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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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

further chromatography. Comparisons were then made of the incorporation ^{14}C into oestrone and oestradiol in each of the sub-groups, under the two lighting regimes.

In the control animals the conversion of androstenedione into oestrone plus oestradiol in the 24hr. group was 2.5 times that in the 12hr./12hr. group. Administration of melatonin did not affect the 24hr. group, the conversion being 0.9 times the control value, but in the 12hr./12hr. group conversion was stimulated to 1.7 times the control value. The findings are in accord with histological evidence, which indicates that ovaries from animals under constant illumination consist almost entirely of follicular cysts with few corpora lutea (Fiske, 1941; Hamilton & Sneddon, 1968). Ovaries from the 24hr. group were very susceptible to an action of DMBA that resulted in almost complete obliteration of steroidogenesis. Melatonin seems to exert some protective action against this effect as the conversion in the 24hr. DMBA plus melatonin group was 0.8 times the control value as compared with 0.0 times for the DMBA group. In the 12hr./12hr. animals, however, DMBA exerted a marked stimulation of conversion of androstenedione into oestrogen, the value being 3.6 times that of the control. When given in conjunction with melatonin their separate stimulatory actions appear to be additive as the conversion was 5.7 times the control.

Oestrone and oestradiol together represented only about one-tenth of the total radioactivity found in the phenolic fraction, the remainder being associated with more polar products. This substantiates the findings of Huang & Pearlman (1962).

The results described indicate that exposure to continuous illumination stimulates conversion of androstenedione into oestrogen. Melatonin and DMBA also stimulate this conversion in rats kept under normal lighting conditions, and it is suggested that these factors might operate by primary stimulation of follicle-stimulating hormone release from the pituitary. Further investigations of this possibility and of the nature of the polar oestrogens produced by the rat ovary are now being made.

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

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Investigation of the effects of light and of the pineal hormone, melatonin, on the induction and growth of hormone-dependent breast tumours in response to administration of 9,10-dimethylbenz[a]-anthracene (DMBA) (Huggins, Grand & Brillantes, 1961) led us to examine in detail the effects of such influences on ovarian steroidogenesis.

Sprague-Dawley rats were maintained under conditions of continuous illumination (24hr. group) or under diurnal conditions of 12hr. light and 12hr. darkness per day (12hr./12hr. group) for 8 months. Each of these groups was divided into four sub-groups: controls; plus melatonin; plus DMBA; and plus DMBA + melatonin; each sub-group contained five animals. Melatonin was administered as a daily subcutaneous injection of 100 μg . from age 43 days, for the duration of the experiment. DMBA was given as a single intragastric instillation of 30mg. in corn oil at age 50 days.

At the end of 8 months, the animals were killed and the left ovaries of each sub-group were pooled, homogenized and incubated with 3 β -hydroxy-[7 α - ^3H]pregn-5-en-20-one (pregnenolone) and [4- ^{14}C]androst-4-ene-3,17-dione (androstenedione). The right ovaries were taken for histological examination.

Incubations were carried out in Krebs-Ringer phosphate buffer, pH 7.4, in a system containing glucose 6-phosphate, glucose 6-phosphate dehydrogenase and NADP.

20 α -Hydroxypregn-4-en-3-one (20 α -hydroxyprogesterone), which is the major progestogen in the rat (Huang & Pearlman, 1962; Weist, 1959), was isolated after the addition of a known quantity of unlabelled authentic material to the incubation extract. This was purified by thin-layer chromatography before and after modification. From the specific activities after the final procedure, the 20 α -hydroxyprogesterone content of the neutral extracts was calculated and expressed as a percentage of the total extract. It was found that the 24hr. conversion was lower than the 12hr./12hr. for all groups. The conversion in the 24hr. control is 0.43 times that of the 12hr./12hr. control, a finding in keeping with the histological evidence that the 24hr. control ovary is composed of large thin-walled follicular cysts (Fiske, 1941; Hamilton & Sneddon, 1968). Melatonin decreases the conversion in both groups, the 12hr./12hr. to 0.72 times that of the control and the 24hr. to 0.76 times that of the control. DMBA has a very similar effect on