isomer in the cat and the 16β isomer in the rat. It has not yet been ascertained whether the radioactivity associated with 16-*epi*-estriol from cat blood and the estriol from rat blood really represents these compounds. Preliminary experiments with separated white and red cells from rat blood have shown the reduction to occur in the latter. Studies are now in progress on detailed aspects of the mechanism involved.

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The conversion of androst-4-en-3,17-dione into testololactone and other substances by incubation in bovine blood

As part of a study of steroidogenesis in perfused bovine adrenals and ovaries, we have incubated androst-4-en-3,17-dione in bovine blood alone and have examined the nature of the transformation products.

[4-^{14}C]Androst-4-en-3,17-dione, (I), was purified first by partition chromatography on a column of diatomaceous earth (Celite 545), using methanol–water (4:1) as the stationary phase (0.67 ml/g) and hexane as the mobile phase, and then by multiple crystallizations in ethyl acetate.

In 1 incubation, 50.0 mg of I, 3000 counts/min/mg, dissolved in 10 ml of propylene glycol, were mixed with 800 ml of oxygenated, citrated whole blood, taken from a pregnant cow at slaughter, to which 20 mg of oxytetracyclinehydrochloride (Terramycin) had been added to inhibit bacterial growth. The mixture was then circulated through the aeration chamber of a perfusion apparatus[1] at 39–40° for 3 h under an atmosphere of 95 % O_2–5 % CO_2. A second incubation was performed in the same manner but for only 160 min. The steroids were extracted from both incubates by isopropyl acetate and the extract residues fractionated by, successively, partition between hexane and methanol–water (7:3); column-adsorption chromatography on silica gel (Grade 62, Davison Chemical Corp.); and column-partition chromatography (chrom. 2) in the same system used to purify I, except that hexane–benzene mixtures, as well as hexane alone, were used for the mobile phase.

Certain of the hexane–benzene (9:1) eluate residues of chrom. 2 were crystallized from ethyl acetate to yield a substance, 69.8 mg, m.p. 173–175°, whose infrared-absorption spectrum was identical with that of androst-4-en-3,17-dione.

The syrups, 11 mg (less 0.4 mg used for tests), eluted before the recovered I, were combined in benzene and rechromatographed (chrom. 3) on an adsorption column of silica gel, developing with benzene–ethyl acetate (97:3). This was a newly-devised, efficient, annular column in which the adsorbent was a 2-mm-thick layer between the outer tube, 18 mm internal diameter, and an inner, sealed tube, 14 mm outer diameter. From certain eluate residues, 1.8 mg, of mobility equal to that of progesterone, a substance, 0.8 mg, m.p. 198–207°, was crystallized in ethyl acetate–neohexane. Recrystallization achieved prisms, 0.22 mg, m.p. 203–208°, 2820 counts per min/mg; ϑ_{max} 1737 and 1709 cm^{-1}; no selective absorption at about 240 mμ in methanol. This substance, II, has not been identified.

The syrups eluted shortly after I in chrom. 2 were combined (24.5 mg) and rechromatographed on a similar partition column (chrom. 4). Certain of the hexane–benzene (9:1) eluate residues, about 0.5 mg, were crystallized in ethyl acetate–neohexane to yield a substance, 0.4 mg, m.p. 209–213°. Recrystallization led to prisms, 0.3 mg, m.p. 204–207°, 3100 counts/min/mg; λ_{max}^{MeOH} 240 mμ; ϑ_{max} 3410, 1650 and 1603 cm^{-1}, whose infrared spectrum was identical with that of epitestosterone.

Later residues, 1.4 mg, eluted by the same solvent, were crystallized in ethyl acetate–neohexane to yield 1.2 mg, m.p. 154–156°, which, on recrystallization, yielded knife-like crystals, 0.6 mg, m.p. 154–156°, 3300 counts/min/mg; λ_{max}^{MeOH} 240.5 mμ; ϑ_{max} 3420, 3380, 1655 and 1610 cm^{-1}. The infrared spectrum was identical with that of testosterone.

Later residues, 1.5 mg, eluted by hexane–benzene (9:1 and 3:1) were crystallized in ethyl acetate–neohexane to yield a substance, 0.7 mg, m.p. 242–246°, which was recrystallized in ethyl acetate to afford prisms, 0.11 mg, m.p. 245–247°, 3140 counts per min/mg; no selective absorption at about 240 mμ; ϑ_{max} 1720, 1711 cm^{-1}. This substance (III) has not been identified.

Later hexane–benzene (3:1) eluates led to residues, 3 mg, from which 2.5 mg, m.p. 207–212°, could be crystallized in ethyl acetate. Three recrystallizations afforded compact, prismatic clusters, 1.0 mg, m.p. 210–213°, 3000 counts/min/mg; λ_{max}^{MeOH} 237 mμ; ϑ_{max} 1720, 1665 and 1613 cm^{-1}, whose infrared spectrum was identical with that of synthetic testololactone (3-keto-13α-hydroxy-13,17-secoandrost-4-en-17-oic acid, lactone)[2].

The isolation of epitestosterone and testosterone was not surprising since LINDNER[3] has already given evidence for their formation from I by incubation with ox or sheep blood. The conversion of I into testololactone, however, a reaction involving oxidative cleavage of the C-13–C-17 bond, was unexpected. This transformation was shown by FRIED *et al.*[4] and PETERSON *et al.*[5] to be effected by microorganisms but this is the first demonstration that such a metabolic pathway occurs in mammalian tissue. It is unlikely that the lactone was produced by bacterial contaminants in the incubated blood since the antibiotic, even at one-fifth the concentration used, is an effective inhibitor of bacterial growth. The extent of conversion in blood—about 8% based on unrecovered I—plus the possibility that II and III may also be lactones indicate that this is a major transformation in blood. It may also occur in endocrine glands and at the site of other carbonyl groups. For example, a peroxidative cleavage of the C-20–C-21 bond may be the route of formation of the etienic acids isolated from bovine adrenal perfusates of deoxycorticosterone[6,7]. Further, FORCHIELLI *et al.*[8] have concluded that progesterone incubated with rat testes can be converted directly into testosterone without going through 17-hydroxyprogesterone. If so, this would be an oxidative cleavage of the C-17–C-20 bond with testosterone acetate as the immediate product, although the acetate was not isolated. Finally, JACOBS *et al.*[9] isolated a substance from pregnant-mare urine which they showed to be a lactone, $C_{19}H_{26}O_3$, but did not identify completely.

It is of interest that testololactone has no androgenic activity[10] and that Δ^1-testololactone, also without androgenic activity[11], produces some objective regression in advanced breast cancer[12].

Our study of the metabolism of steroids in bovine and human blood is being continued.

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