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

the 12hr./12hr. ovary, decreasing the conversion to the same extent as melatonin. The effect of DMBA on the 24hr. ovary is completely unexpected in that the conversion is zero. Melatonin protects against this effect to some extent in that the conversion is 0.23 times that of the DMBA control, but, in the 12hr./12hr. ovary, conversion is stimulated to 2.6 times that of the DMBA control.

It has been suggested that the changes in oestrogen conversion result from an increased secretion of follicle-stimulating hormone. The changes in progestogen conversion described here are in agreement with an alteration in the follicle-stimulating hormone/luteinizing hormone ratio, which is markedly in favour of follicle-stimulating hormone, as a result of exposure to light or melatonin administration.

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Another Pathway of Oestriol Biosynthesis in Pregnancy

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In late pregnancy the major pathway of oestriol biosynthesis involves the secretion of dehydroepiandrosterone sulphate by the foetal zone of the foetal adrenal and hydroxylation of this material at C-16 α by the foetal liver. The placenta converts 16 α -hydroxydehydroepiandrosterone sulphate into oestriol. Other pathways cannot, however, be neglected. Siiteri & MacDonald (1965) demonstrated that the maternal adrenal contributes precursors. Kirschner, Wqvist & Diezfalusy (1966) suggested that 16 α -hydroxy C₂₁ steroids might be involved, by utilizing a pathway that avoids dehydroepiandrosterone (DHA). Shahwan, Oakey & Stitch (1968) observed the conversion of [¹⁴C]-pregnenolone into 16 α -hydroxy[¹⁴C]dehydroepiandrosterone (16 α -OH-DHA) *in vitro* by slices of adrenal tissue from a newborn anencephalic infant. The foetal zone of the foetal adrenal of the anencephalic infant is atrophic and the gland is composed almost entirely of definitive zone tissue.

We now report further results which demonstrate the formation of 16 α -OH-DHA from pregnenolone by a pathway that excludes DHA.

Adrenal tissue (145mg.) obtained within 0.5hr. of death from a newborn anencephalic infant was incubated with [7 α -³H]pregnenolone (19 μ C, 24 μ g.) and [4-¹⁴C]DHA (0.8 μ C, 22 μ g.) in Krebs-Ringer phosphate buffer (5ml., pH 7.3) at 37° with air as the gas phase. After incubation for 5hr., non-radioactive DHA and 16 α -OH-DHA (100 μ g. of each) were added in acetone. A 'neutral steroid' fraction was prepared as described by Shahwan, Oakey & Stitch (1968). After paper chromatography of this fraction in light petroleum (b.p. 40–60°)–toluene–methanol–water (5:5:7:3, by vol.), ³H and ¹⁴C were found associated with carrier 16 α -OH-DHA. Both isotopes remained with this carrier after rechromatography in benzene–methanol–water (10:7:3, by vol.). However, ¹⁴C, but not ³H, was separated from carrier 16 α -OH-DHA during conversion into oestriol by treatment with placental microsomes and reduction of the 16 α -hydroxy-oestrone formed. Therefore, [³H]pregnenolone, but not [¹⁴C]DHA, was a precursor of 16 α -OH-DHA. Consequently, there exists, at least *in vitro*, a pathway for the biosynthesis of an oestriol precursor that excludes DHA.

If such a pathway operates in the foetal adrenal *in vivo* it is unlikely to contribute significantly to oestriol biosynthesis in normal pregnancy. In pregnancy with an anencephalic foetus, however, where oestriol excretion is reduced by 90%, but may still be 100 times that of non-pregnancy, such a pathway could play, relatively, a more important role.

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The Influence of Oestradiol-17 β on Ribonucleic Acid Synthesis in Rat Uterus

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RNA from the uteri of oestradiol-treated and untreated immature rats was fractionated on columns of methylated serum albumin–kieselguhr (MAK), after the administration of radioactive precursors of RNA, and the distribution of radioactivity in the various RNA fractions was examined.

In one series of experiments, 1 μ g. of oestradiol-17 β was administered intraperitoneally to the test animals 1hr. before they were killed; the controls received 0.1ml. of 0.9% (w/v) NaCl. At 30min. before they were killed, both groups received a