Oestrous-Cycle-Dependent Oestradiol Oxidation in Rat Uterus and the Influence of Androst-3-en-17-one

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According to Jensen & Jacobsen (1962), in rat uterus oestradiol is bound by specific receptors, but is not oxidized to oestrone. The oxidation of oestradiol has since been reported in rabbit uterus (Jütting, 1970) and in rat uterus (Lehmann & Breuer, 1968).

In this paper we demonstrate that in rat uterus the oxidation of oestradiol to oestrone is dependent on the oestrous cycle and that the oxidation rate changes in the presence of 5α -androst-3-en-17-one. The influence of this steroid on oestradiol oxidation was studied since it is a specific and effective acceptor of oestradiol hydrogen in different rat tissues (Wenzel & Mützel, 1970).

Materials and methods. [$4\text{-}^{14}\text{C}$]Oestradiol (54.5mCi/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K., [2,4,6,7- ^3H]oestradiol (95Ci/mmol) from NEN Chemicals G.m.b.H., Dreieichenhain, Germany, and 5α -androst-3-en-17-one from Schering A.-G., Berlin 65, Germany.

The procedures for incubating the tissue slices and for extracting the radioactive steroids were the same as those described by Wenzel, Langold & Hallac (1969) and Wenzel & Mützel (1970). The radioactivity on thin-layer chromatograms was measured either directly by the Berthold Dünnschicht Scanner (Stevenson, 1970) or, after scraping the parts of interest off the chromatogram, by liquid-scintillation counting with the Beckman LS 150 Counter.

Results and discussion. The oestrous cycle of adult female rats was followed by vaginal-smear examination for 2–3 weeks. In different stages of the cycle slices of the uteri were incubated *in vitro* with labelled oestradiol. After extraction of the steroids and separation of them by t.l.c. the yield of oestrone was determined.

As shown in Fig. 1 the oxidation of oestradiol to oestrone is oestrous-cycle-dependent both at an oestradiol concentration of 100nm and at the 'physiological' concentration of 0.2nm. The maximum yield of oestrone is found at the first day of oestrus, minimal oxidation of oestradiol occurring at pro-oestrus.

The oxidation of oestradiol at a concentration of 100nm is increased if 5α -androst-3-en-17-one is

added. The enhancement of oxidation by this steroid cannot be detected at an oestradiol concentration of 0.2nm; on the contrary, the androgen inhibits oestrone production. So in this case the oestrous-cycle-dependency of oestradiol oxidation is decreased after addition of 5α -androst-3-en-17-one. Since Yoshinaga, Hawkins & Stoker (1969) have demonstrated an increase of oestrogen release in the

Fig. 1. Oestradiol oxidation in rat uterus in different stages of the oestrous cycle. Slices (100mg) of rat uteri were incubated in 4ml of Ringer phosphate buffer (Umbreit, Burris & Stauffer, 1959), pH 7.4, for 30min at 37°C. Oestradiol concentration: $\square$ and $\blacksquare$, 100nm; $\circ$ and $\bullet$, 0.2nm. The broken lines give the results obtained in the presence of $1.0\mu\text{M}$ - 5α -androst-3-en-17-one. The baseline 0.1–0.2% oestrone formation is due to tissue. Numbers of experiments are given in parentheses.

rat in pro-oestrus and we have found a stimulating effect of oestradiol on the synthesis of 17β -hydroxy steroid oxidoreductase in rat uterus (M. Wenzel & B. Hieronimus, unpublished work), we believe that the increased oxidation of oestradiol during oestrus is the effect of the increased oestrogen secretion in pro-oestrus causing an increased synthesis of 17β -hydroxy steroid oxidoreductase. The oestradiol concentration and therefore the intensity and timing of the hormone effect in the target organ may be regulated by this mechanism.

The effect of 5α -androst-3-en-17-one on oestradiol oxidation (oestradiol concentration 100nm) is similar to that seen in rat liver slices, where oestradiol oxidation was increased up to fivefold after the addition of this steroid (Wenzel & Mützel, 1970). This effect is due to a specific enzymic hydrogen transfer from oestradiol to 5α -androst-3-en-17-one to give 5α -androst-3-en-17-ol. The transfer from oestradiol is 1000 times more effective than the enzymic hydrogen transfer from other substrates

oxidizable by NAD^+ or NADP^+ (Wenzel, 1968; Wenzel & Mützel, 1970).

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- Jensen, E. V. & Jacobsen, H. (1962). *Recent Prog. Horm. Res.* **18**, 387.
- Jütting, G. (1970). *Acta endocr., Copnh.*, **64**, Suppl. no. 145.
- Lehmann, W. & Breuer, H. (1968). *Naturwissenschaften*, **55**, 182.
- Stevenson, R. (1970). *Am. Lab.* May issue, p. 49.
- Umbreit, W., Burris, R. H. & Stauffer, J. F. (1959). *Manometric Techniques*, p. 149. Minneapolis: Burgess Publishing Co.
- Wenzel, M. (1968). *Biochim. biophys. Acta*, **152**, 431.
- Wenzel, M., Langold, M. & Hallac, P. (1969). *J. biol. Chem.* **244**, 4523.
- Wenzel, M. & Mützel, M. (1970). *Hoppe-Seyler's Z. physiol. Chem.* **351**, 1221.
- Yoshinaga, K., Hawkins, R. & Stoker, J. (1969). *Endocrinology*, **85**, 103.