Jones, 1970) prevents transformation of the lymphocytes by phytohaemagglutinin.

Although no change in net phosphate on histones was found during the period of RNA synthesis immediately after stimulation, a change in labelling with [^{32}P]P_i (Cross & Ord, 1970) was detected. It was therefore decided to look at the sizes and specific radioactivities of the ATP and P_i pools immediately after stimulation. Lymphocytes were given a continuous uridine label 30 min before stimulation. In addition a [^{32}P]P_i pulse was administered 30 min before the animals were killed. P_i and acid-hydrolysable phosphate were determined by the method of Berenblum & Chain (1938) and the radioactivity of the butanol extracts was measured. Net values for ATP were confirmed by the firefly luciferase system (Holton, 1959). Intracellular P_i increased immediately after stimulation, returning to control values within 10 min. The specific radioactivity of P_i also showed a correspondingly large increase. It is suggested that this represents an influx of labelled P_i from the medium into the cell as a result of a change in membrane conformation on stimulation. ATP concentrations were lowered during the first 30 min and then rose rapidly above the control value. The specific radioactivity of the ATP reflected this.

Changes in the specific radioactivity of total histone minus histone F1 were directly proportional to changes in the specific radioactivity of the cellular ATP pool. The specific radioactivity of histone F1, however, was not directly related to the specific radioactivity of the ATP pool. It would appear that a rapid increase in labelling with [^{32}P]P_i occurred on this protein within the first 30 min after stimulation, when the rate of RNA synthesis was high. Only a small incorporation of [^{32}P]P_i occurred thereafter.

The changes in histone specific radioactivity and in ATP and P_i concentrations within 30 min of lymphocyte stimulation demonstrate the rapid effect on cellular metabolism produced by transforming agents such as phytohaemagglutinin or cyclic AMP.

M. E. C. thanks the Medical Research Council for a research studentship. We also thank the Cancer Research Campaign for financial support.

- Berenblum, I. & Chain, E. (1938). *Biochem. J.* **32**, 295.
 Cross, M. E. & Ord, M. G. (1970). *Biochem. J.* **118**, 191.
 Forsdyke, D. R. (1968). *Biochem. J.* **108**, 297.
 Hirschhorn, R., Grossman, J. & Weissmann, G. (1970). *Proc. Soc. exp. Biol. Med.* **133**, 1361.
 Holton, P. (1959). *J. Physiol., Lond.*, **145**, 494.
 MacManus, J. P. & Whitfield, J. F. (1969). *Exptl Cell Res.* **58**, 188.
 Monjardino, J. P. P. V. & MacGillivray, A. J. (1968). *Biochem. J.* **108**, 22P.
 Wolff, J. & Jones, A. B. (1970). *Proc. natn. Acad. Sci. U.S.A.* **65**, 454.

Influence of Reaction Medium on the Behaviour of a Hydroxy Steroid-Nicotinamide-Adenine Dinucleotide Oxidoreductase

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Molecules of diverse structures are frequently substrates for hydroxy steroid oxidoreductases, and analogues are often inhibitors. Because of the importance of these phenomena both in general enzymology and steroid biochemistry, it was decided to investigate the recognition and reaction of various steroids by using a crystalline enzyme of microbiological origin (EC 1.1.1.53). Properties are now described of a reaction medium in which various non-polar and most polar steroids are sufficiently soluble to allow detailed study.

The system consists of sodium phosphate buffer, pH 5.2 (0.1 M with respect to phosphate), containing 20% (v/v) of 2-methylpropan-2-ol. Increasing buffer molarity from 0.1 M to 0.2 M decreased the reaction rate with 5 α -androstan-3-one as substrate. Replicates (eight at each of five steroid concentrations) were closer in the 0.10 M than in the 0.15 M or 0.20 M systems, possibly indicating some instability at higher molarities. The high K_m value for 5 α -androstan-3-one (436 μM at 25°C with 115 μM -NADH) made it difficult to discern whether changes in reaction rate arose from small changes in K_m or V_{max} ; K_m for NADH (with 100 μM -5 α -androstan-3-one) increased from 14.8 μM at 0.10 M to 39.8 μM at 0.2 M. Increasing the concentration of 2-methylpropan-2-ol from 18% to 22% decreased the reaction rate fivefold (with 75 μM -5 α -androstan-3-one and 115 μM -NADH).

In the 0.10 M-phosphate-20% 2-methylpropan-2-ol system reaction rates ($\mu\text{mol/min per mg}$) for 90 μM -steroid and 115 μM -NADH were: 5 α -androstan-3-one, 4.8; 5 β -androstan-3-one, 1.0; the corresponding 17 α -hydroxy compounds, <0.1.

In the same reaction medium containing 10% (v/v) of dimethyl sulphoxide instead of 20% 2-methylpropan-2-ol, 5 α - and 5 β -androstan-3-one were substrates, but came out of solution. Reaction of the 5 β -isomer was slower, but exact comparison was not possible. 17 β -Hydroxy-5 α -androstan-3-one and 17 β -hydroxy-5 β -androstan-3-one (90 μM , with 115 μM -NADH) reacted at 3.8 and 0.3 $\mu\text{mol/min per mg}$ respectively, and were much better substrates in this than in the 2-methylpropan-2-ol system.

Reduction of 5 α -androstan-3-one in the 2-methylpropan-2-ol system was inhibited by the hydrocarbon 5 β -androstan-3-one and by 5 α -androstan-17 β -ol. Some implications of these findings are discussed.

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

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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

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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