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, Wiqvist & Diezfalussy (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

mixture of 25 μC of [^3H]uridine and 25 μC of [^3H]guanosine. RNA was extracted separately from the uteri of the control and test groups.

In other experiments, by the dual-labelling procedure of Ellem (1967), the test animals received 1 μg . of oestradiol 90 min. before being killed and the controls were given 0.1 ml. of 0.9% (w/v) NaCl. At the same time, the controls were given 100 μC of [^3H]uridine and the test animals received 25 μC of [^{14}C]uridine. The uteri from both groups were pooled before extraction of the RNA.

No significant differences were observed in the distribution of ultraviolet-absorbing material from the test and control animals. However, in the experiments with ^3H -labelled precursors the total radioactivity recovered from the MAK columns of RNA from treated animals was 72% greater than from the controls. The greatest change occurred in the Q1 RNA peak which showed an increase of 150% over the controls, whereas the ribosomal RNA peak increased by 67%. Smaller increases (30%) occurred in the Q2 RNA peak and in the components eluted with sodium dodecyl sulphate.

Somewhat similar results were obtained in the dual-labelling experiments. After allowing for the difference in specific radioactivities of the precursors, the total ^{14}C recovered was 63% greater than the total ^3H and the largest increase again occurred in the Q1 RNA peak.

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Urease in *Spirodela*

By A. R. COOK (Rowett Research Institute, Bucksburn, Aberdeen) and E. G. BOLLARD (Fruit Research Division, D.S.I.R., Auckland, New Zealand) (introduced by P. N. HOBSON)

Bollard (1966) found that the higher plant *Spirodela oligorrhiza* (a duckweed) could be grown vegetatively in sterile culture and under these conditions it used urea as sole source of nitrogen provided that the pH of the medium was not above 4.5. We have found that in urea-containing media of higher pH plants rapidly became nitrogen-deficient in appearance and made no further growth. Urease could not be detected in plants growing with ammonium sulphate as sole source of nitrogen, but in plants actively growing with urea as a sole source of nitrogen this enzyme was always present. Such an appearance of urease where it is normally absent suggests that, in this species, the enzyme may be induced by urea taken up from the medium. *Spirodela* also grew with the ureide allantoin as source of nitrogen and allantoin-grown plants contained urease. The metabolism of allantoin is likely to produce urea as an intermediate and such

urea produced endogenously might in turn induce urease production.

When *Spirodela* plants were placed in a medium lacking any source of nitrogen they rapidly became nitrogen-deficient in appearance and, after some days, urease activity appeared in such plants, reaching activities as high as in plants growing on urea-containing medium. Some redistribution of cell nitrogen must occur under the stress of nitrogen deficiency and it is likely that some normal catabolic reactions may produce urea endogenously.

It is clear that the activity of urease in plants is markedly affected by the pH of the medium. Plants in media at pH 7 took up urea but no urease appeared in the tissues and consequently such plants did not grow. Growth of plants at a high pH on a medium containing allantoin as a sole source of nitrogen or on a nitrogen-deficient medium lowered or prevented the development of urease activity. When the pH of the medium fell to 3.5 or lower the activity of urease rose to values up to ten times those present in plants growing on urea at about pH 4.3.

It thus appears that in this species urease is normally absent or present at a very low activity. Nonetheless it may be a normal catabolic enzyme that can increase markedly in activity when plants become nitrogen-deficient or when they are supplied with either allantoin or urea. Then what is normally a catabolic enzyme may serve as a primary assimilatory enzyme.

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Activation *in vitro* and Solubilization of Glucuronyltransferase (assayed with Bilirubin as Acceptor) with Digitonin

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Several microsomal enzymes have been activated by treatment with deoxycholate, Triton X-100 or other detergents (Ernster, Siekevitz & Palade, 1962; Dallner, 1963; Snoke & Nordlie, 1967). Recently glucuronyltransferase was shown to behave in a similar way (Lueders & Kuff, 1967; Van Roy & Heirwegh, 1968). With increasing detergent concentrations maximal activation was attained followed by gradual inhibition and loss of enzyme activity. Attempts to solubilize glucuronyltransferase with deoxycholate were unsuccessful (Leventer, Buchanan, Ross & Tapley, 1965; Tomlinson & Yaffe, 1966). In contrast, active non-sedimentable preparations were obtained with digitonin (Pogell & Leloir, 1961).