PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 503rd Meeting of the Society was held at University College London, Gower Street, London, W.C.1, on Monday and Tuesday, 23 and 24 March 1970, when the following papers were presented:

SYMPOSIUM ON 'THE BIOCHEMISTRY OF STEROID HORMONE ACTION'

Oestrogen Action: a Study of the Influence of Steroid Hormones on Genetic Expression

By GERALD C. MUELLER. (*McArdle Laboratory, University of Wisconsin, Madison, Wis. 53706, U.S.A.*)

Acceleration of genetic expression mechanisms is a common feature of the response of target tissues to a wide spectrum of steroid hormones. In each case the interaction of the hormone with a specific receptor system appears to trigger the response. In studies on the mechanism of oestrogen action it is evident that the hormone reacts initially with receptors in the cytoplasm but is rapidly translocated to the nucleus. This translocation, which appears necessary for the ensuing hormone response, is independent of the synthesis of RNA and protein. The hormonal activation of genetic expression, however, requires the synthesis of protein for both the initial activation step and the amplification of the hormone response. Evidence for at least two roles of protein synthesis in the hormone response will be presented. In the attempt to elucidate the manner in which the receptor initiates the hormonal response studies have been carried out on the changing character of the receptor after interaction with the hormone. Characterization studies reveal the oestrogen receptors of the cytoplasm to exist as 9S molecular complexes composed of a 4S oestrophilic (A) unit and a non-oestrogen-binding (B) unit; the latter can be dissociated further to subunits B' and C. Incubation of the native 9S complex at 37°C in the absence of hormone converts it into a sensitized form, which rapidly dissociates on interaction with the hormone even at 0°C. The 4S oestrophilic units, which are derived in this process, fail to reassociate or hybridize with fresh B units obtained by dissociation of the native 9S complexes with 0.3M-KCl. The failure of the system to reassociate appears to be due to the release of the small C subunit, which in turn modifies the character of the oestrophilic A units. The relationship of the modified A units of the cytoplasmic receptor complex to the non-associating oestrophilic unit that is extractable from the nuclei will be discussed in terms of possible roles of the oestrogen-receptor subunits in the control of nuclear RNA polymerase activity.

Interaction of Steroid Hormones with Histones

By M. SLUYSER. (*Department of Biochemistry, Antoni van Leeuwenhoek-Huis, Het Nederlands Kanker Instituut, Amsterdam C, The Netherlands*)

Various steroid hormones stimulate protein synthesis in their target tissues. Evidence that these steroids enhance the synthesis of RNA in these tissues is well documented. This raises the question whether the hormones, or metabolites thereof, interact with chromosomal proteins, thereby increasing the template activity of the chromatin. Studies in this laboratory have shown that steroids attach to histones both *in vitro* and *in vivo*. This appears to be mainly a non-covalent binding to hydrophobic groups, which are located towards the C-terminal regions of the arginine-rich histones. The kinetics of these interactions have been investigated. The results will be discussed in relation to a possible involvement of histones in the mechanism of hormone action.

Steroid Hormones, Ribonucleic Acid Transport and the Regulation of Cytoplasmic Translation

By T. H. HAMILTON. (*Department of Zoology, University of Texas at Austin, Austin, Tex. 78712, U.S.A.*)

Steroid Hormones and Enzyme Induction

By N. LANG. (*Universitäts-Frauenklinik, 53 Bonn den Venusberg, Germany*)

The Effect of Steroids on Transport of Electrolytes through Membranes

By R. FRASER. (*Medical Research Council Blood Pressure Unit, Western Infirmary, Glasgow W1, U.K.*)

The structure and properties of cell membranes appear remarkably stereotyped, irrespective of the locus of the membrane in the cell or of the origin of the cell itself. Among the most universal of the properties of the cell surface is its ability to maintain a high intracellular/extracellular ratio of K⁺

concentration and a low ratio of Na^+ concentration. The mechanism by which these ratios are accomplished and maintained, the sodium pump (Ussing, 1949), is a subject of considerable controversy (see Ling, 1965), and has been studied in many tissues from a variety of animals (see Keynes, 1969), in isolated cells (Richards, Smith, Gibson & Wrong, 1966) and cell membranes such as those obtained from erythrocytes (Garrahan & Glynn, 1967), and in subcellular fractions such as nuclei and mitochondria. The mammalian kidney, because of its role in Na^+ conservation, has been the subject of particularly intense study.

Current theories in common suggest that Na^+ efflux and K^+ influx are components of an active transport system requiring ATP as a source of energy (Hoffman, 1960), one or more unidentified phosphorylated 'carrier' molecules (Cirillo, 1966; Glynn, 1966) and an energy-releasing system, probably the $\text{Na}^+ + \text{K}^+$ -dependent adenosine triphosphatase (Skou, 1964, 1965). Apart from the concentrations of ions on either side of the membrane, the efficiency of the sodium pump depends on the rate at which energy is provided and also on the rate at which ions are able to penetrate the cell surface. Each of these factors is complex and may be analysed further into subcomponents. Energy production, for example, will be governed by the synthesis of the enzyme constituents of the glycolytic pathway and the tricarboxylic acid cycle, by the availability of substrates and cofactors to these pathways and to the efficiency of oxidative phosphorylation. The rate at which ions enter and leave the cell may depend on the permeability of the cell membrane as the result of some as yet unknown structural or enzymic requirement (see, e.g., Sharp & Leaf, 1966; Nutbourne, 1968) or to the quantity of carrier available. The control of such a complex system might conceivably be maintained by altering any one of these factors or possibly several of them.

Electrolyte metabolism in epithelia such as those in the kidney, amphibian bladder and skin, gut and parotid gland is sensitive to control by steroid hormones from the adrenal cortex, notably aldosterone, deoxycorticosterone and corticosterone (Ross, 1965), all of which increase the activity of the pump. The role of aldosterone in other tissues such as salivary glands, sweat glands, lachrymal glands, pancreas and erythrocytes is equivocal (Rettori, Bugyi & Maloney, 1969; Spach & Streeten, 1964; Bott, Blair-West, Coghlan & Wright, 1966). The pump is inhibited by spironolactone (Elmslie, Mulholland & Shields, 1966), cardiac glycosides (Garrahan & Glynn, 1967) and possibly also progesterone. Of these compounds aldosterone is generally considered to be the most important mineralocorticoid and as such has received most attention. Investigation of its mode of action has

taken the form of first establishing that its primary effect is on Na^+ transport, and then to discover which facet of the cell metabolism, orientated towards the sodium pump, is directly altered.

The importance of aldosterone in Na^+ transport has been confirmed in membranes such as amphibian skin by demonstrating its effect on short-circuit current (Crabbé, 1964) and, by using a similar technique, it has also been possible to enumerate those factors, such as substrate concentrations, ion concentrations and inhibitors, that modify the tissue response to the hormone (Sharp & Leaf, 1966). Effects on isolated cells have usually been studied by using labelled Na^+ .

Localization of the intracellular effect of mineralocorticoids is proving more difficult. Reviews of this subject have been published by Sharp & Leaf (1966) and Edelman & Fimognari (1968). Labelled aldosterone is bound by cell membranes, particularly mitochondria and plasma membrane, and by nuclei within a short time of administration *in vivo* (Williams & Baba, 1967), but alteration in Na^+ transport is not detectable until approx. 1 h later. This latent period (Crabbé, 1961) suggests that aldosterone may have some preparative function. For example, nuclear binding has led to the hypothesis that the hormone alters protein synthesis by stimulating DNA-dependent RNA synthesis, which has been considerably strengthened by demonstration of increased orotic acid incorporation into RNA fractions (Forte & Landon, 1968) and by the inhibiting effects of actinomycin D and puromycin (Edelman & Fimognari, 1968). The nature of the new protein synthesized is not known, but may directly affect cell permeability (Sharp & Leaf, 1966) or alter energy metabolism (Edelman & Fimognari, 1968). However, evidence also exists that binding occurs earlier and in greater quantities in mitochondria and in the plasma membrane of the cell (Williams & Baba, 1967). This information, the statement that [^{32}P]phosphate incorporation into ATP precedes that into RNA (Liew & Gornall, 1969) and also the possible effect of aldosterone on Na^+ accumulation by erythrocytes *in vitro* (Rettori *et al.* 1969) may argue against the nucleus as the sole primary focus of action in all tissues. Further study of the intracellular events occurring in the early part of the latent period are necessary to distinguish between nuclear and extranuclear influences.

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Hormone-Receptor Interaction as a Guide to Biochemical Mechanism

By E. V. JENSEN. (*Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Ill. 60637, U.S.A.*)

COMMUNICATIONS

Effect of Bile and Pancreatic Juice on the Absorption of Long-Chain Fatty Acids in the Sheep

By F. A. HARRISON and W. M. F. LEAT. (*Agricultural Research Council Institute of Animal Physiology Babraham, Cambridge CB2 4AT, U.K.*)

In further investigations of the role of pancreatic juice in the digestion and absorption of fat in the ruminant we have studied the absorption into the lymphatic circulation of radioactively labelled stearic acid, palmitic acid and oleic acid. Sheep were suitably prepared with a thoracic-duct fistula and with an intestinal cannula which allowed diversion of the mixed secretions of bile and pancreatic juice. When bile and pancreatic juice flowed normally into the intestine the recovery in the lymph of stearic acid, palmitic acid and oleic acid was 54, 69 and 83% respectively of the radioactivity introduced into the duodenum; 85-95% of the radioactivity was located in the triglycerides. As in non-ruminants, therefore, the absorption of long-chain fatty acids increases in the order stearic acid < palmitic acid < oleic acid (Deuel, 1954).

When the mixed secretions of bile and pancreatic juice were diverted from the intestine, and bile alone infused, the absorption of stearic acid, palmitic acid and oleic acid fell to 0.8, 4.2 and 24.2% respectively of the dose infused. When pancreatic juice alone was infused, with diversion of mixed secretions, less than 1% of the dose was recovered in the thoracic-duct lymph. These results vindicate the conclusions of Heath & Morris (1964) that both bile and pancreatic juice are required for optimum fat absorption in the sheep.

Other experiments involving incubation of

intestinal segments *in vitro* and observations on intestinal loops *in vivo* have shown that, in the absence of pancreatic juice, fatty acids in a micellar form with bile enter the mucosal cell and are re-synthesized rapidly into triglycerides. It appears likely, therefore, that pancreatic juice functions at the luminal stage of digestion, probably by releasing fatty acids from food particles and allowing their solubilization in bile salts; the lysolecithin formed from bile by pancreatic juice may be partly or completely responsible for this action (Leat & Harrison, 1969).

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Base Exchange Reactions of Phospholipid Metabolism in Chick Brain Microsomal Membranes

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The incorporation of nitrogenous bases into phospholipid follows at least two distinct patterns in animal tissue. The first involves the classical Kennedy's pathway and depends on cytidine nucleotides and Mg^{2+} . Its operational existence in nervous tissue has been adequately demonstrated (see Porcellati, Biasion & Pirotta, 1970). The