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

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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

(Gurdon & Woodland, 1968), so one must consider the possibility that a similar situation exists in the uterus. If so, one can ask if the cytoplasmic binding serves a function in addition to that of hormone transportation. Also, although oestradiol-17 β binding is related to biological action, is the converse true that, in order to get oestrogen action, one must have tight binding as in the uterus? No convincing evidence exists for this, but one observation raises the possibility that it might be so. The carcinogenic effect of oestrogen on the hamster kidney is accompanied neither by measurable nuclear binding of the hormone nor its marked retention by the kidney.

These studies were carried out in collaboration with Dr L. Martin, Dr A. W. Steggle and Dr M. Vertes.

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Metabolism of Testosterone and Activity of Metabolites in Prostate Organ Culture

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Organ cultures of prostates of normal young adult rats (Lasnitzki, 1965) were incubated with [³H]-testosterone. After a 24 hr. incubation, radioactivity was somewhat concentrated in the tissue. At least two-thirds of the testosterone was metabolized and among the identified metabolites of the medium and of the tissue the main compound was 17 β -hydroxy-5 α -androstan-3-one (androstanolone), especially in the nuclear fraction of the homogenate. There were also androstanediols, 5 α -androstan-3 β ,17 β -diol and -3 α ,17 β -diols (the former predominantly), and some of the corresponding 17-oxo steroids, but neither 5 β -derivatives nor oestrogens were observed. Unknown non-polar compounds (under study) were also found.

Testosterone and the main metabolites were

tested for their activity at various concentrations in 6-day experiments (two or three changes of medium containing 3.5-70 μ M). Testosterone maintained the epithelium cell height and secretory activity and kept the stroma at minimum. Androstanolone was somewhat more active than testosterone but provoked essentially a hyperplasia (heaping of cells between the basement membrane and the intact superficial secretory cells), which was seen only with very high doses of testosterone. The 5 α -androstan-3 α ,17 β -diol was not very potent, but the 3 β ,17 β -diol was very active, and in a different manner: there was no hyperplasia at all, but a single layer of tall epithelial cells, with considerable secretory activity and much repression of stroma. The corresponding 17-oxo steroids showed a relatively small activity, and the 5 β -metabolites were inactive.

These results suggest that testosterone action may be related to its metabolism in the prostate through the formation of metabolites of different activity.

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The Hormonal Control of Protein Synthesis

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Most hormones can influence protein synthesis in their target organs, but the mechanism by which they do so is not yet understood. At the present time several hypotheses are tenable once it is accepted that not all hormones act in the same way even to produce the same effect and that any one hormone might act by different mechanisms on different aspects of metabolism.

There is an impressive body of evidence implicating 3',5'-cyclic AMP involvement in the response of many metabolic events to hormones (Sutherland, Øye & Butcher, 1965). In a few instances, for example the action of adrenocorticotrophic hormone on the adrenal gland, indirect evidence suggests that cyclic AMP might promote protein synthesis. In most other cases, for example insulin and growth hormone, the evidence suggests that cyclic AMP is not involved in the protein anabolic action of the hormones, although it is involved in the hormonally induced changes in, for example, lipid metabolism (Goodman, 1968).

Some hormones stimulate the synthesis of proteins not previously synthesized by the organism: the most clear-cut examples occur during development. It is possible that, in these cases, de-repression of a portion of the genome has occurred so that new messenger RNA types are synthesized (Karlson, 1963). It seems unlikely, in