We thank Professor I. MacGillivray and Professor S. C. Frazer for interest and encouragement, Professor W. Klyne and Dr D. N. Kirk for reference steroids, C. F. Boehringer und Soehne G.m.b.H. (Mannheim, Germany) for enzyme and the Medical Research Council for a personal grant to W. G.

Oestrogen Synthesis by the Pregnant Baboon: the Contribution of Maternal Circulating Precursors to Urinary Oestrogens

By JOHN D. TOWNSLEY and O. RAY KLING.
(Department of Obstetrics and Gynecology, Ohio State University, Columbus, Ohio 43210, U.S.A.)

A subhuman primate model is required for studies on the endocrinology of human pregnancy. Elevated urinary oestrogen concentrations during human pregnancy reflect increasing placental utilization of foeto-maternal precursors (Siiteri & MacDonald, 1966). We have studied the conversion of circulating precursors into urinary oestrogens by pregnant baboons (*Papio anubis*).

After intra-antecubital vein administration of 25 μ Ci of tracer 3 β -hydroxy[7-³H]androst-5-en-17-one or [7-³H]androst-5-en-17-on-3 β -yl sulphate 73% of the radioactivity appeared in the urine within 72h and negligible amounts thereafter. [4-¹⁴C]-Oestrogens added to urine permitted correction for losses and proof of radiochemical purity of [³H]-oestrogens after their isolation from urine (Brown, 1955). Less than 0.5% of ³H from either precursor was associated with urinary oestrone, oestradiol-17 β and oestriol from non-pregnant animals. The following percentages of the radioactivity of [7-³H]androst-5-en-17-on-3 β -yl sulphate administered to pregnant animals were incorporated into urinary oestrogens: oestrone, 0.84, oestradiol-17 β , 0.14, and oestriol, 0.02, at 93 days of gestation; oestrone, 0.89, oestradiol-17 β , 0.25, and oestriol, 0.07, at 142 days of gestation. Corresponding percentages of the radioactivity of 3 β -hydroxy[7-³H]-androst-5-en-17-one incorporated were: oestrone, 2.82, oestradiol-17 β , 1.08, and oestriol, 0.26, at 113 days of gestation; oestrone, 6.58, oestradiol-17 β , 0.98, and oestriol, 0.39, at 156 days of gestation.

In subsequent experiments tracer [7-³H]androst-5-en-17-on-3 β -yl sulphate plus [¹⁴C]oestradiol-17 β (³H/¹⁴C ratio 5.34) were administered. Within 72h 60% of the ¹⁴C was excreted; 92% of this was associated with oestrone, 7% with oestradiol-17 β and 2% with oestriol. ³H/¹⁴C ratios of urinary oestrone, oestradiol-17 β and oestriol increased from 0.01, 0.08 and 0.06 respectively in a non-pregnant animal to 0.14, 0.13 and 0.16 respectively at 132 days of gestation and 0.23, 0.22 and 0.27 respectively at 168 days of gestation.

Maternal precursors are increasingly utilized for oestrogen synthesis in the pregnant baboon, but oestrone, rather than oestriol, is quantitatively most important. The higher ³H/¹⁴C ratio of oestriol suggests that, as in the human, some oestriol is formed via pathways excluding oestradiol-17 β . The more extensive conversion of 3 β -hydroxy-androst-5-en-17-one than of androst-5-en-17-on-3 β -yl sulphate into oestrogens recalls similar findings with perfused human placentae. Townsley, Scheel & Rubin (1970) have proposed that human placental oestrogen synthesis may be limited by the rate of hydrolysis of conjugated precursors. Baboons appear to be suitable models for exploration of this and other aspects of the endocrinology of human pregnancy.

This work was supported by the John A. Hartford Foundation Inc.

Brown, J. B. (1955). *Biochem. J.* **60**, 185.

Siiteri, P. K. & MacDonald, P. C. (1966). *J. clin. Endocr.* **26**, 751.

Townsley, J. D., Scheel, D. A. & Rubin, E. J. (1970). *J. clin. Endocr.* **30** (in the Press).

Properties of Renal Aldosterone-Binding Proteins

By A. J. MILLS, J. F. WHELDRAKE and L. A. W. FELTHAM (introduced by P. J. A. O'DOHERTY).
(Department of Biochemistry, Memorial University of Newfoundland, St John's, Newfoundland, Canada)

Previous investigators have established that aldosterone binds to protein(s) of both the nuclear and cytosol fractions of kidneys of adrenalectomized rats *in vivo* and *in vitro* (Fanestil & Edelman, 1966; Herman, Fimognari & Edelman, 1968). The present communication describes attempts to characterize these binding protein(s) further and, in particular, investigations of the relationship between nuclear and cytosol binding protein(s).

The ratio of nuclear to cytosol binding was found to be greater in experiments where aldosterone had been injected (*in vivo*) than in experiments where the proteins were assayed by binding of aldosterone *in vitro* as described by Herman *et al.* (1968). This indicates either that the binding protein moves into the nucleus after the injection of aldosterone or that the nuclear and cytosol binding proteins decay at different rates after isolation. It was then found that, if the protein concentration of the binding assay *in vitro* were increased by the addition of bovine serum albumin, the amount of binding by nuclear extract increased but that by cytosol did not. Under these conditions the ratio of nuclear to cytosol binding was the

same *in vitro* as *in vivo*, suggesting that no movement of binding protein(s) from cytoplasm to nucleus had occurred after the aldosterone injection.

The possibility that inactivation of kidney protein(s) might be prevented by aldosterone was investigated by the inclusion of [^3H]aldosterone in the homogenization medium. It was found under these conditions that the amount of aldosterone bound was increased three- to five-fold for the cytosol fractions and up to twofold for the nuclear fraction. However, this binding was inhibited by oestradiol-17 β , unlike the binding occurring after normal isolation. This suggests that, in addition to aldosterone-specific binding protein, kidney also contains an unstable and relatively non-specific aldosterone-binding protein. The amount of the aldosterone-specific component was not significantly increased by homogenization in the presence of aldosterone.

This work was supported by the National Research Council of Canada.

Fanestil, D. D. & Edelman, I. S. (1966). *Proc. natn. Acad. Sci. U.S.A.* **56**, 872.

Herman, T. S., Fimognari, G. M. & Edelman, I. S. (1968). *J. biol. Chem.* **243**, 3849.

Steroid Assays in Human Pregnancy Blood

By P. E. ROSS, J. R. T. COUTTS and M. C. MACNAUGHTON.* (*Department of Obstetrics and Gynaecology, University of Dundee, Dundee, U.K.*)

Little information is available about steroid concentrations in the human foeto-placental unit throughout gestation. This communication describes a method developed to provide such information.

Blood plasma was obtained at various times of gestation from maternal peripheral and uterine veins and umbilical artery and vein. An ethanolic solution containing radioactive tracers of cholesterol, progesterone, pregnenolone, androstenedione, 17 α -hydroxyprogesterone and testosterone was added to the plasma sample (10 ml). The plasma was extracted four times with 15 ml of A.R. ethanol and the combined dried extracts were then applied to a washed paper strip (Whatman 3MM) for chromatography. This chromatogram was developed with *n*-heptane-70% methanol (Yannone, 1968), a system that separates cholesterol, progesterone, pregnenolone and androstenedione into discrete zones but does not resolve 17 α -hydroxyprogesterone and testosterone. After a scanning for radioactivity

(Nuclear-Chicago Actigraph III) all radioactive zones were eluted from the chromatograms. The cholesterol zone was rechromatographed in a hexane (b.p. 67–70°C) adsorption system and then all zones were acetylated with acetic anhydride-pyridine (1:1, v/v). Cholesterol acetate was chromatographed again in the adsorption system; progesterone and androstenedione were rechromatographed in the *n*-heptane-70% methanol system and pregnenolone acetate was developed with hexane (b.p. 67–70°C)-90% methanol.

After elution all of these compounds were ready for quantitative determination. Progesterone 17 α -acetate and testosterone acetate were separated by paper chromatography in hexane (b.p. 60–80°C)-60% methanol. Testosterone acetate was then ready for quantitative determination. Progesterone 17 α -acetate was hydrolysed with sodium hydrogen carbonate (3.8 mM in 50% methanol) and the free steroid was chromatographed in Bush system LT11/A85 (Bush, 1961) before quantitative determination. Recoveries of compounds up to the quantitative step were all in the range 44–65%.

Cholesterol and progesterone were determined quantitatively by g.l.c. (Pye series 104 model 4 gas chromatograph with flame ionization detection) by using the peak-area measurement technique, after chromatography at 230°C in a 7 ft column of 3% XE 60. The extracts were sufficiently clean to allow quantitative determination of these compounds without interference from early eluted material. Proof of identity is based on the results of paper chromatography and g.l.c. on columns containing 3% XE 60, 3% QF 1, 3% SE 30 and 3.5% OV 17. Quantitative determination of the other four steroids is the subject of continuing research.

By using this method it was possible to obtain purified extracts of all six compounds from individual plasma samples. There is good correlation between the results obtained for progesterone by using this method and similar results published by workers who used different methods (Fuchs, Fuchs & Short, 1963; Yannone, McCurdy & Goldfien, 1968).

This work was supported by a grant from the Lalor Foundation to J. R. T. C.

Bush, I. E. (1961). *The Chromatography of Steroids*. Oxford: Pergamon Press Ltd.

Fuchs, F., Fuchs, A.-R. & Short, R. V. (1963). *J. Endocr.* **27**, 333.

Yannone, M. E. (1968). *Am. J. Obstet. Gynec.* **101**, 1054.

Yannone, M. E., McCurdy, J. R. & Goldfien, A. (1968). *Am. J. Obstet. Gynec.* **101**, 1058.

* Present address: Department of Obstetrics and Gynaecology, Royal Maternity and Women's Hospital, Rottenrow, Glasgow C. 2, U.K.