of such animals to utilize glucose, with a consequent rise of gluconeogenesis in the liver. Such a possibility is supported by the observation of Ashmore, Hastings, Nesbett & Renold (1956) that the administration of insulin can restore the blood-sugar level of alloxan-diabetic rats to normal before any change in glucose 6-phosphatase activity can be detected. The observations recorded in the present paper do not support the view that there need be any direct action of insulin on liver glucose 6-phosphatase.

SUMMARY

1. In the intact rat starvation for 24 hr. caused a rise in liver glucose 6-phosphatase concentration but relatively little change in the total glucose 6-phosphatase activity of the liver. This confirms the previous findings of others.

2. When the rat was allowed to eat glucose *ad lib.* after being starved for 24 hr. the liver glucose 6-phosphatase concentration fell almost to the value seen in the normal animal.

3. The changes in enzyme activity observed in the starved rat and in the rat fed with glucose after starvation for 24 hr. were apparent rather than real and can be satisfactorily explained as a result of changes in liver size and composition.

4. When the rat was allowed to eat fat or casein *ad lib.* after starvation for 24 hr. the glucose 6-phosphatase activity/100 g. of liver was the same as that of the starved animal. However, there was a small but significant rise in the total liver glucose 6-phosphatase activity/100 g. body wt. when the starved rat was fed with fat and a highly significant rise when it was fed with protein.

5. Alloxan diabetes caused a rise in liver glucose 6-phosphatase activity, as had been reported previously, and also a rise in kidney glucose 6-phosphatase activity.

6. The view is put forward that if sufficient protein is available, liver glucose 6-phosphatase activity in the rat rises in response to a deficiency of exogenous glucose.

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Metabolism of 11-Oxygenated Steroids

1. INFLUENCE OF THE A/B RING JUNCTION ON THE REDUCTION OF 11-OXO GROUPS

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In an earlier paper, it was suggested that the pattern of 11-oxo steroids and 11β-hydroxy steroids found in human urine might be explained if the well-known oxidation–reduction of steroid 11-oxygen functions *in vivo* was limited to those steroids having a more or less flat α-surface over the A and B rings (Bush, 1956). It was also suggested that this was a reasonable expectation, since reduction at C-11 is stereospecific and must presumably occur by association of the α-side of the steroid with the enzyme responsible for this reaction: steroids with a buckled or 'bent' A/B ring junction would not be likely to combine firmly with such an enzyme, which was already known to act rapidly on Δ^4-3-oxo steroids which have a roughly planar A/B ring system. It was suggested therefore that the substrate specificity of the 11β-dehydrogenase system in man was determined largely by the steric properties of 11-oxygenated steroids, particularly the A/B ring junction and

substituents on the α-side of the molecule. At the same time Hubener, Fukushima & Gallagher (1956) found that, with rat-liver homogenates, reduction of 11-oxo groups occurred rapidly with Δ^4-3-oxo steroids but not with several 3α-hydroxy-5β-(H) steroids (bent A/B junction); they suggested that the reaction was specific for Δ^4-3-oxo steroids although they had not tested the system with any 5α-(H) steroids (flat A/B junction).

This reaction seemed of interest because it appeared likely that adrenal 11-oxo steroids such as cortisone were not themselves biologically active on the tissues affected by the adrenocortical hormones, but were active when administered systemically only because of their rapid and complete reduction by the liver to the related 11β-alcohols (Bush, 1956; Peterson, Pierce, Wyngaarden, Bunim & Brodie, 1957). An alternative possibility in the light of the known reversible oxidation–reduction of steroid 11-oxygen functions was that such hormones exerted their action in a way similar to the coenzymes, in this case by acting as an essential cofactor in a redox system. This suggestion has been developed further by Talalay & Williams-Ashman (1958), who on the basis of experiments with oestradiol and placental *iso*citric dehydrogenase (Villee, 1955), suggest that since most steroid dehydrogenases in animal tissues can use both diphosphopyridine nucleotide and triphosphopyridine nucleotide [DPN^+ and TPN^+] as coenzyme, steroid hormones may in general exert their action by catalysing transhydrogenation from reduced diphosphopyridine nucleotide to triphosphopyridine nucleotide DPNH to TPN^+. They have shown that this catalysis of transhydrogenation is almost certainly the basis of the stimulation of placental *iso*citric dehydrogenase *in vitro* by oestradiol that was observed by Villee, and point out that the same dual requirement for pyridine nucleotide exists for the steroid 11β-dehydrogenase system of liver as for the oestrogen 17β-dehydrogenase. Despite the convincing explanation of Villee's results offered by Talalay & Williams-Ashman on this basis, our results (Bush & Mahesh, 1957, 1958*b*, *c*) and those of others (Glenn, Stafford, Lyster & Bowman, 1957; Huggins, Jensen & Cleveland, 1954) lead us to favour the alternative general theory, namely that the 11β-hydroxy steroid hormones, but not the related 11-ketones, are active *per se* at their site of action, and that the known oxidation–reduction of the 11-oxygen function of such steroids is not itself directly concerned in their mode of action.

Nevertheless, whichever theory is adopted the properties and specificity of systems responsible for oxidation–reduction of steroids at C-11 are of great interest, since they will probably play an important part, directly or indirectly, in determining the pharmacological activity of 11-oxygenated steroids. In the present paper we describe metabolic experiments in human subjects in which the main aim was to examine the part played by the steroid A/B ring junction in determining the substrate specificity of the steroid 11β-dehydrogenase *in vivo*. In addition, several other metabolic pathways of interest have been demonstrated.

METHODS AND MATERIALS

The steroids used in this study were administered to young healthy male subjects going about their normal business. In order to reduce the complications due to the presence of variable quantities of metabolites of endogenous steroids their adrenal secretion was suppressed to a low level by giving prednisone or prednisolone (1:2-dehydrocortisone or 1:2-dehydrocortisol) before and during the administration of the exogenous steroid precursors.

Samples (24 hr.) of urine were collected under toluene and preserved at 2–4°. Extraction was begun within 2–7 days of collection and all extracts and fractions from chromatograms were stored in the refrigerator between manipulations and kept out of 'fluorescent' lighting at all times.

Blood samples were collected in heparinized syringes and centrifuged immediately after collection. The plasma was drawn off and stored in the refrigerator after solid potassium oxalate had been added.

Chromatography was carried out at 25·5° by the procedure of Bush & Willoughby (1957). The solvent systems were: 1, benzene–methanol–water (2:1:1, by vol.); 2, toluene–methanol–water (4:3:1, by vol.); 3*a*, light petroleum–benzene–methanol–water (67:33:80:20, by vol.); 3*b*, light petroleum–toluene–methanol–water (67:33:85:15, by vol.); 4*a*, light petroleum–methanol–water (100:96:4, by vol.); 4*b*, light petroleum–methanol–water (20:17:3, by vol.). The light petroleum had b.p. 100–120°. Unless otherwise stated Whatman no. 2 filter paper was used. R_F values of reference compounds are given in Table 1.

Urine extracts were pre-fractionated by chromatography on strips of Whatman no. 3MM to give three fractions containing steroids of different polarity. Fraction 1 contained the least polar steroids, such as androsterone, fraction 2 steroids of medium polarity (e.g. 3α:11β-dihydroxy-5α-androstan-17-one) and fraction 3 the most polar steroids such as cortisol.

Reagents and materials

These were prepared and purified by the methods of Bush & Willoughby (1957) with the following modifications and additions.

Decolorite (The Permutit Co. Ltd., London, W. 4). This weakly basic resin was obtained as black irregular grains of mesh range −16 to +50. The resin was sieved with tap water to obtain the mesh range 24–50 and fine material removed by elutriation with tap water. The resin was then suspended in 3–4 vol. of N-NaOH and heated to 60–70° in an evaporating basin. It was stirred every 10–15 min. and the alkali decanted and sucked off after 3 hr. Trapped alkali was washed out by suspending in distilled water, stirring well, and decanting the water. This treatment with

Table 1. *Chromatographic properties of metabolites and their derivatives*

Compound	Configuration of 5-(H)	R_F or mobility in cm./hr.	Solvent system	Formula in Fig. 1
11β-Hydroxyandrosterone	α	0·16	3*b*	I
11β-Hydroxyaetiocholanolone	β	0·12	3*b*	I
Acetate of (I)	α	0·39	4*a*	II
Acetate of (I)	β	0·44	4*a*	II
11-Oxoandrosterone	α	0·33	3*b*	VI
11-Oxoaetiocholanolone	β	0·27	3*b*	VI
Acetate of (VI)	α	0·51	4*a*	III
Acetate of (VI)	β	0·55	4*a*	III
Androstane-3:11:17-trione	α/β	0·58	3*b*	V
*allo*Tetrahydrocortisol	α	12·5/20	1	VII
Tetrahydrocortisol	β	9·8/20	1	VII
Diacetate of (VII)	α	0·52	3*b*	VIII
Diacetate of (VII)	β	0·56	3*b*	VIII
*allo*Tetrahydrocortisone	α	16·4/20	1	IX
Tetrahydrocortisone	β	14·4/20	1	IX
Diacetate of (IX)	α	0·60	3*b*	X
Diacetate of (IX)	β	0·64	3*b*	X
Cortolone and β-cortolone	β	4·5/20	1	XI
3α:11β:17α:20ξ:21-Pentahydroxy-5α-pregnane	α	3·5/20	1	IV

NaOH was repeated twice. The resin was then treated similarly four times with N-HCl, washed with water on a Büchner funnel until the filtrate had a pH of 5–6, and suspended in 6–8 vol. of 85% aq. methanol (v/v) in which it was stirred mechanically for 16 hr. Finally the resin was stirred mechanically for 30 min. with excess of water and the water decanted: after a second treatment with water for 30 min. it was stored in a fresh lot of water in the cold room. During the final washing with 85% methanol and water much fine material is released and removed by stirring and decanting. The washed resin is stable for many months in the cold room but is rather friable. Stability is greatly impaired by traces of HCl remaining. This is best tested by examining the pH of the suspending fluid after a few days' storage; it should be 6 or above. Care was also taken not to crush resin particles with the glass stopper of its storage bottle: this produces fines which can be troublesome. It has recently been found that the hydroxide form is also suitable for extracting steroids and their conjugates: this form is conveniently prepared by reversing the order of the treatments with NaOH and HCl.

Sodium borohydride and lithium aluminium hydride. These were used as supplied.

21-*Acetoxy*-3α:17α-*dihydroxy*-5β-*pregnane*-11:20-*dione* (*tetrahydrocortisone* 21-*acetate*). This was supplied by Roussel Laboratories, m.p. 221–223°, and was chromatographically pure to at least 98%, moving 14·5 cm. in 20 hr. in system 1, after saponification.

3α:17α:21-*Trihydroxy*-5α-*pregnane*-11:20-*dione* (allo*tetrahydrocortisone*). Toluene-*p*-sulphonyl (tosyl) chloride (1 g.) was added to a solution of 21-acetoxy-3β:17α-dihydroxy-5α-pregnane-11:20-dione (1 g.) in dry pyridine (8 ml.) and left at room temp. overnight. Excess of reagent was decomposed with ice–water and the steroid extracted with ether–chloroform (1:1, v/v). The extract was dried with anhydrous Na_2SO_4 and evaporated at 45° (or below) under reduced pressure, giving a gum which, on trituration with ether, yielded crystalline 21-acetoxy-3β-tosyloxy-17α-hydroxy-5α-pregnane-11:20-dione (1·1 g.). The toluene-*p*-sulphonate (1·1 g.) was dissolved in acetic acid (30 ml.), heated under reflux for 2 hr. with anhydrous potassium acetate (2 g.) and kept for 24 hr. at room temp. The acetic acid was evaporated under reduced pressure at 45° (or below) and water (50 ml.) was added. The steroid was extracted with ether–chloroform (1:1, v/v) and the extract evaporated to a gum. This was dissolved in 0·067 N-NaOH in 80% aqueous methanol (100 ml.) and left in the dark under nitrogen for 21 hr. After acidification to pH 4·0 with acetic acid, the solution was evaporated under reduced pressure: the residue was dissolved in water (50 ml.) and extracted with ether–chloroform (1:1, v/v). The extract was evaporated and the residue dissolved in benzene for chromatography. A column of 100 g. of silica gel (2·5 cm. diam.; B.D.H. Ltd. 'for chromatography') was used and the steroid adsorbed on it from the benzene solution. The column was washed with benzene (500 ml.), benzene–ethyl acetate (2:1, v/v; 500 ml.), and ethyl acetate–methanol (1:1, v/v; 1500 ml.). The last solvent mixture was evaporated under reduced pressure and yielded 60 mg. of a solid, which was further purified by chromatography on six sheets (25 cm. × 45 cm. long) of Whatman no. 3 MM paper in system 1 for 6 hr. The compound was located with blue-tetrazolium reagent on a 0·3–0·5 cm. wide strip cut out of each sheet and the appropriate zones of each sheet were extracted with ethyl acetate–methanol (2:1, v/v). Crystallization from ether yielded 51 mg. of material, m.p. 214–215° [*allo*tetrahydrocortisone 208–212° (Caspi, Levy & Hechter, 1953)], and chromatographically pure (the material moved 16·4 cm. in system 1 in 20 hr. compared with 16·4 ± 0·4 cm. for known *allo*tetrahydrocortisone on the same sheet).

3α:11β-*Dihydroxy*-5β-*androstan*-17-*one* (11β-*hydroxyaetiocholanolone*). A solution of 3α:17α-dihydroxy-5β-pregnane-11:20-dione (1 g.) in dry tetrahydrofuran (25 ml.) was added dropwise to a suspension of lithium aluminium hydride (0·5 g.) in boiling tetrahydrofuran (100 ml.), the addition taking about 10 min. Further tetrahydrofuran (50 ml.) was added and heating continued for 2 hr. Water (5 ml.) was added cautiously and the mixture brought to pH 4 with H_2SO_4. The solvent was evaporated to a small volume under reduced pressure and 100 ml. of water added. The mixture was extracted thrice with 300 ml. of

methylene chloride and the extract dried over Na_2SO_4 before evaporating under reduced pressure. The crude product was dissolved in 50% (v/v) aqueous acetic acid (300 ml.) and shaken with sodium bismuthate (20 g.) for 1·5 hr. in the dark. Aqueous sodium metabisulphite (100 ml., 10% w/v) was added and the mixture shaken for 30 min. After 3N-NaOH (500 ml.) had been added the steroid was extracted with ether–chloroform (1:1, v/v) and the extract washed with 3N-NaOH and water. On evaporation a white solid was obtained which crystallized from ethanol in prisms [m.p. 236–238°; 11β-hydroxyaetiocholanolone, m.p. 237–239°, Sarett (1948)]. The material was chromatographically pure and had an R_F in system 3*b* of 0·12 (11β-hydroxyaetiocholanolone 0·12 on the same sheet).

3α-*Hydroxy*-5β-*androstane*-11:17-*dione* (11-*oxoaetiocholanolone*). This was supplied by Roussel Laboratories, m.p. 188–189°. The R_F in system 3*b* was 0·27 (11-oxoaetiocholanolone from the M.R.C. Reference Collection had values of 0·27 on the same sheet).

3α:11β-*Dihydroxy*-5α-*androstan*-17-*one* (11β-*hydroxyandrosterone*). This was prepared by the method of Klyne & Ridley (1956) from 3β:17α-dihydroxy-5α-pregnane-11:20-dione. The m.p. was 198–200° and the R_F in system 3*b*, 0·16, in agreement with a reference standard (M.R.C. Steroid Collection).

3α-*Hydroxy*-5α-*androstane*-11:17-*dione* (11-*oxoandrosterone*). This was prepared by the method of Klyne & Ridley (1956) from the 21-acetate of the above starting material, and had m.p. 148–150°. The R_F in system 3*b* was 0·33, identical with a standard prepared from the above M.R.C. reference standard.

Chemical methods

Extraction of urinary steroids. Procedure 1 was identical with that described by Bush & Willoughby (1957).

Procedure 2 was that described briefly by Bush & Gale (1957). Urine (0·05 of a 24 hr. sample; 70–100 ml.) is percolated at 3 ml./min. through a column of Decolorite (1 cm. × 22 cm.) containing 15 ml. of resin (when measured under gravity packing in water) and the column is then washed with 40 ml. of water at the same rate. The free and conjugated steroids are then eluted with 80 ml. of methanol–aq. NH_3 solution (sp.gr. 0·88) (19:1, v/v). The eluate is evaporated under reduced pressure, with a wide-bore capillary (approx. 1 mm.) leak and a high-capacity condensing system with acid traps for about 5 min. with no external heating, and the evaporation then continued with the flask on a water bath at 40–45°. The residue is then dissolved in 20 ml. of 0·5M-sodium acetate–acetic acid buffer at pH 4·8, 0·5 ml. of succus entericus from *Helix pomatia* (about 40 000 Fishman units of glucuronidase) are added (Henry & Thevenet, 1952) and the mixture is incubated for 15–16 hr. at 45°. This solution was then extracted as in procedure 1.

Extraction of plasma. The heparinized and oxalated plasma was diluted with an equal volume of water, and 0·05 vol. of N-NaOH added (Short, 1957). The mixture was then extracted twice with 3 vol. of ether–ethyl acetate (2:1, v/v), the extracts were combined and washed once with 0·05 vol. of N-NaOH, and once with water. This extract was acidified with two drops of acetic acid, filtered through about 2 g. of anhydrous Na_2SO_4 and evaporated under reduced pressure at 35–40° with a very fine capillary leak. The flask was removed from the water bath and cooled under the tap as soon as the extract was reduced to 0·3 ml. or less, and the residue transferred with ethyl acetate and methanol to a conical centrifuge tube for evaporation with an air jet. This residue was run on chromatograms as described below.

Estimation of urinary α-ketols. This was carried out by the method of Bush & Willoughby (1957).

Estimation of urinary 17-oxo steroids. Fraction 2 from the pre-fractionation chromatogram was eluted and 0·8 of it deposited over the full width of a 2·5 cm. × 57 cm. strip of Whatman no. 2 paper in a zone about 6 cm. long at one end of the strip. The material was then concentrated by running up to the origin line with ethyl acetate–methanol (2:1, v/v) and the strip run, together with standards, in system 3*b* for 12–15 hr. after 5 hr. equilibration. This time of equilibration is longer than that used for sheets and has been found necessary to avoid 'edging' which makes quantitative estimation by the scanning method difficult. The strips were dried for 30–40 min. in the fume cupboard.

The dried strips were then dipped once over alkaline *m*-dinitrobenzene and heated as described by Bush & Willoughby (1957). The strips were left exposed to room air for 5 min. and then packed in groups of five, sandwiched between two strips of Whatman no. 2 paper (5·0 cm. × 56 cm.) which had been treated with alkaline *m*-dinitrobenzene and dried, shortly after each group of five chromatograms had been treated with the reagent. The exposure to air for 5 min. reduced the background colour; the sandwiching between the blank sheets of treated filter paper prevented the colour reaction with 17-oxo steroids from fading, thus allowing sufficient time for scanning the strips. The scanner and the filter used were the same as those used for the blue-tetrazolium method above.

Fraction 1 from the pre-fractionation chromatogram was treated exactly as fraction 2 except that the strips were run in system 4*a*, for 14 hr. after 5 hr. equilibration.

Estimation of Δ^4-3-*ketones.* This was carried out by the modified NaOH fluorescence reaction with aq. alkali (Bush, 1953*a*) but omitting triphenyl tetrazolium from the reagent. One-fifth of fraction 3 from the pre-fractionation chromatogram was run in system 2 for 6–7 hr. to obtain a slightly overrun chromatogram, in parallel with known standards in quantities ranging from 0·2 to 2·0 μg. Only cortisol was estimated since other Δ^4-3-oxo steroids usually found in urine were not detectable owing to suppression of adrenocortical secretion with prednisone. This method is based on visual matching with standards and has an error of ±15% in the optimum range.

Chromatographic identification of metabolites. The methods were similar to those described by Bush & Willoughby (1957). The following additional methods and modifications were used.

Oxidation with sodium bismuthate. On occasion it was found that considerable oxidation of 11β-hydroxyl groups to 11-oxo groups had occurred with this reagent. This varied considerably with different samples of bismuthate but could be reduced to a certain extent by carrying out the oxidation for 1 hr. with 25 mg. of sodium bismuthate in 1 ml. of 50% acetic acid (v/v). When the preservation of 11β-hydroxyl groups was essential to the identification, samples of bismuthate were used which had been tested

previously to show that they did not oxidize 11β-hydroxyl groups.

Selective reduction of 11-*oxo groups*. Reactive ketone groups were protected by making the ethylene ketals, after which the 11-oxo group was reduced with sodium borohydride (Appleby & Norymberski, 1955) and the 11-hydroxy compound (almost entirely 11β-) regenerated by hydrolysing the ketal with acid. The dry residue containing 20–50 μg. of steroid was refluxed with benzene (10 ml.), ethylene glycol (0·2 ml.) and toluene-*p*-sulphonic acid (5 mg.) for 3·5 hr. and the mixture evaporated under reduced pressure at 40–45°. Aq. NaOH (0·2 N, 10 ml.) was added and the ketal extracted by shaking twice with ethyl acetate (30 ml.). The extract was washed twice with 5 ml. of water, dried by filtering through 1 g. of anhydrous Na_2SO_4, and then evaporated under reduced pressure, to dryness. The dry product was refluxed with tetrahydrofuran (10 ml.), sodium borohydride (15 mg.), and NaOH (2·5%, w/v; 1·0 ml.) for 10 hr. Water (10 ml.) was then added and the tetrahydrofuran evaporated under reduced pressure at 40–45°. The aqueous residue was extracted with ethyl acetate as above, washed, dried and evaporated. The dry reduction product was dissolved in acetic acid (2·0 ml.) and heated on a boiling-water bath for 30 min. Water (10 ml.) was then added and the mixture extracted twice with ethyl acetate (30 ml.). The extract was washed with saturated $NaHCO_3$ solution until evolution of gas had ceased, washed twice with water (5 ml.) and dried with anhydrous Na_2SO_4. The extract was evaporated as above (Antonucci, Bernstein, Lenhard, Sax & Williams, 1952; Allen, Bernstein & Littell, 1954).

This reaction gives an additional and highly specific indication of the presence of an 11-oxo group in a steroid, when used in conjunction with chromatographic techniques. Ketal formation can also be used for the demonstration of reactive ketone groups in the same way that acetylation reveals esterifiable hydroxyl groups. In this respect it is superior to thiosemicarbazide (Bush, 1953*b*), since the ketals are less polar (Reineke, 1956) and more soluble than comparable steroid thiosemicarbazones and thus usually more suitable for chromatography.

EXPERIMENTAL AND RESULTS

The procedure for experiments *A*–*E* was to give prednisone (5 mg. at 8.00 a.m., 12.30 p.m. and 8.00 p.m.) by mouth to a male subject for 3 days. On the second and third days 24 hr. urine samples were collected between 10.00 a.m. and 10.00 a.m. The second day's urine, sample 1, served as control. The third day's urine was designated sample 2. On the third day the compound under investigation was given at 10.00 a.m. In experiments *F* and *G* prednisolone (5 mg. at 8.00 a.m. and 8.00 p.m.) was given instead of prednisone and urine was collected on the third (sample 1) and fourth (sample 2) days. The dose was given on the fourth day at 10.15 a.m. in experiment *F* and at 9.00 a.m. in experiment *G*. Doses and results are given in Table 2 and the derivatives prepared for identification and their properties are summarized in Fig. 1 and Table 1.

Metabolism of 11β-*hydroxyaetiocholanolone* (*Experiment A*)

Routine estimation of urinary steroids was carried out by extraction procedure 1. One-twentieth of the urine sample of the third day was also extracted with ether–ethyl acetate without previous extraction and hydrolysis of the conjugates, and the steroid content of the extract estimated as above. This extract contained 10 μg. of 11-hydroxyaetiocholanolone (= 0·2 mg./24 hr.).

Identification of 11β-*hydroxyaetiocholanolone*. Fraction 2 from the pre-fractionation chromatogram of the extract of the urine of the third day was eluted and an amount equivalent to 0·01 of the 24 hr. sample was run on Whatman no. 2 paper in system 3*a* for 16 hr. spread over a width of 10 cm., with 11β-hydroxyaetiocholanolone in small amounts alongside the urinary material. The major component of the urinary fraction (detected on a thin strip cut from the sheet) and 11β-hydroxyaetiocholanolone both moved 11·5 cm. and gave violet colours with alkaline *m*-dinitrobenzene. The remaining part of the sheet was cut into small pieces and the zone containing the main component extracted with ethyl acetate–methanol (2:1, v/v) and the extract evaporated at 30–40° with a jet of air.

About 20 μg. of the material was run in system 3*b* for 3·5 hr. on the same sheet as a sample of 11β-hydroxyaetiocholanolone. Both substances had an R_F of 0·12, after detection with alkaline *m*-dinitrobenzene.

About 20 μg. of the urinary material and an equal amount of 11β-hydroxyaetiocholanolone were each oxidized with chromic acid and the products run in system 3*b* for 3·5 hr. A substance with an R_F of 0·58 was detected with alkaline *m*-dinitrobenzene, giving a blue–violet colour developing partly in the cold, identical in colour-reaction and position with the product from the reference substance (5β-androstane-3:11:17-trione).

About 60 μg. of the urinary material and a similar amount of 11β-hydroxyaetiocholanolone were each acetylated and the two products run in system 4*a* for 3·5 hr. A thin strip from the sheet was cut, taking a fraction of the lanes in which each product had been deposited and treated with alkaline *m*-dinitrobenzene. The acetate of the urinary material and of 11β-hydroxyaetiocholanolone both gave similar colours and had R_F values of 0·44. The remainders of the acetates of the urinary material and the reference steroid were extracted from the appropriate zones of the last chromatogram with ethyl acetate–methanol (2:1, v/v) and oxidized with chromic acid. The products were run in system 4*a* for 3·5 hr. and a part of the chromatogram was treated with alkaline *m*-dinitrobenzene.

Table 2. *Excretion of steroids after the administration of precursors*

In each experiment urine sample 1 is the control 24 hr. sample and sample 2 is the urine collected for 24 hr. after administration of the dose. Results are expressed in mg./24 hr., fully corrected. All subjects were males.

Expt.	*A*		*B*		*C*		*D*	*E*	*F*		*G*	
Age of subject (years)	29		24		21		29	24	33		24	
Precursor given	11β-Hydroxyaetio-cholanolone		11-Oxoaetio-cholanolone		11-Oxoaetio-cholanolone		11β-Hydroxy-andro-sterone	11-Oxo-andro-sterone	Tetrahydro-cortisone 21-acetate		*allo*Tetra-hydrocortisone	
Dose (mg.)	100		45		45		50	50	60		50	
Urine sample	1	2	1	2	1	2	2	2	1	2	1	2
Urine vol. (ml.)	2400	1340	1060	1600	1500	1020	3100	1000	840	740	700	560
Steroid excreted												
Tetrahydrocortisol	1·9	0·81	0·43	0·77	0·53	0·39	0·47	0·64	1·3	2·1	0·71	0
Tetrahydrocortisone	1·5	0·90	0·72	1·3	0·80	1·1	0·37	0·53	0·86	17	0·73	0
*allo*Tetrahydrocortisol	0	0	0	0	0·23	0	0	0	0·78	1·0	0·64	13
*allo*Tetrahydrocortisone	0	0	0	0	0	0	0	0	0	0	0	1·5
Cortisol	0·29	0	0·11	0	0·057	0	0	0	0·021	0·022	0·014	0
11β-Hydroxyaetiocholanolone	0·11	47	0·16	0·93	0·27	0	0	0	0·24	0·26	0·21	0
11-Oxoaetiocholanolone	0·29	0·70	0·16	31	0·31	18	0	0	0·23	1·3	0·17	0
11β-Hydroxyandrosterone	0·31	0	0·23	0	0·43	0	31	6·3	0·20	0·25	0	1·2
11-Oxoandrosterone	0	0	0	0	0	0	0·87	21	0	0	0	0
Dehydro*epi*androsterone	0·90	1·3	0·13	0·15	0·08	0·35	0·62	0	0	0	0	0
Aetiocholanolone	1·0	0·74	0·41	0·87	0·71	1·1	2·0	0·53	3·0	2·7	2·4	1·9
Androsterone	0·33	0·40	0·36	0·83	0·93	0·61	2·5	0·79	3·5	2·5	2·1	2·1

Both the urinary material and the reference product gave pinkish colours developing partly in the cold, and had an R_F of 0·55, as with known 11-oxoaetiocholanolone 3-acetate. The remainders of the acetates on this chromatogram were extracted separately as above and each product was saponified. The products were then run in system 3*b* for 3·5 hr. and detected with alkaline *m*-dinitrobenzene. Both gave pinkish colours in the cold and had R_F values of 0·27, as did 11-oxoaetiocholanolone. This identification is summarized in Fig. 1 and Table 1.

Metabolism of 11-*oxoaetiocholanolone* (*Experiments B and C*)

11-Oxoaetiocholanolone was identified in fraction 2 of the urinary extract by the same general procedure used for the identification of 11β-hydroxyaetiocholanolone above.

Metabolism of 11β-*hydroxyandrosterone* (*Experiment D*)

The urine samples were extracted by procedure 2. 11β-Hydroxyandrosterone was identified by a procedure similar to those used above.

Metabolism of 11-*oxoandrosterone* (*Experiment E*)

11β-Hydroxyandrosterone was identified as above and 11-oxoandrosterone by preparing the derivatives shown in Fig. 1 and Table 1.

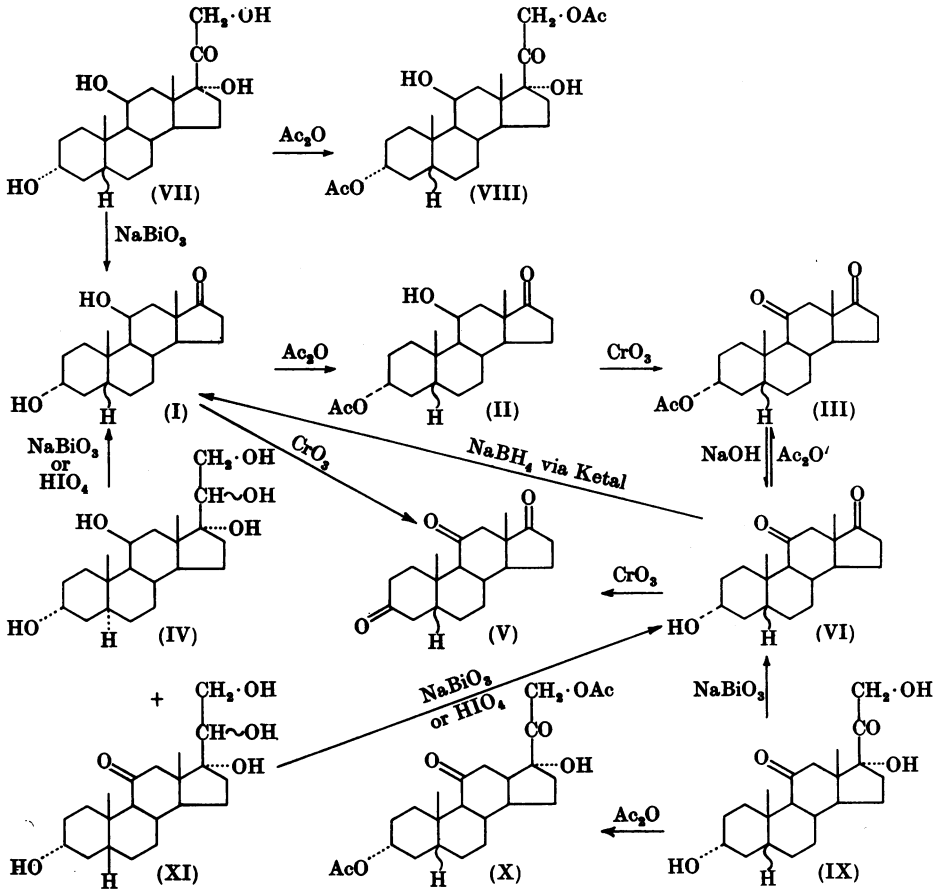

Fig. 1. Degradations used in the chromatographic identification of urinary steroids. (I), 3α:11β-Dihydroxy-5α/β-androstan-17-one; (IV) 3α:11β:17α:20α/β:21-pentahydroxy-5α-pregnane; (V) 5α/β-androstane-3:11:17-trione; (VI) 3α-hydroxy-5α/β-androstane-11:17-dione; (VII) 3α:11β:17α:21-tetrahydroxy-5α/β-pregnan-20-one; (IX) 3α:17α:21-trihydroxy-5α/β-pregnane-11:20-dione; (XI) 3α:17α:20α/β:21-tetrahydroxy-5β-pregnan-11-one. 5α/β means that both 5α-(H) and 5β-(H) isomers were degraded by the same routes. This is indicated in the figure by leaving the 5-H configuration ambiguous. Differentiation of these isomers is described in the text and summarized in Table 1. 20α/β means that such isomers were not separated but that probably both were present (Fukushima *et al.* 1955).

Metabolism of tetrahydrocortisone (Experiment F)

The urine was extracted by procedure 2. Plasma was separated from a blood sample drawn at 1.15 p.m. The concentrations of free steroids were extremely low and the results given below have a higher probable error. Tetrahydrocortisone was identified by the derivatives shown in Table 1 and by oxidation with sodium bismuthate to 11-oxoaetiocholanolone, which was identified as above. 3α:11β:17α:21-Tetrahydroxy-5β-pregnan-20-one (tetrahydrocortisol) was identified by the derivatives shown in Fig. 1 and Table 1 and by oxidation with sodium bismuthate to 11β-hydroxyaetiocholanolone, which was identified as described above.

11-Oxoaetiocholanolone was identified as above.

3α:17α:20:21-Tetrahydroxy-5β-pregnan-11-one (cortolone and β-cortolone, Fukushima *et al.* 1955) was detected when fraction 3 was run in system 1 for 20 hr. and a thin strip from the sheet cut out and treated with the periodate–Zimmermann reaction (Bush, 1955). The remainder was eluted and oxidized with sodium bismuthate to 11-oxoaetiocholanolone, which was identified as above.

Plasma steroids. Plasma (25 ml.) was extracted as above (Methods) and half of the extract run for 16 hr. in system 1. Treatment with alkaline blue-tetrazolium gave a blue spot in the position of tetrahydrocortisone estimated by eye to contain 0·9 μg. (uncorr.). No tetrahydrocortisol was found (<0·5 μg.). The other half of the extract was oxidized with sodium bismuthate and the product run for 6 hr. in system 3*b*. On treatment of the chromatogram with alkaline *m*-dinitrobenzene 0·8 μg. (uncorr.) of 11-oxoaetiocholanolone was found (visual estimation). No 11β-hydroxyaetiocholanolone was found (<0·3 μg.).

Metabolism of allo*tetrahydrocortisone (Experiment G)*

Blood was drawn from the antecubital vein at 12 noon. The urines were extracted by procedure 2. *allo*Tetrahydrocortisol was identified by preparation of the derivatives shown in Table 1 and Fig. 1, and by oxidation with sodium bismuthate to 11β-hydroxyandrosterone. 11β-Hydroxyandrosterone was identified as above.

On examination of fraction 3 from the second urine sample a compound running just behind cortolone was detected with the periodate–Zimmermann reaction. In system 1 it moved 3·5 cm. in 20 hr. at 25·5°. On oxidation with sodium bismuthate it yielded 11β-hydroxyandrosterone. These properties suggest very strongly that the original metabolite was the 5α-epimer of cortol or β-cortol (or both) (Fukushima *et al.* 1955), i.e. 5α-pregnane-3α:11β:17α:20α:21-pentol and its 20β-epimer.

Plasma steroids. These were estimated as in Expt. *F*. Amounts of 0·6 μg. (uncorr.) of *allo*tetrahydrocortisol and 0·6 μg. (uncorr.) of 11β-hydroxyandrosterone were found on the chromatograms. The related 11-ketones were not found (<0·4 μg. of *allo*tetrahydrocortisone and <0·2 μg. of 11-oxoandrosterone).

DISCUSSION

The results of these experiments confirmed that reduction of steroid 11-oxo groups in man is determined largely by the stereochemistry of the substrate, particularly the A/B ring junction, and not by any other obvious property of particular groups in ring A. Thus little if any reduction of their 11-oxo groups occurred with 11-oxoaetiocholanolone and tetrahydrocortisone, both of which have the buckled A/B ring junction typical of 5β-(H) steroids. On the other hand, the 11-oxo groups of their 5α-epimers (11-oxoandrosterone and *allo*tetrahydrocortisone) with flat A/B ring junctions were extensively reduced, especially with the latter steroid. This is summarized in Fig. 2.

It is more difficult to estimate the quantities of minor metabolites derived from the exogenous precursors because of the day-to-day and individual variation of the endogenous contribution to the metabolites. In order to minimize this source of error, we have calculated the probable endogenous contribution to each metabolite under consideration by multiplying the quantity excreted in the control sample by the ratio (excretion in sample 2)/(excretion in sample 1) with respect to an independent group of metabolites known from other work to have excretion rates which, in any individual, are closely correlated with the excretion rate of the metabolite under consideration. In considering the excretion of 11β-hydroxyaetiocholanolone in Expt. *B*, for instance, the figure for sample 1 is 0·16 mg./24 hr. (Table 2). Since this metabolite is normally closely correlated with the summed excretion rates of tetrahydrocortisone and tetrahydrocortisol (Dorfman, 1954; Roberts & Szego, 1955), we note that this sum is 1·15 mg./24 hr. in sample 1 and 2·07 mg./24 hr. in sample 2; multiplying 0·16 by the ratio 2·07/1·15 gives the probable endogenous contribution to the 11β-hydroxyaetiocholanolone in sample 2, namely 0·29 mg./24 hr. Since the observed figure in sample 2 was 0·93 mg./24 hr., it is probable that 0·64 mg. was derived from the exogenous precursor. Similarly, in considering the excretion of tetrahydrocortisol in Expt. *F*, *allo*tetrahydrocortisol was taken as the independent metabolite, giving a correction factor of 1·0/0·78 and the probable endogenous contribution to tetrahydrocortisol in sample 2 as $1{\cdot}3 \times 1{\cdot}0/0{\cdot}78 = 1{\cdot}7$ mg./24 hr. In Expts. *D* and *E* the control urines were not

analysed because of losses in the collection and values of 0·16 mg./24 hr. (mean of all other samples) for 11β-hydroxyandrosterone and of 0·0 mg./24 hr. for 11-oxoandrosterone were assumed.

This type of calculation is the basis for the figures given in Table 3, where the probable yield of metabolites from the exogenous precursors is expressed in each case as the percentage of the dose administered. While this procedure improves upon the more usual method of basing calculations on the uncorrected values of the control samples, it does not avoid the error due to day-to-day variation of the relative proportions of the endogenous metabolites. Thus the exogenous contribution to tetrahydrocortisol in sample 2 of Expt. *F* seems of questionable significance, while the apparently

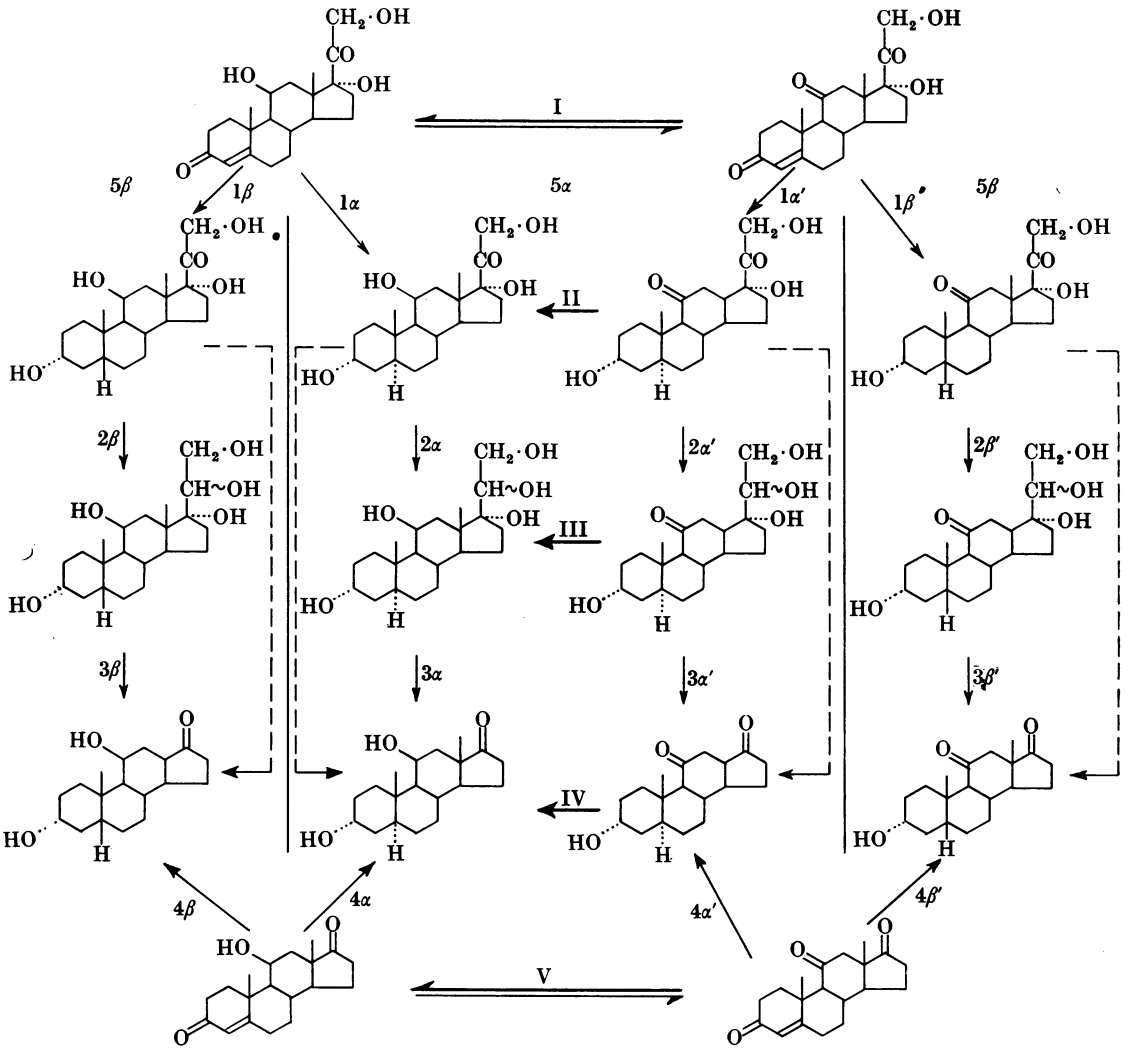

Fig. 2. Summary of the probable main pathways of metabolism of cortisol and 11β-hydroxyandrost-4-ene-3:17-dione condensed to single lines of 5α-(H) and 5β-(H) metabolites. It is known from other work that reaction 2 may precede 1; and it is possible that reaction 3 may precede 2, but not 1 to any great extent (Dorfman, 1954). Routes II and IV were demonstrated here for the first time; also routes 2β′, 2α and/or 2α′, and 3α and/or 3α′. Other routes have been demonstrated previously or are reasonable inferences from our results (Dorfman, 1954; Lieberman & Teich, 1953; Roberts & Szego, 1955). For simplicity reactions 2 and 3 have been drawn in sequence; the possibility that the 20-hydroxy steroids are not obligatory intermediates in the metabolic pathway from C_{21} to C_{19} steroids is indicated by the dashed arrows.

significant increase in excretion of 11β-hydroxyaetiocholanolone in Expt. *B* is rendered doubtful by the absence of this substance from sample 2 of Expt. *C*. On the other hand, the exogenous origin of most of the 11β-hydroxyandrosterone in sample 2 of Expt. *G*, and of 11-oxoaetiocholanolone in sample 2 of Expt. *F*, seems beyond reasonable doubt.

While the assumptions underlying the above procedure are well supported by other work on steroid metabolism, a stricter test of the significance of the supposed minor metabolites of the exogenous precursors can be made by assuming complete independence of the excretion rates of the different metabolites and applying Student's *t* test (Fisher, 1946). These calculations confirm the above conclusions, even though the assumption of independence of excretion figures is certainly an exaggeration, and rules out the possibility of applying any correction to the values of the control samples (i.e. samples 1). Thus the excretion of 11-oxoaetiocholanolone in Expt. *F*, sample 2, is 5·6 times the mean excretion in the samples available for comparison (*A* 1, *B* 1, *C* 1, *F* 1, *G* 1), giving a value for *t* of 15·8 ($P < 0.025$). The excretion of 11β-hydroxyandrosterone in Expt. *G*, sample 2, is 7·5 times the mean excretion of the nine samples available for comparison (i.e. excluding *D* and *E*), giving a value for *t* of 6·4 ($P < 0.05$). In this case the difference between samples 1 and 2 can also be compared with a reasonable number of controls; in Expt. *G* it is +1·2 mg./24 hr., as against a mean for Expts. *A*, *B*, *C* and *F* of −0·23 mg./24 hr., giving a value for *t* of 7·0 ($P < 0.05$). The *t* values for the excretion of 11-oxoaetiocholanolone in Expt. *A* and for that of 11β-hydroxyaetiocholanolone in Expt. *B* are 6·4 and 6·9 ($P < 0.05$). Other minor metabolites gave *t* values of negligible significance ($P = 0.1$–0.4).

The 20-hydroxy metabolites described in Expts. *F* and *G* were not estimated quantitatively. The amount of cortolone found in the control sample was about one-tenth of that in sample 2; *allo*cortol was not detectable in the control sample.

Only with *allo*tetrahydrocortisone was complete suppression of endogenous adrenocortical secretion observed, as judged by complete disappearance of metabolites of cortisol from the urine. This contrasts with the experiments of Savard & Goldfaden (1954), who reported that tetrahydrocortisol had suppressed the endogenous adrenocortical secretion of two post-menopausal women. However, it is not clear from their paper how sensitive were their methods for estimating cortisol, nor were there details of control specimens of urine from their subjects. In view of the known biological activity of *allo*tetrahydrocortisol (Hechter, 1955; Silber, personal communication) and the inactivity of

Table 3. *Percentage recovery of identified metabolites and unchanged precursors from urine*

Recoveries are given as percentage of dose based on the fully corrected figures of Table 1. In Expt. *F* the dose and yield of metabolites are expressed in terms of the free steroid; the 21-acetate was administered. The urines were samples 2 (see Table 1).

Experiment						*A*	*B*	*C*	*D*	*E*	*F*	*G*
Precursor given						11β-Hydroxyaetiocholanolone	11-Oxoaetiocholanolone	11-Oxoaetiocholanolone	11β-Hydroxyandrosterone	11-Oxoandrosterone	Tetrahydrocortisone	*allo*Tetrahydrocortisone
Dose (mg.)						100	45	50	50	50	54	50
Steroid excreted					Group at C-11							
Tetrahydrocortisol					·OH	—	—	—	—	—	⩽0·72	—
Tetrahydrocortisone					:O	—	—	—	—	—	28	—
*allo*Tetrahydrocortisol					·OH	—	—	—	—	—	—	26
*allo*Tetrahydrocortisone					:O	—	—	—	—	—	—	3·0
11β-Hydroxyaetiocholanolone					·OH	47	⩽1·6	0	—	—	0	—
11-Oxoaetiocholanolone					:O	⩽0·55	69	36	—	—	1·9	—
11β-Hydroxyandrosterone					·OH	—	—	—	62	12·0	0	2·4
11-Oxoandrosterone					:O	—	—	—	1·7	42	—	—
Total recovery of identified metabolites						—	⩾71	36	64	54	⩾33	31

tetrahydrocortisol, our results are intelligible in view of the observed reduction of *allo*tetrahydrocortisone to *allo*tetrahydrocortisol and its appearance in the blood stream, although its concentration in plasma was low (about 7 μg./100 ml.; corr.).

Estimation of plasma steroids was rather unsatisfactory because of the low concentrations present. However, the compounds found suggested that the plasma ratios of 11-oxo/11β-hydroxy forms were similar to the ratios observed with the urinary metabolites. Identification was based on the chromatographic properties of the α-ketols themselves and of their oxidation products with sodium bismuthate. Since the normal plasma concentrations of these α-ketols are too low to be detectable by the above methods the identifications were considered satisfactory.

It might be objected that these observations admit of other interpretations, since the urinary metabolites of a steroid precursor are the result of many competing processes of transfer and enzymic action; or that reduction had occurred with the 5β-(H) steroids but that the 5β-(H)-11β-alcohols had been metabolized to undiscovered metabolites, or conjugated in some way not amenable to hydrolysis by our methods. There are no reasonable grounds at present for accepting such an explanation of our findings. Furthermore, the recovery of recognized metabolites (or unchanged precursor) was high (Table 3) and in Expts. *A* and *D* the related 11β-hydroxy steroids were themselves administered and recovered in comparable yield from urine by the same methods. Another possible objection might be that the redox potentials of the 11-oxygen function differed in the 5α-(H) and 5β-(H) steroids, so that although oxidation–reduction occurred the 11-ketones of the latter predominated in the steady-state. This interpretation is not only extremely unlikely, but disproved in the case of the C_{19} precursors in which administration of the 11β-alcohols yielded only the unchanged precursors in the urine.

While it is reasonable to expect that enzymic reduction of 11-oxo groups to 11β-hydroxyl groups by an enzyme specific for Δ^4-3-oxo steroids would be impossible, or at most extremely slow, with 5β-(H) steroids (A/B junction bent) it is not certain that a single enzyme is responsible for this reaction. There is still no evidence to exclude the possible existence of several enzymes capable of reducing the 11-oxo groups of 3α-OH-5α-(H) steroids and others, but until proved otherwise it seems reasonable to adopt a working hypothesis that the hepatic system is in fact based upon one steroid 11β-dehydrogenase, or at most a set of 11β-dehydrogenases with very similar properties of their active regions in those parts which are opposed to the A and B rings in the enzyme–substrate complex. Thus there is direct or indirect evidence showing that the reaction occurs *in vivo* or *in vitro* with steroids containing almost every known natural side chain at C-17 as well as with 17-oxo steroids (Burton, Keutman & Waterhouse, 1953; Burstein, Savard and Dorfman, 1953*a*, *b*; Savard, Burstein, Rosenkrantz & Dorfman, 1953; Hubener *et al.* 1956). On the other hand, there is similar evidence showing that with a variety of side chains at C-17 and with 17-oxo steroids the rules for substrate specificity are the same as far as deformations or substitutions in the AB ring system are concerned.

It may also be objected that rules of substrate specificity cannot be deduced from experiments *in vivo* since differences in distribution and transport of these steroids may be the basis of the differences observed in their metabolism. Two arguments seem to remove this objection from serious consideration: First, our results with 11-oxoaetiocholanolone and tetrahydrocortisone, and previous work with cortisone itself and 2α-methylcortisone, parallel very closely the observations of others with rat-liver homogenates, and with microsome fractions from rat liver, where differences in distribution and availability of the substrates are minimized (Glenn *et al.* 1957; Hubener *et al.* 1956). Secondly, very large differences in the solubility and other properties of such steroid substrates which would be expected to influence their transport and distribution have made no difference in the striking effects observed with differences in the AB ring system (e.g. C_{19} and C_{21} steroids, except for the rather inefficient reduction of 11-oxoandrosterone); on the other hand steroids with similar properties, and known to be metabolized, conjugated, and excreted at similar rates and by similar pathways, still show gross differences in reduction of 11-oxo groups according to the steric properties of their AB ring systems (e.g. cortisone, tetrahydrocortisone and *allo*tetrahydrocortisone).

The findings reported here are a reasonable explanation of the pattern of 11β-hydroxy and 11-oxo steroid metabolites found in normal human urine which was remarked upon previously (Bush, 1956). The suggested pathways of metabolism are generalized in Fig. 3. However, A. A. Sandberg & R. Slaunwhite (personal communication) have suggested that 11β-hydroxy-Δ^4-3-oxo steroids are preferentially reduced to 5α-(H)-3α-ols and 11-oxo-Δ^4-3-oxo steroids to 5β-(H)-3-ols. It will be shown in subsequent papers that this effect is definitely observable but it appears at present to be too small to account for the normal urinary pattern.

Thus it is clear from our results that the 5β-(H)-3α:11β-dihydroxy steroids are neither reduced nor oxidized appreciably at C-11. It follows that 5β-(H)-3α:11β-dihydroxy steroids must be derived

from the reduction of an 11β-hydroxy-Δ⁴-oxo steroid precursor. Even if all the *allo*tetrahydrocortisol (5α-H) in human urine were derived from direct reduction of cortisol, and none via reduction of *allo*tetrahydrocortisone, it would appear from the usual predominance of tetrahydrocortisol (5β-H) that reduction of C_{21}-11β-hydroxy-Δ⁴-3-oxo steroids to the 5β-(H)-3α-ols was favoured over their reduction to 5α-(H)-3α-ols (Dorfman, 1954; Bush & Willoughby, 1957).

Further work on oxidation–reduction of steroid 11-oxygen functions is in progress, some of which has been reported briefly (Bush & Mahesh, 1958*a–c*). To avoid possible confusion it is worth noting here that, as suggested by Morris & Williams (1953) among others, there is some evidence that a second system exists, more widely distributed in the peripheral tissues, in which the steady state is normally such as to produce a net oxidation of 11β-hydroxy steroids to the related 11-ketones. The substrate specificity of this second system is probably rather different from the hepatic system. Thus 9α-fluorocortisone was reduced completely to 9α-fluorocortisol after oral administration, but although it was metabolized by pathways otherwise identical with those of cortisone, the corresponding 9α-fluoro-11-ketones were not found (Bush & Mahesh, 1958*b*). More significantly the oxidation of endogenous cortisol (i.e. the small amount secreted before complete suppression of adrenocortical secretion had occurred) was inhibited, so that its normally predominant 11-oxo metabolites were only excreted in traces, while the normally less plentiful 11β-hydroxy metabolites were excreted in amounts which were low but far greater than those of the related 11-ketones. In this instance it would be unreasonable to suppose that the oxidative, but not the reductive, reaction

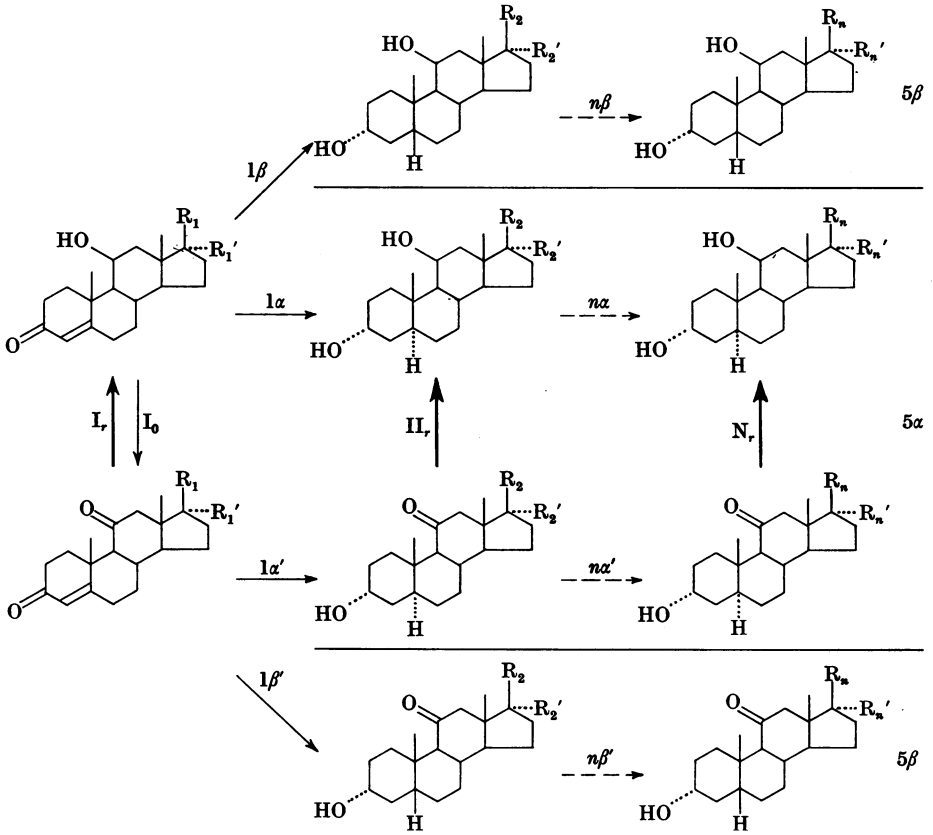

Fig. 3. Hypothetical generalization of the routes of metabolism shown in Fig. 2. Present work suggests that reactions I_r, II_r and N_r will not occur when large α-substituents at C-1, C-2, C-9 and C-12 are present; substituents at other positions, or 1-, 2- and 12-β substituents, probably do not stop these reactions. These suggestions are based partly on ordinary stereochemical reasoning and partly on results obtained with 2α-methyl steroids and 1:2-dehydro steroids (Bush & Mahesh, 1957, 1958*a*, *c*). The representation of substituent groups by R_1, R_2, etc. does not imply that these are necessarily altered in the course of the reactions.

due to the hepatic enzyme was inhibited by 9α-fluorocortisol, and one is left with the strong supposition that the system normally responsible for the well-known net oxidation of steroid 11β-hydroxyl groups has properties differing from those of the reducing system of the liver. This supposition is now under further examination.

SUMMARY

1. An earlier hypothesis that reduction of steroid 11-oxo to 11β-hydroxy groups *in vivo* was largely dependent on the stereochemistry of the A and B rings has been tested by studying the metabolism of various 3α-hydroxy steroids.

2. 11-Oxoandrosterone, 11β-hydroxyandrosterone, 11-oxoaetiocholanolone, 11β-hydroxyaetiocholanolone, tetrahydrocortisone and *allo*tetrahydrocortisone were given by mouth in doses of 40–100 mg. to young male volunteers, together with prednisone or prednisolone to suppress endogenous adrenocortical secretion. Steroids in blood plasma and urine were estimated and identified by chromatographic methods.

3. The proportions of the major metabolites of 11-oxoandrosterone (5α-H) and of *allo*tetrahydrocortisone (5α-H) the 11β-hydroxy (reduced) form were 23 and 91% respectively. No 11β-hydroxy metabolites of the other steroid precursors (5β-H) were detected (< 2% of their major metabolites).

4. The C_{21} 3α-hydroxy steroids gave rise to small but significant amounts of the related 17-oxo steroids and 20ξ-hydroxy steroids.

5. It is suggested that the normal pattern of 11-oxo and 11β-hydroxy steroids in human urine, in which little or no 11-oxo-5α-(H) steroids can be found while the 11-oxo-5β-(H) metabolites are more plentiful than 11β-hydroxy-5β-(H) metabolites, is largely accounted for by the ease with which the 11-oxo-5α-(H) metabolites are reduced to the related 11β-alcohols.

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