although an increase in urinary corticoids (Porter-Silber chromogen) after giving ACTH has been reported by Borrell (1958). Glenn & Heftmann (1951) and Tewell & Freeman (1954) also reported that no increase in the excretion of 17-oxo steroids in dog urine could be found after intramuscular administration of ACTH.

As pointed out by Klyne & Wright (1959), it could be possible that animal urines contain unknown interfering constituents that make the Zimmermann reaction unreliable. However, four different procedures for hydrolysis and extraction, which might yield extracts with differing amounts of interfering substances, all failed to yield Zimmermann-positive material. Dehydroepiandrosterone added to urinary extracts could be determined quantitatively, so the absence of Zimmermann chromogen in urinary extracts was not due to interference with colour development. The development of the atypical yellow chromophore required *m*-dinitrobenzene as well as potassium hydroxide.

The apparent absence (or extremely low concentration) of 17-oxo steroids in cat urine could reflect (a) a quantitative difference from other species in the biosynthesis of C₁₉ steroids, (b) different pathways of metabolism of C₁₉ and C₂₁ steroids to compounds not containing 17-oxo groups, or (c) a difference in the route of excretion for 17-oxo steroids. The failure by cats to excrete 17-oxo steroids after the administration of testosterone propionate indicates that either explanation (b) or (c) is responsible for the experimental results, whether or not biosynthesis of C₁₉ steroids is also lower in the cat. Taylor & Scratcherd (1960, 1962) have reported that the major pathway for the excretion of metabolites of progesterone and corticosterone is via the bile and the faeces.

SUMMARY

1. Cats do not appear to excrete 17-oxo steroids in the urine.

2. Hydrolysis and extraction of urine, followed by the Girard separation and the application of the

Zimmermann reaction, gave rise to atypical yellow colours in both ketonic and non-ketonic fractions.

3. After the administration of adrenocorticotrophic hormone and of testosterone propionate, it was still not possible to demonstrate typical Zimmermann chromogens in cat urine.

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