The formation and hydrolysis of 16α, 17α-epoxy-oestratriene-3-ol by rat-liver tissue

When oestratetraeneol ($\Delta^{1,3,5(10),16}$-oestratetraene-3-ol) is administered to human subjects, a considerable increase in the urinary "oestriol" fraction is observed[1,2]. To obtain more detailed information on the mechanism of this reaction it was decided to study the metabolic transformations of oestratetraeneol during incubation with rat-liver slices.

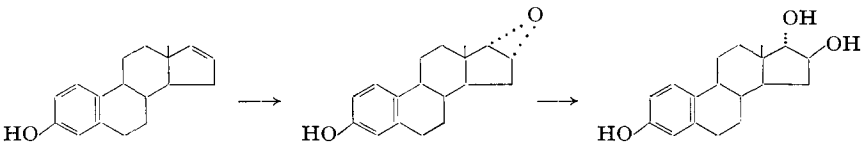
200 mg of rat-liver slices were incubated with 100 μg of oestratetraeneol in 5 ml Krebs phosphate–saline for 30 min at 37°. After incubation the medium and the tissue were extracted with ether–chloroform (3:1, v/v) and the extracts subjected to paper chromatography in the system formamide–chloroform. The area corresponding in mobility to the oestriol/16,17-*epi*-oestriol fraction was eluted and the eluate rechromatographed in the system benzene–methanol–water (100:55:45). After spraying with FOLIN AND CIOCALTEU reagent[3] a phenolic metabolite was detected which showed the same mobility (11.7 cm/24 h) as 16,17-*epi*-oestriol ($\Delta^{1,3,5(10)}$-oestratriene-3,16β, 17α-triol).

In a second experiment the area expected to contain 16,17-*epi*-oestriol was eluted, the eluate evaporated and then subjected to sublimation under atmospheric pressure. The sublimed crystals had the same m.p. (248°) and the same absorption curve with the H_2SO_4–water reaction[4] and with the KOBER reaction[5] as authentic 16,17-*epi*-oestriol.

These experiments showed that 16,17-*epi*-oestriol is a metabolite of oestratetraeneol, and the question arose whether the *trans* glycol is formed directly from the parent compound or via an intermediate. On chemical grounds it appeared highly probable that a 16,17-epoxide is such an intermediate. Accordingly, the 16α,17α-epoxy-oestratrieneol (16α,17α-epoxy-$\Delta^{1,3,5(10)}$-oestratriene-3-ol) and the 16β,17β-epoxy-oestratrieneol (16β,17β-epoxy-$\Delta^{1,3,5(10)}$-oestratriene-3-ol) were incubated with rat-liver slices. The incubation of the 16α,17α-epoxide yielded only one phenolic metabolite which proved to be identical with 16,17-*epi*-oestriol in the reactions described above. When the 16β-17β-epoxide was incubated with rat liver two phenolic compounds were isolated and subsequently identified as oestriol and 16,17-*epi*-oestriol.

In view of these findings it seemed reasonable to assume that 16α,17α-epoxy-oestratrieneol might be the intermediate in the reaction leading from oestratetraeneol to 16,17-*epi*-oestriol; an attempt was therefore made to isolate the 16α,17α-epoxide. Oestratetraeneol was incubated with rat-liver slices, and the extracts were chromatographed in the system propylene glycol–cyclohexene. This system is most suitable for the separation of oestratetraeneol (mobility 19.2 cm/6 h) and 16α,17α-epoxy-oestratrieneol (mobility 3.7 cm/6 h) from one another. The areas corresponding in mobility to 16α,17α-epoxy-oestratrieneol were eluted, the eluates of 5 experiments combined and rechromatographed in the same system. FOLIN AND CIOCALTEU reagent produced a blue spot which showed the same mobility as 16α,17α-epoxy-oestratrieneol. In a second experiment the material was sublimed, and the m.p. of the crystals obtained was identical with that of 16α,17α-epoxy-oestratrieneol (214–215°). For further identification the metabolic product was treated with acetic acid and then hydrolysed with methanolic KOH. This procedure yielded only 16,17-*epi*-oestriol, as it does with authentic 16α,17α-epoxy-oestratrieneol.

The results reported here show that epoxidation occurs when a steroid containing an isolated double bond is incubated with liver tissue, and that the epoxide thus formed is subsequently hydrolysed to a *trans* glycol (Fig. 1). So far, steroid epoxidation has only been observed in molds[6]. The present investigation demonstrates the presence in animal tissue of a steroid epoxidase and of a hydrolase attacking the epoxide.



Oestratetraene-3-ol 16α,17α-epoxy-oestratriene-3-ol 16,17-*epi*-oestriol

Fig. 1. Metabolic transformations of oestratetraene-3-ol during incubation with rat-liver slices.

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Evidence for the reversibility of the reaction catalyzed by adenosine 5′-phosphosulfate reductase

Sulfate in the form of adenosine 5′-phosphosulfate is reduced to sulfite by extracts of *Desulfovibrio desulfuricans*[1]. The enzyme, APS reductase, occurs in the "sulfate-reducing bacteria" and also in the Thiobacilli, that oxidize reduced compounds of sulfur to sulfate. A scheme has been proposed for the oxidation of thiosulfate to sulfate involving APS as an intermediate[3] based on consideration of: (1) the experiments of SANTER[2], who observed that during the oxidation of thiosulfate in the presence of ^{18}O-labeled orthophosphate, ^{18}O is transferred to the sulfate produced in the oxidation, (2) the requirement of phosphate or arsenate for the complete oxidation of thiosulfate, and (3) the presence of certain enzymes concerned with the metabolism of inorganic sulfur compounds in extracts of these organisms. The critical reaction in this scheme is the formation of APS from AMP and sulfite, *i.e.*, the reversibility of the reaction catalyzed by APS reductase. In the present communication, direct

Non-standard abbreviations: APS, adenosine 5′-phosphosulfate; de-AMP, deoxy-AMP; de-GMP, deoxy-GMP; UMP, uridine 5′-phosphate; P_i, inorganic orthophosphate.