(100) *n*-propanol (45) acetic acid (15) H_2O (10). A single hydroxamic acid spot was found after spraying the paper with $FeCl_3$ with the same R_F value (approx. 0.6) as a sample of the hydroxamic acid prepared from methyl cholate.

These results suggest that cholyl-CoA is an intermediate in the synthesis of conjugated bile acids. Since this work was started BERGSTRÖM AND GLOOR[2] have reported the synthesis of taurocholic acid in rat liver slices and homogenates.

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Conversion of 19-hydroxy-Δ^4-androstene-3,17-dione to estrone by endocrine tissue

Ever since the similarity in structure of the steroidal estrogens and androgens was recognized in the middle thirties, speculations as to the biological conversion of C_{19} into C_{18} steroids have been advanced[1]. The most direct experimental foundation for such a hypothesis was arrived at in the observation of an increase in the urinary estrogens after the administration of androgens. The possibility that these androgens functioned indirectly by stimulating the production or release of the estrogens, however, could not be ruled out[2]. The recent failure to detect a substantial incorporation of injected radioactive cholesterol (a precursor of androgens) into the urinary estrone of the pregnant mare[3], left the problem wide open[4].

With the discovery of an enzyme in mammalian tissue capable of hydroxylating androstenedione at the angular methyl group which is absent in the estrogens, the possibility of a biosynthesis of the natural estrogens from non-aromatic steroids became more plausible. The elimination of an oxygenated methyl group is compatible with biological concepts and such a course was consequently postulated[5]. Subsequently the conversion by human ovary of radioactive testosterone to estradiol, deduced by the use of a carrier dilution technique, was announced[6]. The utilization of acetate in the production of estradiol and estrone by dog ovaries[7] and by human term placenta[8] has also been reported recently.

In the present experiments, 400 μg of steroid were incubated, while shaking at 37.5° for 4 h in air, with 0.80 g of tissue slices suspended in 3.0 ml of aqueous medium (pH 7.3) containing the following additions: phosphate buffer 0.09 *M*, sodium fumarate 0.012 *M*, nicotinamide 0.02 *M*, and diphosphopyridine nucleotide 0.0006 *M*. The incubated slices were homogenized and the total incubate deproteinized with acetone. The filtered extract was brought to dryness by lyophilization and the residue re-extracted with fresh acetone. This second extract was dried and separated into three fractions on a silica gel adsorption column. The solvent mixture used in the first fraction (5% hexane in benzene) eluted lipid material only and was not processed further. The second fraction (40% ether in benzene) contained substances of the polarity of estrone and estradiol, and the third fraction (25% isopropanol in ethyl acetate) the more polar compounds such as Δ^4-androsten-19-ol-3,17-dione.

The second fraction was placed on a 3–4 cm wide paper strip (Whatman No. 1) which was impregnated with a solution of propylene glycol in 90% aqueous methanol (40:60 by volume) and blotted after 3 minutes. When chromatographed at 31° with propylene glycol saturated toluene[9], the R_F for estrone was 0.20 and for estradiol 0.03. These zones were eluted and suitable aliquots rechromatographed on paper at 31° (after a 4 h equilibration) with a two phase mixture of benzene-hexane-methanol-2 *N* ammonium hydroxide (65:35:15:15)[10]. In this system the R_F for estrone was 0.85 and for estradiol 0.65. Evidence for the presence of estrogens was considered acceptable when the very sensitive Turnbulls blue reagent for phenolic substances[11] produced a notable color at the correct location (sensitivity 0.5 μg/sq. cm). The quantity of phenolic estrogens could be easily estimated visually by comparing the color intensities with those of known amounts (accuracy $\pm$ 10%).

Of the tissues tested, human placenta appeared to convert 19-hydroxy-Δ^4-androstene-3,17-dione (I) to estrone (II) most efficiently. A production of II was also observed with cow ovarian follicular fluid (1.5 ml of fluid in a buffered and balanced salt solution with 400 μg of steroid) and to a lesser extent with cow adrenals. Rabbit placenta, removed after 15 days gestation period, and ovaries of rabbits which had been stimulated with gonadotropic hormone 7 h before sacrifice, did not show a pronounced conversion.

Human placentae were obtained by Caesarean section from healthy women after gestation periods of 7 months to term. Slices were incubated 1–3 hours after collection. A conversion of I $\rightarrow$ II amounting to 2–6% was observed. The formation of estradiol (III) was of a smaller order of magni-

tude. Control experiments with tissues incubated alone showed only a minimal content of these estrogens of a few tenths of micrograms, being in the range of the corrected figures as indicated *e.g.* by MITCHELL AND DAVIS[12].

For the purpose of a further characterization of II, ten incubations were pooled and processed essentially as described. The estrone fraction from the paper chromatogram in the toluene system was further purified on a hyflo super cel partition column (1.1 × 12 cm, 0.8 ml of stable phase per gram of celite) at 20° using the two phase mixture benzene-hexane-methanol-2 *N* sodium hydroxide (60:40:5:5)[13]. II was eluted at a rate of 12.5 ml/h in the 340 to 360 ml of mobile phase. Upon heating a dried aliquot of these eluates in 90% sulfuric acid at 80° for 1 h, the solution turned green and fluoresced.

Final identification was achieved by spectroscopic measurements. The heated sulfuric acid solution showed maxima at 299 and 450 mμ and an inflection near 275 mμ. When diluted with the equal volume of absolute ethanol[14] the spectrum was considerably changed; maxima appeared now at 271, 455, and 508 mμ. The combined eluates were dried and pressed with potassium bromide in a micro-die[15] to a transparent prism which absorbed in the infrared maximally near 3400 cm^{-1} (hydroxyl), 1722 cm^{-1} (pentacyclic carbonyl), 1621 and 1580 cm^{-1} (aromatic C=C). All these physical data (including the retention volume of the chromatography) were identical with those determined with authentic estrone.

Of the other steroids incubated with human placenta, Δ^4-androstene-3,17-dione (IV) also showed a conversion to II, although only to an extent of $^1/_2$–$^1/_8$ the transformation observed in corresponding experiments with I. No testosterone (V) was recovered after its incubation; it was found to be converted in major part to IV. (The presence of an active 17β-dehydrogenating system in human placenta has been previously noted in incubations with III[16].) If any transformation to II occurred with $\Delta^{1,4}$-androstadiene-3,17-dione (VI) and 19-nor-Δ^4-androstene-3,17-dione (VII), it was lower than that with IV.

The present experiments thus would strongly indicate that the bioconversion of androgens to estrogens proceeds via a $C_{(19)}$-hydroxylated intermediate. At what oxidative level this angular group is split off, will be the subject of further investigations. Similar enzymic mechanisms may be operative in ejecting as one-carbon fragments the quaternary methyl groups of lanosterol in its biological transformation[17] to cholesterol.

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