lead to decreased cytochrome *P*-450 concentrations and low enzyme activity. Response to phenobarbitone (Marshall & McLean, 1969a) and DDT (McLean & McLean, 1966) is greatly decreased. However, some enzymes are increased under these circumstances (Szepesi & Freedland, 1968). This suggests that, in conditions of dietary protein deficiency, the control of protein synthesis is altered to divert the flow of available amino acids into those proteins essential for survival.

When rats are maintained on phenobarbitone and are changed from a low-protein diet to a high-protein diet, a curious phenomenon is observed. In the first 48 hr. after the change of diet there is an enormous increase in liver weight and liver protein. The synthesis of serum albumin increases rapidly also (Kirsch, Frith, Black & Hoffenberg, 1968). However, there is no increase in cytochrome *P*-450 or associated enzyme activities until about 96 hr. after the change in diet. This again suggests that

there is a system of priorities that switches the flow of amino acids in response to nutritional status.

This work was supported by a grant from the Medical Research Council.

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STEROID BIOCHEMISTRY GROUP MEETING (HELD IN CONJUNCTION WITH THE SOCIETY FOR ENDOCRINOLOGY) ON 'HORMONAL ACTION AT THE CELLULAR LEVEL'

Oestrogen-Receptor Interaction in Target Tissues

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

As first suggested by their striking affinity for oestradiol, both *in vivo* and *in vitro*, oestrogen-responsive tissues, such as uterus, vagina, anterior pituitary and certain mammary tumors, contain unique amounts of specific oestrogen-binding components, which have been called 'oestrogen receptors' or 'oestrophiles'. Strong but reversible association of hormone with receptor, without chemical transformation of the steroid molecule, appears to be a primary step in the uterotrophic process and can be specifically blocked, both *in vivo* and *in vitro*, by such oestrogen inhibitors as nafoxidine (Upjohn 11100), Parke-Davis CI-628 or clomiphene. The interaction of oestradiol with target tissues *in vivo* involves two distinct phenomena: uptake, which is not saturable even with hyperphysiological amounts of administered hormone, and retention, which becomes saturated as the dose exceeds the physiological value.

Centrifugal fractionation experiments, confirmed by radioautographic studies, demonstrate two sites of oestrogen binding in uterine cells. Most of the hormone resides in the nuclei, from which it can be extracted by 0.3M-KCl as a macromolecular complex sedimenting at about 5s. A smaller but

significant amount of hormone is bound to a macromolecular substance of apparent extranuclear origin, present in considerable excess in the high-speed supernatant fraction, which shows a sedimentation coefficient of about 8s. The 8s oestradiol-receptor complex forms directly on addition of oestradiol to uterine cytosol, but the 5s complex is not produced by treatment of either nuclei or nuclear extract with oestradiol in the absence of the cytosol. Both receptor substances are proteins containing thiol groups vital to complex-formation. The 8s oestradiol-receptor complex shows a marked tendency to form large aggregates with other proteins of the cytosol and also to form a well-defined complex with ribonuclease. In 0.3M-KCl, the 8s protein dissociates reversibly into 4s subunits; if Ca^{2+} is also present, the 4s subunit does not revert to the 8s form or to larger aggregates when salt is removed. The Ca^{2+} -stabilized 4s unit, which represents the oestradiol-binding moiety of the 8s protein, has been purified about 5000-fold by salt precipitation, gel filtration and ion-exchange chromatography.

A variety of experimental evidence suggests that the interaction of oestradiol with uterine tissue involves a two-step mechanism in which the hormone associates first with the 8s protein, causing it to interact with the nucleus by a temperature-dependent process that consumes 8s receptor. Whether this phenomenon involves specific transfer of the hormone to some pre-existing nuclear

component or whether the 8s oestradiol-receptor complex, or its 4s subunit, is itself transformed into the 5s complex is not yet certain. It appears that the role of the oestrogen is to activate the 8s 'uptake receptor' to interact with the nucleus; as a working hypothesis it is proposed that this interaction involves the binding of the oestrogen-activated receptor protein to specific inhibitors, either of gene expression or of other nuclear function, that are uniquely present in the hormone-dependent tissue.

Oestradiol-17 β Binding: some Unresolved Problems

By R. J. B. KING. (*Department of Hormone Biochemistry, Imperial Cancer Research Fund, Lincoln's Inn Fields, London W.C.2*)

Work by several groups has considerably advanced our knowledge about the processes by which oestrogens enter target cells, are transported to the nucleus and are then attached to a non-histone protein of the chromatin (Jensen *et al.* 1968; King, 1967; Gorski, Toft, Shyamala, Smith & Notides, 1968). However, a number of questions remain to be answered. Is there more than one cytoplasmic binding protein? Coupled with this is the question of the relevance of studies with immature or ovariectomized animals to the situation in intact animals. In uteri from immature rats only a 9s binding protein is present, whereas uteri from mature animals also contain a smaller binding protein (about 4s). This is lost after ovariectomy, it does not appear to be a blood component and it does not bind other steroids. Does this hormone-sensitive protein play a role in the transport of oestrogen into the nucleus or in some other non-nuclear function of the hormone? In human endometrium, more oestrogen enters the nucleus during the follicular than the luteal phase of the cycle despite the lower endogenous concentrations of oestrogen in the latter (Brush, Taylor & King, 1967). This suggests that endogenous hormones influence oestrogen binding by ways other than simple competition for binding sites.

What is the mechanism whereby oestradiol-17 β is transported into the nucleus? The cytoplasmic binding protein is required for specific oestradiol-17 β binding within the nucleus, but is the oestradiol-17 β transferred from the cytoplasmic protein to a smaller nuclear protein or is the cytoplasmic component partly degraded during its passage into the nucleus? The nuclear and cytoplasmic proteins have at least two features in common in that they are both acidic and are both precipitated by polycations. Radioautographic evidence suggests

that some oestradiol-17 β is bound in the region of the nuclear membrane (Inman, Banfield & King, 1965). Do these binding sites play a part in the transport mechanism? Also, if the cytoplasmic protein enters the nucleus, is new cytoplasmic receptor synthesized before more oestradiol-17 β can enter the nucleus?

Is oestradiol-17 β binding the same in all types of target cell? There is a marked difference in salt-extractability of nuclear receptors from different cells (King, 1967), and Rochefort & Baulieu (1969) have shown differences in sedimentation profile of oestradiol-17 β -binding proteins from endometrium of different species. Even within the uterus it is not yet certain which cell types contain which proteins. Both epithelial and stromal cells contain the 9s component, but the stromal cells also contain a smaller component (about 4s). It has been suggested that binding in the stroma is more labile than in the epithelium (Alberga & Baulieu, 1965; King, 1967), and, if so, does this mean that different 9s receptors exist in different cells? This also highlights the absence of information as to what controls, if any, exist for the synthesis and turnover of receptor proteins. The decrease in uterine oestradiol-17 β binding after neonatal androgen administration suggests that, under some conditions, the hypothalamus may be important. The results with human endometrium quoted above and with rat uterus reported by McGuire & Lisk (1968) indicate a possible involvement of other hormones. Also, virtually nothing is known about what determines the dissociation of the oestradiol-17 β -receptor complex *in vivo*.

What structural features of the receptor proteins determine the ability to bind oestrogen? The disruptive effects of urea and acid are compatible with but by no means prove that hydrophobic bonds are involved. It is also noteworthy that the number of oestradiol-17 β -binding sites decreases in the order albumin > haemoglobin > lysozyme > ribonuclease, which is compatible with the idea that internal hydrophobic pockets in the protein are required, but the association constant of oestradiol-17 β with these proteins is much lower than with the specific receptors.

Finally, what is the relationship of oestradiol-17 β binding to the mode of action of the hormone? There is ample evidence that oestrogen binding is related to a biological effect of the hormone, but several questions remain unanswered. The association with an acidic protein in the chromatin is in accord with recent work suggesting that tissue specificity for DNA transcription is controlled by non-histone proteins (Paul & Gilmour, 1968), but is this the only possible action of oestradiol-17 β ? Nuclear transplantation experiments show that cytoplasm can markedly influence nuclear function