hormones and this has been studied from two aspects: (1) the ability of nuclei to accept cytoplasmic receptor; (2) the number of cytoplasmic receptor sites.

The nuclear oestradiol-17 β receptor is an acidic protein, but very little is known about the acceptor molecule for this receptor. No association of nuclear receptor with DNA was detected by sucrose-density-gradient analysis. The receptor does complex with histones, and it seems likely that the acceptor is a basic protein (King, Gordon & Steggles, 1969). When uterine nuclei were incubated with [6,7- ^3H]oestradiol-17 β -labelled supernatant at 37°C, ^3H entered the nucleus and could be extracted with 0.4M-KCl. At 0°C, very little ^3H entered the nucleus. Liver nuclei took up less ^3H at 37°C than did uterine nuclei. The ability of nuclei from mature uteri to accept [6,7- ^3H]oestradiol-17 β (expressed per mg of either nuclear DNA or protein) under these conditions decreased within one day of ovariectomy. Liver nuclei were not affected.

Ovariectomy of mature rats also decreased the number of cytoplasmic receptors as measured by the protamine method (Steggles, Vertes & King, 1969). One week after ovariectomy the number of cytoplasmic sites either per uterus or per mg of DNA has fallen by about half. One week after hypophysectomy the value fell to 20–30% of the controls. Adrenalectomy had no effect. None of these operations affected the number of receptor sites when expressed per mg of protein, which suggested that the number of sites decreased in proportion to the loss of cytoplasmic protein and that hypophysectomy had a greater effect than ovariectomy.

King, R. J. B., Gordon, J. & Steggles, A. W. (1969). *Biochem. J.* **114**, 649.

Steggles, A. W. & King, R. J. B. (1970). *Steroids*, (in the Press).

Steggles, A. W., Vertes, M. & King, R. J. B. (1969). *Biochem. J.* **115**, 48P.

Metabolic Clearance Rates and Conversions of Oestradiol-17 β in the Sheep

By J. R. G. CHALLIS, F. A. HARRISON and R. B. HEAP. (*Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.*)

The metabolic clearance rate of oestradiol-17 β and its interconversions with oestrone have been measured in non-pregnant women (Longcope, Layne & Tait, 1968). We have begun to investigate the metabolic clearance rate of oestradiol-17 β in non-pregnant and pregnant sheep.

Tritiated oestradiol-17 β (50 and 100Ci/mmol) was chromatographically purified and infused at a constant rate (about 1 $\mu\text{Ci}/\text{min}$) into a jugular vein of both conscious and anaesthetized sheep. The blood concentration of labelled oestradiol-17 β reached equilibrium after 2½h, and samples were taken from the opposite jugular, haemolysed and stored at -15°C. After thawing at 4°C unlabelled oestradiol-17 β , oestradiol-17 α , oestrone, 16-oxo-oestradiol-17 β and oestriol were added to the samples, extracted with diethyl ether, partitioned to obtain a phenolic fraction and separated by repeated t.l.c. Specific radioactivities were measured before and after formation of derivatives (acetates and heptafluorobutyrate; Challis & Heap, 1969).

The whole-blood metabolic clearance rate of oestradiol-17 β in a non-pregnant anaesthetized sheep was 2117 litres/day (39 litres/day per kg body wt.). In an anaesthetized pregnant sheep (day 144 of pregnancy) this value had almost doubled. Three labelled metabolites, oestradiol-17 α , oestrone and 16-oxo-oestradiol-17 β , were identified in an extract of the 'free' and 'protein-bound' oestrogens; very low radioactivity was found in the oestriol fraction. In these and two experiments on a conscious pregnant sheep the conversion ratio of oestradiol-17 β and its labelled metabolites was calculated (conversion ratio, CBB: 100 $\times$ isotope concentrations of product/precursor). In the non-pregnant sheep the conversions into oestrone and oestradiol-17 α were similar (13 and 14% respectively). In mid-pregnancy (day 92) the conversion into oestradiol-17 α had increased, but a few days before parturition it had fallen to 4.4%, whereas the conversion into oestrone had increased to 18.9%. The conversion into 16-oxo-oestradiol-17 β was greater than 15%.

These preliminary experiments indicate that in the sheep, as in the human, the metabolic clearance rate of oestradiol-17 β exceeded the estimated splanchnic blood flow, suggesting considerable extrahepatic oestrogen metabolism, particularly in late pregnancy. Significant changes were observed in the relative concentrations of circulating labelled metabolites. The ratio of CBB oestrone to CBB oestradiol-17 α was about 1.0 in the non-pregnant sheep, but greater than 4.0 just before parturition.

J. R. G. C. gratefully acknowledges a Science Research Council Studentship.

Challis, J. R. G. & Heap, R. B. (1969). *Biochem. J.* **112**, 36P.

Longcope, C., Layne, D. S. & Tait, J. F. (1968). *J. clin. Invest.* **47**, 93.