and other evidence suggest that the transformation of the complex is prerequisite to the uptake and thus rate-limiting in the case of the slow uptake. The transformation was found to be favoured by dilution of the supernatant and by high ionic strength. The transformed and the untransformed type of the complex were also different with respect to their partition coefficient in an aqueous polyethyleneglycol-Dextran phase system and their behaviour during adsorption with dextrancoated charcoal, where great losses of the transformed complex were observed. The uptake of complex on chromatin was found to be unsaturable in the concentration range studied (0·005–1 nM). No steroid-binding activity could be found on chromatin, which had been incubated with supernatant in the absence of hormone, indicating that receptor without hormone is not taken up on the chromatin.

7. **Glucocorticoid receptors in cortico-sensitive and cortico-resistant thymocyte subpopulations,** D. DUVAL, J. P. DAUSSE and M. DARDENNE, INSERM U7 and INSERM U25, Hôpital Necker, 75015 Paris, France

The various lymphoid cells of mice thymus do not have the same sensitivity to glucocorticoids. The thymocytes located in the cortex are destroyed by glucocorticoids whereas those located in the inner medulla are not affected by steroid administration. In order to know whether this variation in sensitivity to corticoids is related to a difference between the steroid receptors of the cells, the following investigations have been performed. In a first series of experiments, adrenalectomized C_{57} BL_6 mice were injected with 10 mg/day of hydrocortisone hemisuccinate for two days. The binding of [^{3}H]-dexamethasone to the cortico-resistant cells and to the thymocytes extracted from the thymus of untreated animals was studied in parallel. Three days after the second injection of hydrocortisone, the thymus of steroid-treated animals contains only 8% of the number of cells per thymus found in untreated animals. However, these cells had the same number of binding sites per 10^6 cells as those of intact thymocytes. These results suggest that there is no difference between the cytosolic receptors of cortico-sensitive and cortico-resistant thymocytes. In a second series of experiments, thymocytes of untreated mice were separated into various subpopulations by centrifugation on discontinuous gradient (BSA concentration varied from 10 to 35%). This procedure allowed the separation of thymocytes into four bands. The thymocytes present in the various bands had the same number of receptors for [^{3}H]-dexamethasone and the same affinity for the hormone. However, a marked difference was observed in the action of the steroid, on *in vitro* incorporation of [^{3}H]-uridine. No effect was observed in the lighter fraction whereas incubation of cells from the heavier fraction with dexamethasone for 4 h at 37°C resulted in a 70% inhibition of uridine uptake as compared to the control in the absence of steroid. Intermediary values were observed in the two other bands. Recent experiments performed on lymphoma cells and on human lymphoblasts suggest the existence of a relationship between the sensitivity to steroids and the number of specific receptors for glucocorticoids. It appears from our results, that the determination of steroid binding in lymphoid cells may not be sufficient to assess the biological activity of glucocorticoids in these cells.

8. **Differences in corticosterone and dexamethasone binding to putative receptor sites in rat limbic brain and pituitary,** RONALD DE KLOET, Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, Utrecht, The Netherlands

The interaction of a natural and synthetic glucocorticoid with rat limbic brain and pituitary has been investigated in an attempt to relate binding with endocrine and behavioral effects of such steroids. Experiments are discussed on *in vivo* and *in vitro* high affinity binding to purified cell nuclei and soluble macromolecules. Adrenalectomized rats (3 to 7 days) have been used perfused at sacrifice with 6% Dextran-saline. [^{3}H]-Corticosterone shows a pronounced regional distribution pattern in rat brain with hippocampus cell nuclei showing the highest preference for the natural glucocorticoid. The extremely potent synthetic glucocorticoid dexamethasone is taken up by brain cell nuclei but does not show a distinct regional difference. In contrast the cell nuclei of the anterior pituitary have a marked preference for [^{3}H]-dexamethasone. Kinetic measurements on the interaction with the soluble macromolecules have suggested the presence of more than one population of specific corticoid binding sites in brain and pituitary. In an attempt to purify the soluble putative receptor sites, the pituitary appears to contain intracellularly a transcortin-like macromolecule and a presumptive receptor site able to bind both [^{3}H]-corticosterone and [^{3}H]-dexamethasone. The latter macromolecule complexed with the [^{3}H]-steroids appears after activation (15', 25°C) to be implicated in the binding to calf thymus DNA absorbed to cellulose. Three binding components can be distinguished in the soluble hippocampus [^{3}H]-corticoid complexes after column chromatography via DE-52 anion-exchanger. The elution pattern of the column differs clearly for the two steroids. 85% of the [^{3}H]-dexamethasone complex is eluted at 0·15M KCl against 49% of the [^{3}H]-corticosterone complex. The differences observed in corticosterone and dexamethasone binding support the notion on a dissociation in endocrine and behavioral effects of such steroids.

C. **Receptor interactions with the genome,** C. E. SEKERIS, Institute for Cell research, German Cancer Research Center D-6900 Heidelberg, Germany

Evidence has accumulated during the last decade indicating that the interaction with the genetic material of receptor-steroid hormone complexes, formed in the cytoplasm of target cells, is an indispensable event triggering the action of the hormones on macromolecular synthesis [1, 2, 3]. The physical chemical aspect of this interaction will be discussed as well as its possible implications for transcription.

After *in vivo* administration of tritium labeled hormone, radioactivity can be recovered associated with the chromatin fraction isolated from the respective target tissue, the amount of steroid recovered depending on the system under investigation, the dose, the time period of application, and the method of chromatin isolation among other factors [4, 5]. Similar results have been obtained in *in vitro* studies with isolated cells or subcellular fractions.

The basic question of whether the cytoplasmic receptor is also intranuclearly translocated and similarly associates with the genetic material during the passage of the hormone from the cytoplasm to the nucleus, has not been unequivocally answered. The possibility that on its way to the chromatin the hormone is passed on, on the level of the nuclear membrane, to nuclear receptors, should be kept in mind and further experimentally tested.

In favor of the translocation of the cytoplasmic receptor is the observed depletion of the receptor in the cytosol after *in vivo* administration of the respective

hormone and the concomitant increase in extracted hormone-receptor complexes from the nucleus [4, 6]. However, no clear-cut demonstration has been presented that the receptor isolated from the nucleus and the cytoplasmic receptor are the same protein or possess a precursor-product relationship. Such a demonstration can be given only after purification of the receptor proteins to homogeneity is accomplished, a formidable task, still unattained at the moment of completion of the present review. Indirect data from *in vitro* experiments with cells and subcellular fractions also suggest that the cytoplasmic receptor, after accepting the steroid hormone and after activation, is translocated to the nucleus. This has been inferred from the decrease in the cytosol receptor binding capacity during incubation of the cells with hormone parallel to the appearance of the hormone in the nucleus. Furthermore, during dissociation of the receptor-hormone complex, receptor capacity reappears in the cytosol [6, 7, 8]. Although alternative explanations are possible, the data suggest a shuttle of the cytoplasmic receptor between the cytoplasm and the nucleus.

The nature of the "activation" of the receptor by salts or heat, in the presence or absence of hormone, is still unknown. It very probably involves allosteric modification of the receptor molecule [9, 10] which may have been subjected to limited proteolysis or acquirement of further components during this process [2, 3, 11, 12, 13].

DNA seems to be the chromatin component primarily involved in the intranuclear binding of the receptor [2, 14]. DNase treatment of nuclei abolishes binding of the receptor–hormone complex and leads to release of previously bound complex in some systems [15, 16, 17]. In other reported cases, however, no such release can be observed [18, 19]. The activated receptor, irrespective of whether it carries hormone, also binds to isolated native, much less to denatured, DNA. Binding is observed with equal low affinity to eucaryotic and procaryotic DNA as well as to poly [d(AT)] but not to RNA [2, 20] in a nonsaturable way. These data and certain limitations of the experimental methods used [20, 21] cast doubts on the validity of the *in vitro* binding studies involving DNA and nuclear fractions. Two completely varying sets of results have been obtained in such studies. One group of workers has reported a complete lack of specificity of nuclear binding of the cytosol receptor, attachment being observed both with nuclei from target and non-target cells. Furthermore, no saturation of nuclear binding sites could be attained [21, 22, 23]. Contradictory experiments implying saturation of a limited high affinity sites have also been reported [24, 25].

In fact, recent experiments with oviduct nuclei suggest [26] the existence of two acceptor sites for the cytoplasmic or progesterone receptor, one with low affinity (K_d 10^{-8}M) and high (8000 mol/nucleus) capacity and one with high affinity (K_d 10^{-11}M) and low (40 mol/nucleus) capacity. The nonhistone chromosomal proteins seem to be instrumental in conferring specificity to the binding reaction, although the exact role of these proteins in this process is quite obscure [27].

The presence of a large number of low affinity nuclear binding sites for cytoplasmic receptors could be an experimental artefact. Disregarding this possibility, it is interesting to speculate on the analogy of the existence of both specific, high affinity binding sites as well as nonspecific, low affinity regions on bacterial and phage DNA for repressor proteins with the possible presence of the two "acceptor" sites in chromatin for cytoplasmic receptors. As Lin and Riggs [28] have discussed, the bacterial regulatory molecules attach to the low affinity sites on the DNA and search for the high affinity sites by sliding along the DNA. This one-dimensional sliding is an important part of the binding mechanism and could also be relevant for the binding of the cytoplasmic receptor to the specific high affinity chromatin sites (see also 26). In their recent extrapolation of regulatory mechanisms prevailing in bacterial systems to eucaryotic nuclei, Lin and Riggs [28] conclude that an increase in the concentration of the intranuclear regulatory component could be one of the evolutionary modifications in the eucaryotic regulatory system necessary for efficient control, taking into account the concentration of the DNA in cell nuclei. The theoretically deduced number of regulatory molecules per nucleus for one gene or group of genes regulated by the same molecule is ∿15000, which approximates the number of unspecific acceptor sites for the cytosol receptor.

In most of the hormone dependent systems examined, increased nuclear RNA synthesis accompanies the translocation of the hormone into the nucleus [4, 29, 30]. The stimulation of transcription could be a result of changes in the capacity of chromatin to support RNA synthesis (more initiation sites, removal of elongation or termination blocks) changes in the amount or activity of the DNA dependent RNA polymerases (direct activation or by way of modulation of regulatory molecules) among others.

The evidence suggesting a role of the hormone receptor complex on the various steps of transcription has been mostly indirect. Direct experiments have also been performed by measuring the rate of transcription of isolated nuclei or chromatin in the presence either of hormone, hormone–receptor complex or activated receptor alone [31, 32, 33, 34, 35,36]. Although these experiments in general tend to support a modulation of transcription by the hormone–receptor complex or the activated receptor, they are still too crude to be conclusive and should be further refined. A necessary prerequisite is the need of purified receptor, proteins, a more thorough understanding of the complex mechanism of transcription in eucaryotic systems and a more sophisticated analysis of the transcription products. The question of whether the hormone, the receptor or the hormone–receptor complex is the inducer can only be answered when all the components of the *in vitro* system have been characterized and the eucaryotic transcription systems attain a degree of dependability at least comparable to that existing in the case of the translation systems in current use.

In summary, recent concepts on the intracellular fate of the steroid–hormone receptor complex are reviewed. A general scheme for all steroid hormones has emerged involving binding of the hormone to cytoplasmic receptors, allosteric modification of the hormone–receptor complex, its translocation into the nucleus and binding to the genetic material. Although DNA seems to be the primary acceptor of the hormone–receptor complex, chromosomal proteins very probably play a significant role as regards affinity and specificity. The interaction of the complex with the genome leads to increased chromatin transcription. However, the elucidation of the molecular mechanism of this effect must await further progress in the field of *in vitro* transcription with eucaryotic systems.

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9. The effect of antiestrogens on chromatin associated estrogen receptors and egg yolk protein synthesis in the rooster liver, MICHAEL GSCHWENDT, Deutsches Krebsforschungszentrum, Heidelberg, Germany

Recently we have demonstrated and partially characterized estrogen-binding sites on the liver chromatin from roosters. The binding capacity of the chromatin is increased several-fold after estrogen treatment of the roosters (M. Gschwendt and W. Kittstein, 1974, *Biochim. biophys. Acta* **361**, 84–96). Antiestrogens, like nafoxidine (Upjohn) and CI-628 (Parke–Davis) are known to inhibit the uterine estrogenic response. On the other hand they are also weak estrogens in the uterus. Since chicken liver and uterus respond quite differently to estradiol, it was of interest to investigate the effect of antiestrogens on chicken liver. Nafoxidine and CI-628 reduce the stimulating effect of estradiol on the estrogen-binding capacity of the liver chromatin from roosters. They show the ability, however, to increase the estrogen-binding sites on the liver chromatin themselves to some extent. *In vitro* both antiestrogens compete with [^{3}H]-estradiol for the binding sites on the liver chromatin. The relative affinities of nafoxidine and CI-628 are 0·008 and 0·014, respectively. The anti-estrogens inhibit the estrogen-induced synthesis of egg yolk proteins and fail to induce this estrogen-specific protein synthesis by themselves. Thus in the chicken liver antiestrogens are purely antiestrogenic, as far as the specific effect on yolk protein synthesis is concerned, whereas in the uterus an estrogenic response is also observed. Therefore antiestrogens might become a valuable tool for the investigation of mechanistic differences between a rather pleiotypic (uterus) and a specific (chicken liver) estrogenic response.

10. Impaired nuclear translocation and regulation: a possible explanation of anti-estrogenic activity, M. M. BOUTON and J. P. RAYNAUD, Centre de Recherches Roussel-Uclaf, 93230 Romainville, France

The molecular impacts of estrogen action, in particular at the nuclear level, have been investigated in an attempt to elucidate the differences in activity between two stereoisomers: moxestrol (11β-methoxy-ethynyl-estradiol) and RU 16117 (11α-methoxy-ethynyl-estradiol). Moxestrol is a highly potent estrogen (5–10 times more uterotrophic than estradiol in the Rubin test); RU 16117 is an extremely weak estrogen (1/100 EII) and, on the contrary, antagonizes the action of estradiol in a dose ratio of 10:1. Neither distribution nor metabolism explain the differences. Non-specific binding is weak in the plasma and negligible in the uterus; neither compound binds specifically in the plasma. No differences have been detected in the formation of the cytosolic steroid–receptor complex. Both compounds bind to the mouse uterus cytoplasmic receptor with approximately the same affinity ($1/K = 4 \times 10^{-9}$M) as measured by equilibrium dialysis and the association rate constants are the same ($5 \times 10^4 M^{-1} S^{-1}$) as measured by the Dextran-coated charcoal technique. However, the RU 16117-receptor complex dissociates 20 times faster. Both complexes are translocated into the nucleus, but translocation by RU 16117 is slower and quantitatively less. From these results, it would appear that the two steroid–receptor complexes do not have the same capacity to induce a response at the genome level, as has moreover been sub-