To eliminate interference from cytochrome *P*-450, cytochromes were examined by using the difference spectra after reduction with succinate in the presence of cyanide (Cammer & Estabrook, 1967b). The absorption bands with maxima at 605 and 445nm, 561 and 428nm and 552 and 419nm indicated the presence of cytochromes $a+a_3$, b and $c+c_1$ respectively. Human chorionic gonadotrophin increased the concentrations of these cytochromes from 0.08, 0.05 and 0.07 to 0.15, 0.12 and 0.14 nmol/mg of mitochondrial protein respectively. Estimation of cytochrome *P*-450 (Omura & Sato, 1964) showed that human chorionic gonadotrophin increased the concentration from 0.11 to 0.24 nmol/mg of protein.

Difference spectra (Cammer & Estabrook, 1967b) indicated that succinate in the absence of cyanide would reduce cytochrome *P*-450 in ovary mitochondria. This was confirmed by the 450nm absorption maxima after CO treatment.

Rat ovary mitochondria apparently contain a dual cytochrome system similar to that of the adrenal gland. The cytochrome concentrations are of the same order as those of the adrenal gland except for the substantially lower content of cytochrome *P*-450. In ovary the cytochrome activity is modified by human chorionic gonadotrophin.

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The Effect of Angiotensin II on the Biosynthesis of Aldosterone by Ox Adrenocortical Tissue in Continuous-Flow Experiments *in vitro*

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Attempts to demonstrate the effect of angiotensin on aldosterone biosynthesis *in vitro* have yielded conflicting results.

Kaplan & Bartter (1962) demonstrated a stimulation of aldosterone formation by ox, but not by rat, adrenal tissue. Kaplan (1965) confirmed this. In both cases aldosterone was assayed by a double-labelled-derivative method. Müller (1966) observed a stimulation of aldosterone production by rat adrenal sections from endogenous precursors, but not from added radioactively labelled cholesterol or C_{21} steroids. The effect was observed only with very large amounts of angiotensin.

Lommer & Wolff (1965) incubated ox adrenal slices with ^{14}C -labelled cholesterol and progesterone, and failed to show any effect of angiotensin.

For the present work a g.l.c. method for the determination of aldosterone in media from continuous-flow (superfusion) experiments was developed. The biosynthesis of aldosterone was observed during superfusion of the outer slices of ox adrenal glands (mainly zona glomerulosa tissue) with Krebs-Ringer bicarbonate solution containing glucose alone. In control experiments, the rate of production of the hormone reached a maximum during the first hour and thereafter declined steadily. Angiotensin (Hypertensin CIBA) introduced into the system after 2h caused a significant increase in the aldosterone production rate. The advantages of superfusion techniques in this type of investigation will be discussed.

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Studies on Adenyl Cyclase and Adenosine Triphosphatase in Rat Corpus Luteum

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Adenyl cyclase has been demonstrated in bovine luteal slices by Marsh, Butcher, Savard & Sutherland (1966). As part of a programme of investigation into effects of luteinizing hormone on rat corpus luteum we have investigated some aspects of this enzyme and the adenosine triphosphatase with which it is contaminated in the 600g pellet, the main subcellular location of the cyclase.

3':5'-Cyclic AMP was assayed by a modification of the procedure used by Jungas (1966). This involved isolation of 3':5'-cyclic [8- ^{14}C]AMP produced from [8- ^{14}C]ATP during incubation. Recovery was 50-55%.

Unless an ATP-regenerating system is present NaF must be added if cyclase activity is to be seen (Rall & Sutherland, 1958). Maximal activity of the cyclase is seen when the 600g pellet from 80mg of tissue is present in incubation mixtures: further increase inhibits production of 3':5'-cyclic AMP. Conversion of ATP into 3':5'-cyclic AMP is 0.6%, but after 40min of incubation only 5% of the substrate ATP remains. Adenyl cyclase activity is linear with time to a maximum of 40min at the optimum pH 7.5-8.0.

Luteal adenyl cyclase activity is dependent on Mg^{2+} , requiring a 2:1 Mg^{2+} /ATP ratio before the activity begins to level off. Mn^{2+} was only 30% as effective as Mg^{2+} ; Ca^{2+} and Zn^{2+} were inhibitory. The adenosine triphosphatase could not be inhibited