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

by lowering the Mg^{2+} concentration but was inhibited by EDTA. No other adenosine triphosphatase inhibitors were as effective as NaF and none of them permitted the detection of adenyl cyclase activity. An excess of GTP over substrate ATP, in the presence of excess of Mg^{+} and NaF, greatly increased the cyclase activity.

Attempts to separate the adenyl cyclase and adenosine triphosphatase physically, the value of other selective inhibitors and the possible function of NaF in the luteal cyclase assay will be discussed.

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Metabolism of Mevalonic Acid in the Liver of the Dogfish *Scyliorhinus caniculus*

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Certain elasmobranch fish appear to be unique in that their livers contain relatively high amounts of squalene, which has recently been implicated in mechanisms of buoyancy control (Corner, Denton & Forster, 1969). The present work is concerned with the biosynthesis of squalene and its possible further metabolism in the liver of the dogfish *Scyliorhinus caniculus*. Diplock & Haslewood (1965) have reported that labelled acetate is incorporated both *in vivo* and in liver homogenates into squalene, but not sterols, in the liver of the lesser spotted dogfish (*Scyliorhinus caniculus*).

Electron-microscopic examination of livers of dogfish revealed the presence of very large droplets of oil that appear to be bounded by a single membrane. The conventional microsomal fraction of liver contains small numbers of small oil droplets. Fractionation of homogenates of dogfish liver yields an oil accounting for approx. 40% of the wet weight of the liver. Squalene and cholesterol account for 5 and 2% respectively of the weight of the oil.

At 1-5 h after injection of $[2-^3H]$ mevalonate into the liver of *Scyliorhinus caniculus* considerable radioactivity is present in the non-saponifiable lipids in the oil fraction. Essentially all the radioactivity is present in material with the chromatographic properties of squalene and no radioactivity was detected in cholesterol. Significant radioactivity is found in squalene in the microsome fraction. Radio-

activity is also present in bile to a minor extent and appears to be associated with bile salts.

Isolated microsomes from dogfish livers actively incorporate $[2-^3H]$ mevalonate into non-saponifiable lipids at 20°C when supplemented with ATP- and NADPH-generating systems and the soluble fraction from a rat liver homogenate. No incorporation occurs in the absence of the generating systems, or when fish soluble fraction replaces rat soluble fraction. When assayed at 20°C the activity of the soluble fraction from fish liver in metabolizing mevalonate to its phosphorylated derivatives is very much less than the activity of the comparable fraction from rat liver. Of the radioactivity found in non-saponifiable lipids derived from incubating fish microsomes, rat soluble fraction, ATP, NADPH and radioactive mevalonate, 90% is present in squalene. Only the *R*-stereoisomer of $[4-^3H]$ mevalonate is incorporated into squalene.

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Fatty Acid Synthetase from the Liver of the Plaice, *Pleuronectes platessa*

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The synthesis of fatty acids *de novo* has been studied in a variety of vertebrate species, and fatty acid synthetase multienzyme complexes have been isolated from the livers of pigeon (Bressler & Wakil, 1961; Hsu, Wasson & Porter, 1965) and rat (Burton, Haavik & Porter, 1968). Fish species have been reported to incorporate $[^{14}C]$ acetate into fatty acids *in vivo* (Mead, Kayama & Reiser, 1960), but the total synthesis of fatty acids from acetyl-CoA and malonyl-CoA has not been demonstrated.

We now report the purification of a fatty acid synthetase from the liver of the plaice, *Pleuronectes platessa*, by procedures similar to those used in the isolation of the analogous enzymes from avian and mammalian liver. The purified enzyme preparation sediments essentially as a single component in the analytical ultracentrifuge, having $s_{20,w}$ 12.6 S when dissolved in 0.2 M-phosphate buffer, pH 6.5, and behaves in a similar manner to urease (mol.wt. 4.8×10^5) on both gel filtration and sucrose-density-gradient centrifugation.

Examination of the products of incubation of the enzyme with $[2-^3H]$ acetyl-CoA, malonyl-CoA and NADPH showed radioactivity to be associated with palmitic acid and stearic acid in the ratio 3:2. No unsaturated fatty acids were produced. The fatty acids could be extracted into pentane without