COMMUNICATIONS

New 3 α -Hydroxy Steroid-Nicotinamide-Adenine Dinucleotide Oxidoreductase Activity in Crystalline Enzyme Preparations

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3 α -Hydroxy steroid-NAD oxidoreductase activity is now described in crystalline preparations of 20 β -hydroxy steroid-NAD oxidoreductase (EC 1.1.1.53), from strains of *Streptomyces* (Hübener, Sahrholz, Schmidt-Thomé, Nesemann & Junk, 1959).

3 α -Hydroxy steroid-NAD oxidoreductase activity extended to both 5 α -androstande and 5 α -pregnane compounds. No 17-hydroxy steroid oxidoreductase activity was detected, and androstande compounds were used to study the requirements for reaction at the 3 position.

3 α -Hydroxy steroid-NAD oxidoreductase activity was found only towards compounds of the 5 α -series, and a C-1-C-2 double bond prevented reaction in a 5 α -H compound. No 3 β -hydroxy steroid-NAD oxidoreductase activity was found. Michaelis constants were in the region of 0.1mM for the steroids, and 10 μ M for NADH. No reaction was detected with NADPH as cofactor. This 3 α -hydroxy steroid oxidoreductase activity differs from, and is more specific than, that reported for other enzymes (EC 1.1.1.52 and EC 1.1.1.50).

Substitution of an 11-, 16- or 17-oxo group, or an 11 β - or 17 β -hydroxyl group, or introduction of a C-16-C-17 double bond into the skeleton of 5 α -androstan-3 α -ol or the corresponding -3-one altered the kinetic constants but did not prevent reaction.

The ratio of 3 α - to 20 β -hydroxy steroid-NAD oxidoreductase activity was very closely similar for different batches of crystalline enzyme, suggesting that the activities might reside in the same molecule. Kinetic results relevant to the identity of the active centres will be presented and discussed. One explanation of these results would be that the centre for 20 β activity is independent of that for 3 α activity, and that at least two distinct types of 3 α activity dependent upon different centres may be present. Although this is not the only conceivable explanation, it is probably the simplest consistent with the data.

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Hübener, H. J., Sahrholz, F. H., Schmidt-Thomé, J., Nesemann, G. & Junk, R. (1959). *Biochim. biophys. Acta*, **35**, 270.

Influence of Light and 9,10-Dimethylbenz[a]-anthracene on Rat Ovarian Steroidogenesis: Phenolic Steroids

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Endocrine organs have been shown to be markedly affected by alteration of environmental light conditions (Wurtman, Roth, Altschule & Wurtman, 1961), and it has been suggested that such effects might be mediated by an indole derivative, melatonin, which is produced uniquely in the pineal gland (Lerner, Case & Takahashi, 1960).

In the experiments to be described, Sprague-Dawley rats were kept under standardized lighting conditions for 8 months. One group was subjected to continuous illumination (24 hr. group), the other group being kept under conditions of 12 hr. light and 12 hr. darkness per day (12 hr./12 hr. group).

Rats in each of these two groups were divided into four sub-groups, each containing five animals. The first group consisted of control animals. Rats in the second group received 100 μ g. of melatonin daily by subcutaneous injection, from age 43 days until termination of the experiment. The third group of animals were given a single intragastric instillation of 30 mg. of 9,10-dimethylbenz[a]anthracene (DMBA) in corn oil at age 50 days. The fourth group of animals were given both melatonin and DMBA in the same dosages and at the same times as those in the second and third groups.

At the end of 8 months the animals were killed and the left ovaries of each sub-group were pooled, homogenized and incubated in a Krebs-Ringer phosphate buffer, pH 7.4, in the presence of a NADPH-generating system with the precursors [4-¹⁴C]androst-4-ene-3,17-dione (androstenedione) and 3 β -hydroxy[7 α -³H]pregn-5-en-20-one (pregnenolone).

Then 100 μ g. each of 3-hydroxyoestra-1,3,5(10)-trien-17-one (oestrone) and oestra-1,3,5(10)-trien-3,17 β -diol (oestradiol) was added to each phenolic extract in order to assess recoveries at each stage of purification by the Kober reaction (Brown, 1955). Oestrone and oestradiol were isolated by thin-layer chromatography and purified by methylation and