binding" of high affinity with a $K_d(4°C)$ of $3·3 \times 10^{-10}$M.

It is concluded that in the experimental conditions used (intact animals), the formation of nuclear [^{3}H]-estradiol complexes is temperature dependent and can occur either through an intermediate cytosol complex or by the "two-step" mechanism being carried out inside the nucleus which the [^{3}H]-estradiol can reach by direct diffusion.

3. **The binding of 18-hydroxydeoxycorticosterone (18-OH-DOC) of 18-hydroxyprogesterone (18-OH-progesterone) of a new urinary 18-hydroxy-steroid and a set of fluorinated steroids to mineralo-corticoid and glucocorticoid receptors in the rat kidney,** M. PALEM-VLIERS, P. GENARD and H. VAN CAUWENBERGE, Department de Clinique et de Pathologie Médicales (Professeur H. VAN CAUWENBERGE), Hôpital de Bavière, Université de Liège, Liege, Belgium

Cytosol prepared from the rat kidney slices of adrenal-ectomized male rats was incubated 30 min at 25°C with 2×10^{-9}M [^{3}H]-aldosterone, 2×10^{-8}M dexamethasone, and increasing concentrations of unlabeled aldosterone, 18-OH-DOC, 18-OH-progesterone and a new urinary 18-hydroxy-steroid (compound *x*). 2×10^{-8}M dexa-methasone included in all flasks were required to prevent [^{3}H]-aldosterone binding to glucocorticoid sites. Bound and free ^{3}H steroid were separated with G_{50} Sephadex column. $1·5 \times 10^{-14}$ mol of [^{3}H]-aldosterone were bound per mg of protein, this value was taken as control (100%). The apparent KDiss for the mineralocorticoid receptors were $1·4 \times 10^{-7}$M for 18-OH-DOC, 9×10^{-7}M for 18-OH-progesterone. 2×10^{-5}M *cp x* were able to compete with aldosterone for the cytosolic receptor. The apparent KDiss of *cp x* for the mineralocorticoid receptor was 4×10^{-6}M. Our preliminary results have shown that *cp x* had a weak affinity for mineralocorticoid receptors sites; this affinity was less than that obtained with 18-OH-progesterone. *cp x* has a affinity for kidney gluco-corticoid binding sites labelled by [^{3}H]-dexamethasone. The affinity of some fluorinated steroids for the mineralo- and gluco-corticoid was also estimated.

4. **Binding proteins for androgens and estadiol in rat perineal and skeletal muscles,** ROLAND R. TREMBLAY, JEAN Y. DUBE and R. LESAGE. Department of Endocrinology, Laval University Hospital Center, Quebec, Canada

The presence of specific binding proteins for androgens and estrogens has been previously demonstrated in target tissues; however, until recently, there has been perplexity as to whether muscles would contain such highly specific binding proteins or receptors. The aim of our work was therefore to study some of the characteristics of androgens (testosterone (T) and dihydrotestosterone (DHT)) and estradiol-17β (E_2) binding proteins in rat levator ani/bulbocavernosus muscle complex (LA/BC) and in thigh muscle (TM). Specific *in vitro* binding of T, DHT and E_2 was demonstrated in the cytosol (30,000 *g* supernatant) of LA/BC and TM by gel filtration through Sephadex G-25 columns. Animals were castrated 24 h prior to the experiment. In LA/BC cytosol, T, DHT and E_2 were bound with high affinity (Ka = 1·9, 0·5 and $0^3 \times 10^9 M^{-1}$ respectively). In TM cytosol, T and E_2 had similar affinities (Ka = 1·1 and $2·3 \times 10^9 M^{-1}$ respectively), whereas DHT had a lower affinity for its receptor (Ka = $5·0 \times 10^7 M^{-1}$). The number of binding sites for T, DHT and E_2 in LA/BC cytosol was respectively 7·5, 14·5 and 12·0 fmol/mg prot., while it was significantly lower, 1·8, 5·3 and 4·2 fmol/mg prot. in thigh muscles. Moreover, competition experiments strongly suggested the conclusion that the binding of the 3 steroids in these sites was due to different proteins. A fundamental difference is therefore demonstrated between the muscles where T, as opposed to DHT in the prostate, is the steroid bound with high affinity to the cytosol receptor.

5. **Response of the immature rat to androgen and estrogen following treatment on day one of life with estrogen, testosterone or an estrogen antagonist,** LEONARD J. LERNER and ADRIANA VITALE, Lepetit Research Labs., Milan, Italy

Administration of androgen or estrogen to the newborn rat has been shown to profoundly influence the endocrine system and sexual behaviour of the matured animal. It was of interest to determine if alteration of the hormonal environment in the newborn animal could alter its respon-siveness to hormones at later stages in its development. Newborn male and female rats were administered single subcutaneous injections of estradiol benzoate (EB) (10 μg), testosterone propionate (TP) (0·5 mg) or the antiestrogen MER-25 (ethamoxytriphetol) (2 mg) within 24 h after birth. At 21 days of age the females were sub-cutaneously administered EB at daily doses of 0·1 or 1 μg, or TP at daily does of 1 or 5 mg for 3 days and on the following day, body, uterine and ovarian weights were determined. At 21 days of age the males were sub-cutaneously administered EB at daily doses of 1 or 10 μg, or TP at daily doses of 1 or 5 mg for 7 days and on the following day, body, testis, epididymis, seminal vesicle and ventral prostate weights were determined. Body weights of the rats were unaltered by any of the treat-ments. EB or TP on day 1 of life reduced ovarian weight on day 24 by 50% regardless of subsequent treatment. Early treatment with EB reduced the later response of the uterus to EB or TP. Early treatment with TP reduced later uterine response to TP only. Early treatment with MER-25 did not alter uterine response to EB or TP. Testis weight was significantly decreased by administration of EB or TP regardless of type of treatment at birth, how-ever, early treatment with TP magnified the reduction of the size of this organ. The weights of the epididymes, seminal vesicles and ventral prostates and their responses to the steroids were not altered by treatment of the new-born rat with any of the compounds. This study indicates that sex steroid treatment of the newborn rat can alter the response of the prepuberal animal to EB or TP at some hormone target tissues.

6. **Transformation of glucocorticoid receptor complex from rat thymocytes and its subsequent uptake on chromatin in a cell-free system,** PETER A. ANDREASEN, Institute of Experimental Hormone Research, Norre Allé 71, 2100 Copenhagen O, Denmark

The uptake of glucocorticoid receptor complex from rat thymocytes on isolated chromatin from the same tissue has been studied. A thymocyte 100,000 *g* supernatant was prepared and made 40% with respect to glycerol. Tritiated glucocorticoid receptor complex was formed by incubation of [^{3}H]-triamcinolone acetonide or [^{3}H]-dexamethasone with the supernatant at −5°C. When the supernatant was incubated with chromatin at 4°C, an uptake of complex on the chromatin was found. A rapid uptake was seen after incubation of the diluted supernatant at 4°C prior to the addition of the chromatin, whereas a slow uptake was seen without preincubation. This indicated a transformation from one form of the complex to another during the preincubation. However, the total uptake was not changed by preincubation. This

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