formation increases with decreasing 4S EBP concentration. The 4S EBP is weakly associated with other macromolecules or inhibitors in buffers of low ionic strength. Nevertheless, dilution of the cytosol with buffer of low ionic strength favors dissociation of the 4S EBP-macromolecular complex and thereby increases the fraction of the 4S EBP available for the activated dimer formation. The rate at which the 4S EBP forms the active dimer is very temperature dependent; the high energy of activation ($\sim$20 kcal mol^{-1}) suggests marked conformational changes are necessary [8, 9].

The monomer-dimer equilibrium model for receptor activation by steroidal hormones. We favour the following hypothesis for estrogen receptor activation, which may be a useful model for other steroidal hormones. The 4S EBP (monomer) with no or limited biological activity, by an estradiol- and temperature-mediated conformational change, dimerizes to form the 5S EBP. Even higher polymers may occur *in vivo*. This receptor model is analogous to that for a number of enzymes whose activity is modulated by a ligand- and, frequently but not always, a temperature-mediated association-dissociation process [10–12]. This molecular model is different from the site-site interaction of an allosteric protein that has been suggested by some investigators for the steroid hormone receptors. As a consequence of being a protein whose activity is regulated by a monomer-dimer equilibrium, the biological activity of the receptor is dependent upon the equilibrium constant between the monomer and dimer in the (a) absence or (b) presence of the steroid hormone, (c) the concentration of monomeric units in the cell, as well as (d) the concentration within any cellular compartment (e.g., cytoplasmic vs nuclear).

The activated state of the estrogen receptor, the dimer (5S EBP), is not dissociated during sucrose gradient centrifugation in buffers containing 0·4 M KCl at pH 7·4. Nevertheless, other steroidal hormone receptors may be readily dissociated as a consequence of any number of physiochemical forces that would drive their monomer-dimer equilibrium toward the monomeric state. These dissociating forces would include: (a) the equilibrium constant, (b) weak bonding forces between monomeric units, (c) high ionic strength buffers (0·4 M KCl), (d) lowered temperature (0–4°C), (e) receptor dilution, and (f) duration of centrifugation. Obviously, methods other than centrifugation may be necessary to examine the monomer-dimer equilibrium hypothesis for some steroidal hormone receptors.

Although the 5S EBP is invariably associated with estrogen–receptor activation, the possibility that dimerization is coincidental and unnecessary has not been demonstrated. Additional studies supporting a dimer model of estrogen–receptor structure have been reported by Jungblut and his associates [13] and Yamamoto and Alberts [14]. (This research was supported by National Institutes of Health Grant HD06707.)

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1. **Complementation analysis of steroid hormone action,** U. GEHRING, K. R. YAMAMOTO and G. M. TOMKINS, Biochemistry Department, University of California Medical Center, San Francisco, California 94143 and Institut für Biologische Chemie der Universität, D 69 Heidelberg, Germany

Wild type S49 mouse lymphoma cells are growth arrested and killed by physiological doses of glucocorticoids and hormone resistant variants can be isolated. The cause of steroid resistance has been traced to defects in the intracellular steroid receptor molecule and 3 types of receptor alterations have been identified among a variety of resistant clones: r^- (receptor negative, no steroid binding), nt^- (nuclear transfer negative), and nt^- (increased nuclear transfer). Wild type (*wt*) cells have been hybridized with examples of each class of variant and the resulting hybrids are steroid sensitive although to a lesser extent than the *wt* X *wt* hybrids. This can be attributed to the lower concentration of wt receptor in these hybrids. Hybrids formed between various types of resistant cells are steroid unresponsive. No evidence of hybrid receptor molecules has been obtained. These results suggest that the steroid and nuclear binding domains of the receptor molecule are within the same complementation group.

2. **Mechanism of cytosol and nuclear ^{3}H-estradiol binding in fetal kidney of guinea-pig,** C. SUMIDA and J. R. PASQUALINI, Foundation for Hormone Research, 26 Blvd. Brune, 75014 Paris, France

In previous studies the presence of specific estradiol (E_2) receptors in the fetal kidney of guinea-pig (40–55 days of gestation) was demonstrated: *in vivo*, after subcutaneous, *in situ*, administration of 7×10^{-10} mol of [^{3}H]-estradiol (0·19 μg) per fetus or *in vitro*, after incubation of [^{3}H]-estradiol (4×10^{-8}M) with kidney cell suspensions. Cytosol fraction is obtained after centrifugation at 250,000 *g* and the nuclear extracts are obtained by successive extractions with: (a) 0·1M Tris–HCl-0·0015M EDTA (Ext. a); (b) 0·3M NaCl-0·01M Tris–HCl (Ext. b) and (c) 1M NaCl-0·01M Tris–HCl (Ext. c).

In the cytosol fraction the K_d(4°C) for the [^{3}H]-estradiol macromolecule complex is $2{\cdot}5 \times 10^{-10}$M. [^{3}H]-Estradiol macromolecule complexes of the cytosol, obtained after incubation of this fraction with [^{3}H]-estradiol (4×10^{-8}M), when incubated with purified nuclei showed that the formation of [^{3}H]-estradiol nuclear complexes was 2–3 times greater at 37°C than at 4°C. Purified nuclei incubated with [^{3}H]-estradiol can also form specific complexes in all the nuclear fractions. In another series of experiments, the incubation of purified nuclei with the cytosol fraction showed no increase in nuclear [^{3}H]-estradiol specific binding. Incubations of the 1M NaCl nuclear extracts (Ext. c) which contained 90–95% of the nuclear DNA showed "direct

binding" of high affinity with a $K_d(4°C)$ of $3{\cdot}3 \times 10^{-10}$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-hydroxysteroid and a set of fluorinated steroids to mineralocorticoid 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 adrenalectomized 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 dexamethasone 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. $1{\cdot}5 \times 10^{-14}$ mol of [^{3}H]-aldosterone were bound per mg of protein, this value was taken as control (100%). The apparent K_{Diss} for the mineralocorticoid receptors were $1{\cdot}4 \times 10^{-7}$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 K_{Diss} 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 glucocorticoid 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 $2{\cdot}3 \times 10^9 M^{-1}$ respectively), whereas DHT had a lower affinity for its receptor (Ka = $5{\cdot}0 \times 10^7 M^{-1}$). 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 responsiveness 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 subcutaneously 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 subcutaneously 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 treatments. 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, however, 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 newborn 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