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