A Method for the Quantitative Determination in Urinary Extracts of C_{21} 17:20-Dihydroxy-20-methylsteroids

By R. I. COX

Department of Biochemistry, University of Edinburgh

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Hyperactivity of the adrenal cortex in human subjects due to malignant neoplasm, hyperplasia or stimulation by adrenocorticotropic hormone (ACTH) has frequently been observed to be associated with the urinary excretion of considerable amounts of various C_{21} 20-methylsteroids (see Table 1).

Table 1. *20-Methylsteroids, probably of adrenal origin, isolated from human urine*

Compound	Associated condition of subjects	References
Pregnane-3α:17α:20α-triol	Adrenal hyperplasia	Butler & Marrian (1937, 1938)
	Adrenal hyperplasia	Mason & Kepler (1945)
	Adrenocortical tumour	
Pregnane-3α:17α-diol-20-one	Adrenal hyperplasia and adrenocortical tumour	Lieberman & Dobriner (1945)
	Adrenal hyperplasia	Miller & Dorfman (1950)
Pregn-5-ene-3β:17α:20α-triol	Adrenocortical tumour	Hirschmann & Hirschmann (1950)
Pregn-5-ene-3β:17α-diol-20-one	Adrenocortical tumour	Hirschmann & Hirschmann (1947)
Pregnane-3α:20α-diol-11-one	Adrenal hyperplasia	Lieberman, Fukushima & Dobriner (1950)
Pregnan-3α-ol-11:20-dione	Adrenal hyperplasia	Lieberman *et al.* (1950)
Pregnane-3α:17α-diol-11:20-dione	After administration of ACTH and cortisone to patients with neoplasia	Lieberman *et al.* (1951)

Such urinary 20-methylsteroids may arise directly from related 20-methylsteroids present in the adrenal glands, but there is good reason to suppose that they may in part arise by metabolic reduction at C-21 of 21-hydroxysteroids secreted by the glands. The evidence that such metabolic reduction of 21-hydroxysteroids to 20-methylsteroids, i.e. to 21-deoxysteroids can occur in the human body appears to be conclusive. Thus Cuyler, Ashley & Hamblen (1940) and Horwitt, Dorfman, Shipley & Fish (1944) isolated pregnane-3α:20α-diol from the urine of human subjects after the administration of 11-deoxycorticosterone acetate; Mason (1948) isolated pregnane-3α:20α-diol-11-one after the administration of 11-dehydrocorticosterone; while more recently Lieberman, Hariton, Stockem, Studer & Dobriner (1951) have isolated pregnane-3α:17α-diol-11:20-dione after administering cortisone acetate. It has been suggested that the principal C_{21} adrenocortical hormone secreted by the adrenal glands in man may be 17-hydroxycorticosterone (Reich, Nelson & Zaffaroni, 1950; Hechter, 1950; Pincus, Hechter & Zaffaroni, 1951). In view of this possibility and because of the widespread therapeutic use of 17-hydroxy-11-dehydrocorticosterone (cortisone), it was thought that a specific method for the quantitative determination in urine of C_{21} 17:20-dihydroxy-20-methylsteroids which might be formed from these two hormones (and from 17-hydroxy-11-deoxycorticosterone) by metabolic reduction of the C-21 hydroxyl group, would be of some value and interest.

As is well known, C_{21} steroids with either α-ketol or α-glycol side chains give almost quantitative yields of formaldehyde on oxidation with periodic acid, and this reaction has been widely employed for the quantitative determination in suitably prepared urine extracts of the so-called 'formaldehydogenic' steroids (Lowenstein, Corcoran & Page, 1946; Corcoran & Page, 1948; Daughaday, Jaffe & Williams, 1948). On oxidation with periodic acid, C_{21} 17:20-dihydroxy-20-methylsteroids, and steroids of no other type, should yield acetaldehyde. Accordingly, the possibility was envisaged of determining both formaldehydogenic and acetaldehydogenic steroids in urinary extracts by periodate oxidation with separation and estimation of the formaldehyde and acetaldehyde thus produced. Talbot & Eitingon (1944) have estimated the 17-ketosteroids formed after periodate oxidation of urine extracts, thus measuring the combined total of C_{21} 17:20:21-trihydroxysteroids and C_{21} 17:20-dihydroxy-20-methylsteroids.

With pregnane-3α:17α:20α-triol (Butler & Marrian, 1937, 1938) preliminary experiments showed that nearly quantitative yields of acetaldehyde as determined colorimetrically in sulphuric acid with 4-hydroxydiphenyl (Miller & Muntz, 1938; Koenemann, 1940; Barker & Summerson, 1941) could be obtained after oxidation with periodic acid. In further experiments in which mixtures of pregnane-3α:17α:20α-triol and 11-deoxycorticosterone or 17-hydroxy-11-deoxycorticosterone were oxidized with periodic acid, it was found possible to remove the acetaldehyde quantitatively from a reaction mixture containing glycine without loss of formaldehyde, by aeration into a bisulphite trap (cf. Shinn & Nicolet, 1941). The formaldehyde could then be recovered quantitatively in the usual way by distillation (Daughaday *et al.* 1948).

To test out the potentialities of the procedure for the determination of acetaldehydogenic steroids in urine, recovery experiments were carried out in which known amounts of pregnane-3α:17α:20α-triol were added to both unwashed and sodium hydroxide-washed chloroform extracts of urine from normal men. These extracts were obtained from fresh urine, from urine after incubation with ox-spleen β-glucuronidase at pH 4·5, and from urine extracted after standing at room temperature at pH 1 for 24 hr. In every experiment 'blank' determinations were carried out on a portion of the extract with no added pregnanetriol.

Reasonably satisfactory recoveries were obtained in those experiments in which the triol was added to sodium hydroxide-washed chloroform extracts of urine. In those in which unwashed extracts were employed, the recoveries were irregular, indicating possibly the presence of material interfering in some way with the periodate oxidation of the triol. It may be noted that Hollander, Di Mauro & Pearson (1951), studying the recovery in periodate oxidations of 11-deoxycorticosterone added to urine extracts, concluded that the oxidation could be inhibited by substances present in crude extracts.

The 'blank' experiments all indicated the presence of acetaldehydogenic material in the extracts, and it is noteworthy that from the various extracts the amounts of acetaldehyde obtained were roughly of the same order as those of formaldehyde and, like the latter, were increased both by glucuronidase and acid hydrolysis of the urine. It is also noteworthy that there was little or no increase in chloroform extractable acetaldehydogenic material over that obtained from untreated urine when incubation with glucuronidase was carried out in the presence of saccharate. Since the latter is a competitive inhibitor of β-glucuronidase (Karunairatnam & Levvy, 1949), this supports the suggestion that acetaldehydogenic material released during enzymic incubation was present as a β-glucuronide. It is pertinent to note that Mason & Kepler (1945) isolated from adrenal carcinoma urine a mixture of steroid conjugates which yielded pregnane-3α:17α:20α-triol after incubation with a crude rat-liver glucuronidase preparation.

As far as can be judged from recovery experiments with pregnane-3α:17α:20α-triol, the procedure developed should provide a satisfactory method for determining acetaldehydogenic steroids in sodium hydroxide-washed chloroform extracts of untreated urine, and of urine hydrolysed enzymically or with acid. It is not claimed that this procedure provides a means for the quantitative determination of such steroids in urine, since no demonstrably quantitative methods have yet been developed for the hydrolysis of the conjugated acetaldehydogenic steroids which must be assumed to be present in urine, or for the extraction of the free steroids after hydrolysis. In fact, in these respects the method is neither better nor worse than the widely employed procedures for the determination of urinary reducing or formaldehydogenic steroids, the difficulties involved in which have been recently summarized by Marrian (1951).

EXPERIMENTAL

Apparatus. The apparatus used is shown in Fig. 1. *A* is the oxidation tube; *B* contains aqueous $NaHSO_3$ solution in which acetaldehyde, carried over from the oxidation mixture in the air stream, is trapped. A second bisulphite trap has not been found necessary under the conditions used.

Pregnane-3α:17α:20α:-triol, m.p. 245–247° (Butler & Marrian, 1938) has been used throughout as the reference acetaldehydogenic steroid. All steroid samples were dried *in vacuo* over P_2O_5 before weighing. Standard solutions were prepared in redistilled ethanol.

Method. Ethanolic solutions of pure steroids or of suitably prepared urine extracts are evaporated in oxidation tubes *A* (Fig. 1) to about 1 ml. under a rapid stream of filtered air on a water bath at about 80°. Evaporation to dryness is carried out *in vacuo* with warming on a water bath. The residues are moistened with 0·10 ml. glacial acetic acid (distilled off CrO_3). The tubes are warmed slightly in a water bath and rotated to wet any residue adhering to the sides of the tube. Equal volumes of 0·12 M-periodic acid in 0·4 N-H_2SO_4 (A.R.) and 1 % glycine in 0·4 N-H_2SO_4 (A.R.) are freshly mixed, and 1 ml. portions of the mixture pipetted into the oxidation tubes. The trap tubes *B*, each containing 2 ml. 1 % $NaHSO_3$ solution, are immediately connected up. A slow stream of air, purified by passage through conc. H_2SO_4 (A.R.) and a soda-lime tower is blown through the sets of tubes arranged in parallel. The rate of aeration is about 5 ml./min.

After 45 min. of oxidation with aeration, the air flow is stopped and the tubes are disconnected. The formaldehyde retained in the oxidation mixture is estimated as in the method of Daughaday *et al.* (1948), using 3 ml. 6 % (w/v) $SnCl_2$ (A.R.) solution to reduce the correspondingly larger excess of periodic acid present.

The bisulphite traps are made up to 3 ml. by washing down the ends of the connecting tubes with distilled water,

and 1 ml. samples of the solutions pipetted into dry 150 × 25 mm. test tubes. The colorimetric estimation of acetaldehyde in these samples is then carried out according to Barker & Summerson (1941), except that 7·0 ml. portions of conc. H_2SO_4 (A.R.) are added to the samples, and colour development is carried out at 25°.

The absorption of the solutions is determined with a Hilger Spekker absorptiometer using Ilford no. 605 filters.

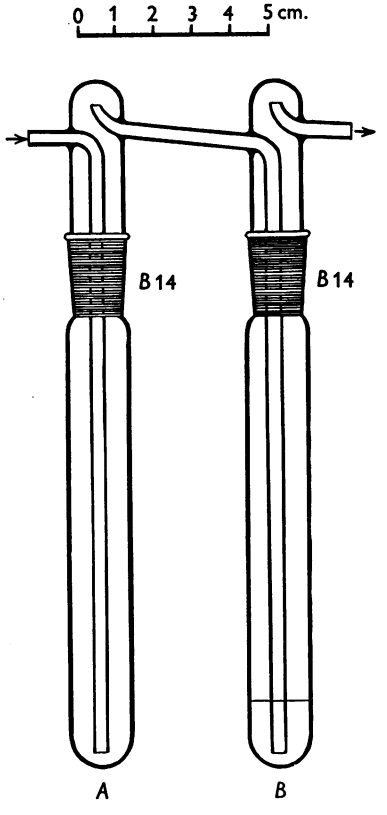

Fig. 1. Apparatus used for oxidation with aeration. *A*, oxidation tube; *B*, bisulphite trap graduated at 3·0 ml. Arrow indicates direction of air stream.

Colorimetric estimation of acetaldehyde

The method of Miller & Muntz (1938), as modified by Koenemann (1940) and Barker & Summerson (1941), must be rigorously standardized to obtain reproducible results. Particular attention should be paid to the purity of the A.R. H_2SO_4. For instance, Russell (1944) has shown that presence of nitrates or nitrites in the sulphuric acid affects the colour development. Therefore each bottle of H_2SO_4 should be checked before use and those giving poor colour development discarded.

Also to be noted is the advice of Barker & Summerson (1941) against the use of chromic acid mixtures for cleaning apparatus used in the acetaldehyde estimations, because of the difficulty of removing interfering traces of the cleaning agent.

Optimum conditions for the oxidation of pregnane-3α:17α:20α-triol

The effects of varying periodic acid concentration, time of aeration and acidity of the oxidation mixture on the yield of acetaldehyde obtained by periodate oxidation of pregnanetriol are illustrated in Tables 2–4. Apart from the variable studied, other conditions in these experiments were as described in the method. The oxidation-aerations have been carried out at room temperature, i.e. 13–21° and within this range the recoveries of acetaldehyde have not been affected by temperature.

Table 2. *Effect of periodic acid concentration on oxidation of pregnane-3α:17α:20α-triol*

(58·0 μg. pregnanetriol samples oxidized.)

Concn. of periodic acid (M)	Acetaldehyde found (μg.)	Equivalent amount of triol (μg.)	Recovery (%)
0·24	7·80	59·5	103
	7·80	59·5	103
0·12	7·61	58·0	100
	7·80	59·5	103
0·04	7·87	60·0	103
	6·69	51·0	88
0·01	5·50	42·0	72
	6·69	51·0	88

Table 3. *Effect of oxidation aeration time on yield of acetaldehyde from pregnane-3α:17α:20α-triol oxidized with* 0·06 M *-periodic acid*

(25·1 μg. pregnanetriol samples oxidized.)

Time aerated (min.)	Acetaldehyde found (μg.)	Equivalent of triol (μg.)	Recovery of triol (%)
5	0·11	0·9	4
10	0·45	3·4	13
15	0·62	4·7	19
20	1·13	8·6	34
25	2·29	17·4	69
30	3·07	23·4	93
40	3·32	25·2	100
50	3·27	24·9	99

Table 4. *Effect of acidity of oxidation mixture on yield of acetaldehyde from pregnane-3α:17α:20α-triol in periodate oxidation*

(25·1 μg. pregnanetriol samples oxidized.)

Normality of H_2SO_4 in oxidation mixture	Acetaldehyde found (μg.)	Equivalent amount of triol (μg.)	Recovery of triol (%)
0	0·87	6·7	27
0·01	2·31	17·6	70
0·05	2·97	22·8	90
0·1	2·76	21·1	84
0·2	3·21	24·5	98
0·4	3·35	25·6	102
0·8	3·23	24·7	98

Table 5 shows the recoveries of acetaldehyde after oxidation of pregnanetriol with 0·06 M-periodic acid in 0·4 N-H_2SO_4 with aeration for 45 min. as in the method finally adopted.

Oxidation of mixtures of acetaldehydogenic and formaldehydogenic steroids

Preliminary experiments with aqueous solutions of acetaldehyde and formaldehyde showed that a satisfactory separation of the aldehydes at levels of 10 μg. or less was possible under the conditions used for the oxidation and estimation of pregnanetriol. These conditions would be expected to permit quantitative formation of formaldehyde from formaldehydogenic steroids. It was therefore probable that the acetaldehyde and formaldehyde formed on periodate oxidation of mixtures of C_{21} steroids with various types of C-17 side chains could be separated and hence 10–100 μg. amounts of steroids estimated quantitatively.

Table 5. *Recovery of acetaldehyde on oxidation of pregnane-3α:17α:20α-triol by the method finally adopted*

(Oxidation with 0·06 M-HIO_4 in 0·4 N-H_2SO_4.)

Pregnanetriol oxidized (μg.)	Acetaldehyde found (μg.)	Triol equivalent to acetaldehyde found (μg.)	Recovery (%)
58·0	7·38	56·3	97
43·5	5·58	42·6	98
29·0	3·82	29·2	101
17·4	2·16	16·5	95
14·5	1·78	13·6	94

From mixtures of pregnane-3α:17α:20α-triol and 11-deoxycorticosterone or 11-deoxy-17-hydroxycorticosterone the amounts of acetaldehyde and formaldehyde recovered were consistent with the respective amounts of steroids present (Table 6).

Urinary studies

Collection of urine. Urine specimens were collected over 24 hr. with 1–2 ml. $CHCl_3$ as preservative and were analysed as soon after collection as possible.

Preparation of urine extracts. Extracts prepared from unhydrolysed and hydrolysed urine samples have been studied. Two methods have been used for hydrolysis of urine samples. (1) Acidification of urine: a measured portion of 50–100 ml. of a 24 hr. specimen was acidified to pH 0·95–1·1 (glass electrode) with 10 N-H_2SO_4. After 24 hr. at 25° the acidified urine was extracted with $CHCl_3$. For 'hydrolysis curves' larger portions of 24 hr. specimens were acidified to allow a number of samples to be withdrawn for analysis at various time intervals. (2) Incubation of urine with β-glucuronidase preparations: the crude β-glucuronidase was prepared (cf. Kerr & Levvy, 1951) by suspending finely minced fresh ox spleen in acetate buffer, pH 5·2, and standing at 37° for 4 hr. After centrifuging off tissue debris, the supernatant was made 60% saturated with $(NH_4)_2SO_4$. The precipitate was dialysed free of $(NH_4)_2SO_4$, and in further fractionations the material precipitated between 25 and 50% saturated $(NH_4)_2SO_4$ was retained. After dialysis this crude enzyme preparation was assayed, using phenylglucuronide as substrate, and expressing the activity in 'glucuronidase units' (G.U.) where 1 G.U. liberates 1 μg. phenol in 1 hr. under the conditions given by Kerr, Graham & Levvy (1948).

For incubation with enzyme, 25–50 ml. of a 24 hr. urine specimen was acidified to pH 4·5 with acetic acid and 10 ml. 0·1 M-acetate buffer, pH 4·5, added. A solution containing enzyme in the proportion of 10 000 G.U./24 hr. specimen was added and the mixture incubated at 37° for 24 hr. An enzyme blank with 25–50 ml. of water in place of urine was carried out simultaneously. Blank values have been consistently less than 10% of the corresponding estimations.

Neutralization of urine prior to extraction. For comparative purposes it is desirable to extract urine samples after

Table 6. *Recovery of acetaldehyde and formaldehyde from periodate oxidation of mixtures of pregnane-3α:17α:20α-triol (triol), 11-deoxycorticosterone (DOC) and 17-hydroxy-11-deoxycorticosterone (S)*

Steroid oxidized (μg.)			Acetaldehyde found (μg.)	Formaldehyde found (μg.)	Steroids (μg.) equivalent to CH_3CHO and H.CHO found		Steroids oxidized (%)	
Triol	DOC	*S*			Acetaldehydogenic	Formaldehydogenic	Acetaldehydogenic	Formaldehydogenic
58·0	—	36·0	7·42	2·76	56·7	31·9	98	89
34·8	—	36·0	4·50	2·76	34·4	31·9	99	89
11·6	—	36·0	1·60	3·13	12·2	36·1	105	100
52·7	—	54·0	6·05	5·04	46·2	58·1	88	108
31·6	—	54·0	3·96	4·90	30·2	57·0	96	106
21·1	—	54·0	2·88	4·30	21·8	49·6	103	92
10·5	—	54·0	1·40	4·57	10·7	52·8	102	98
52·7	—	72·0	6·66	5·84	50·8	67·4	96	94
31·6	—	72·0	3·49	5·78	26·6	66·7	84	93
21·1	—	72·0	2·97	5·73	22·8	66·1	108	92
52·7	63·2	—	6·33	5·25	48·3	57·7	92	91
31·6	63·2	—	4·05	5·46	30·9	60·0	98	95
10·5	63·2	—	1·47	5·73	11·2	63·0	107	100
52·7	63·2	—	6·75	5·67	51·5	62·4	98	99
31·6	15·8	—	4·50	1·43	34·4	15·7	104	99
21·1	15·8	—	2·70	1·48	20·6	16·3	98	103
10·5	15·8	—	1·35	1·38	10·3	15·2	98	96
52·7	31·6	—	6·75	2·70	51·5	29·7	98	94
31·6	31·6	—	3·96	2·65	30·2	29·2	96	92
10·5	31·6	—	1·37	2·81	10·5	30·9	100	98

various pretreatments under similar conditions. In most experiments reported here, urine samples, prior to extraction, were brought to pH 6·9–7·1 (glass electrode) using 2N-Na_2CO_3 and 0·5M-K_2HPO_4. The samples were stirred vigorously during the addition of alkali.

Extraction of urine. Urine samples were extracted three times with 2 vol. of $CHCl_3$. Using these proportions, emulsions were rarely obtained. The $CHCl_3$ extracts were washed once with 0·1 vol. 0·1N-NaOH and twice with 0·1 vol. water. The water washings were washed once with 2 vol. $CHCl_3$, this $CHCl_3$ being added to the main $CHCl_3$ extract. The $CHCl_3$ extracts were dried with the minimum amount (about 2 g./100 ml.) of anhydrous Na_2SO_4 (A.R.) for 20 min. After filtering off the Na_2SO_4 the $CHCl_3$ extracts were evaporated to dryness under reduced pressure in a water bath at about 60°. The residues were transferred with three 2 ml. portions of redistilled ethanol to the oxidation tubes *A* (Fig. 1). The subsequent procedure is detailed in the Method section (p. 340).

Recovery experiments on urine extracts. The recoveries of pregnane-3α:17α:20α-triol added to $CHCl_3$ extracts of unhydrolysed, acid and enzymically hydrolysed urine were studied, in addition to the recovery of pregnanetriol added to the urine itself (added in 0·5–1·0 ml. ethanol) with subsequent extraction of the urine and purification of extracts. Recoveries were calculated from the increase in acetaldehyde obtained from extracts containing added pregnanetriol over that given by extracts without added triol.

The recoveries of pregnanetriol added to $CHCl_3$ extracts of urine where no alkali washing was carried out, varied from urine to urine (Table 7). With alkali-washed extracts recoveries were consistent, and are illustrated in the results obtained for the recovery of pregnanetriol added to urine itself with subsequent $CHCl_3$ extraction (Table 8).

The urine 'blank' values, i.e. acetaldehyde obtained from urine extracts without added triol, are a measure of the acetaldehydogenic material extractable from urine and may indicate the presence of C_{21} 17:20-dihydroxy-20-methylsteroids. The blank values given in Tables 7 and 8 are expressed as mg. pregnanetriol/24 hr.

In Table 9 are compared 'blank' values obtained from untreated urine, acid hydrolysed and enzymically hydrolysed urine from normal male subjects. The values obtained after acidification or incubation with β-glucuronidase preparations are considerably higher than those from untreated urine (cf. formaldehydogenic steroids in urine (Heard, Sobel & Venning, 1946; Paterson, Cox & Marrian, 1950; Kinsella, Doisy & Glick, 1950; Cox & Marrian, 1951). The amount of acetaldehydogenic material extractable after acidification of urine from normal males increases with time. The increase is rapid for a few hours, after which very little change is observed up to 24 hr. after acidification. ($CHCl_3$

Table 7. *Irregular recovery of pregnanetriol added to unwashed chloroform extracts of normal male urine*

		Pretreatment of urine					
		(1) None		(2) Acid hydrolysis		(3) Enzymic hydrolysis	
	Triol added (μg.)	Triol recovered (%)	Urine 'blank' (mg. as triol/ 24 hr.)	Triol recovered (%)	Urine 'blank' (mg. as triol/ 24 hr.)	Triol recovered (%)	Urine 'blank' (mg. as triol/ 24 hr.)
Urine I	0	—	0·98	—	2·15	—	3·90
	0	—	0·97	—	2·02	—	4·12
	10	19	—	102	—	35	—
	25	8	—	54	—	69	—
	50	62	—	52	—	59	—
Urine II	0	—	0·86	—	2·63	—	3·64
	0	—	0·94	—	2·79	—	3·88
	10	82	—	95	—	117	—
	25	103	—	101	—	90	—
	50	100	—	93	—	77	—

Table 8. *Recovery of pregnanetriol added to urine from normal male subjects. (Chloroform extracts alkali-washed)*

(31·5 μg. triol added to each sample.)

Subject	Urine 'blank' (value as mg. triol/day)	Recovery of added triol (%)
A	0·33	96
	(0·26)*	—
B	0·48	94
	0·49	95
C	0·40	93
	0·40	93
D	0·26	90
	0·29	93

* Loss during processing.

Table 9. *Amounts of acetaldehydogenic material found in urine of normal male subjects*

(All values expressed as mg. pregnanetriol/24 hr.)

Urine sample	Treatment of urine prior to chloroform extraction: Untreated urine	Acidification to pH 1, with immediate extraction	Acidification to pH 1; extracted after 24 hr.	Incubated at 37°, pH 4·5, with β-glucuronidase
	(*A*) Chloroform extracts alkali-washed			
I	0·33	0·44	0·92	—
II	0·28	0·43	—	—
III	0·36	—	—	1·58
IV	0·47	—	—	—
V	0·48	0·93	0·80	1·94
VI	0·12	0·19	0·29	0·66
VII	0·28	0·32	0·45	—
	(*B*) Chloroform extracts not alkali-washed			
VIII	0·92	2·96	2·80	3·60
IX	0·72	3·20	3·40	4·80

extracts were alkali-washed.) As yet, insufficient study has been made of such 'hydrolysis curves' to permit of interpretation, but they indicate the presence in urine of conjugates hydrolysed by acid at 25°. With regard to the acid stability of acetaldehydogenic steroids, it has been found that pregnane-3α:17α:20α-triol is stable to pH 1 at 25° for 24 hr.

DISCUSSION

Estimation of acetaldehyde formed by periodate oxidation of urine extracts is more specific and sensitive than the estimation of 17-ketosteroids formed (Talbot & Eitingon, 1944). As C_{21} 17:20:21-trihydroxysteroids as well as C_{21} 17:20-dihydroxy-20-methylsteroids yield 17-ketosteroids on periodate oxidation, these types of steroids are estimated together in the latter method. It is desirable for metabolic studies that these two types, which include both 21-hydroxy- and 21-deoxy-steroids, should be estimated separately. In addition to the general disadvantages of the 17-ketosteroid methods the Talbot & Eitingon (1944) method estimates the relatively small increase of 17-ketosteroids after periodate oxidation over that present before oxidation.

It has been found that neutral chloroform extracts of normal male urine contain substances which liberate acetaldehyde when oxidized with periodic acid under the same conditions necessary for quantitative oxidation of 17:20-dihydroxy-20-methylsteroids.

The presence of some types of conjugates was indicated by the increased amounts of acetaldehydogenic material extractable from urine after treatment with acid or β-glucuronidase preparations. Although C_{21} 17:20-dihydroxy-20-methylsteroids have been isolated from urine of patients with hyperactive adrenals, proof of the presence of such compounds in normal urine must await isolation studies. This work is now in progress.

Some of the hydrolysis techniques used in the study of urinary formaldehydogenic steroids have been applied to the study of acetaldehydogenic substances. These relatively mild hydrolysis conditions were employed to avoid excessive destruction of the sensitive C-17 side chain (in particular the tertiary hydroxyl at C-17). The value of enzymic hydrolysis with β-glucuronidase (especially when carried out on urine directly) for quantitative assay of urinary cortical steroids or their metabolites seems doubtful at present, when there is not adequate evidence that such enzymic hydrolysis is complete.

Acetaldehyde gives a brown coloration with chromotropic acid (McFadyen, 1945) and may interfere in the estimation of formaldehyde if present in relative excess over the latter. Where estimation of the acetaldehyde is not desired, it may be removed by aeration from the formaldehyde distillates in the Daughaday (1948) method if 1% aqueous glycine is used in place of the ethanolic sulphite solution.

SUMMARY

1. A method for estimating C_{21} 17:20-dihydroxy-20-methylsteroids in urine, based on periodic acid oxidation, is described.

2. The method allows of the simultaneous estimation of acetaldehydogenic and formaldehydogenic steroids.

3. A urinary excretion of unconjugated acetaldehydogenic material equivalent to 0·1–0·5 mg./day of C_{21} 17:20-dihydroxy-20-methylsteroids was found for seven normal males.

4. The presence of conjugated acetaldehydogenic material in normal male urine is also indicated.

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The Metabolism of Subcutaneously Injected [^{15}N] Urea in the Cat

By H. L. KORNBERG and R. E. DAVIES

Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield 10

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The experiments described in this paper arose from a study of the physiological role of gastric urease. In order to throw light on this problem, an investigation was undertaken into the fate of [^{15}N]urea in the cat.

Although Luck (1924) demonstrated the presence of urease in the stomach, urea has been widely regarded solely as an end product of mammalian nitrogen metabolism. This view was supported by experiments of Schoenheimer (1942) and Bloch (1946) who fed [^{15}N]urea to rats and found no more than a slight incorporation of ^{15}N into the proteins of liver and intestine. They believed that this could be interpreted as the result of bacterial decomposition of urea in the gut. More recently, Leifer, Roth & Hempelmann (1948) injected [^{14}C]urea into mice and recovered 20·8% of the injected ^{14}C in the carbon dioxide of the expired air. The breakdown of [^{14}C]urea in mice has been confirmed by Jones (1950) and by Skipper *et al.* (1951). Kornberg, Davies & Wood (1952) made analogous observations on cats.

In the study of the metabolism of [^{15}N]urea it must be borne in mind that the ammonia produced by hydrolysis of urea is largely reconverted to urea in the liver. Hence the amounts of ^{15}N incorporated into other nitrogenous materials give only minimum values for the urea breakdown. In order to arrive at an estimate of the actual breakdown, information is required on the conversion of [^{15}N]ammonia to [^{15}N]urea under the conditions of the experiment.

Although previous workers have studied the fate in the animal body of [^{15}N]ammonia after oral administration of [^{15}N]ammonium citrate (Foster, Schoenheimer & Rittenberg, 1939; Rittenberg, Schoenheimer & Keston, 1939; Sprinson, 1948; Sprinson & Rittenberg, 1949), and after intravenous injection of a single dose of [^{15}N]ammonium glutamate (Berenbom & White, 1950), their results did