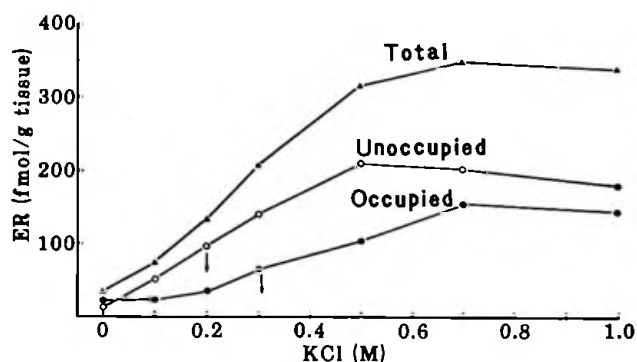


We have previously shown that estrogen receptors (ER) in the testis of the spiny dogfish *S. acanthias* remain associated with nuclei during tissue processing due to an unusually high affinity for chromatin (Callard *et al.*, The Bulletin 22:43, 1980; Callard and Mak, P.N.A.S., in press). Nonetheless, both occupied and unoccupied ER are present in nuclear extracts. This prompted us to re-evaluate the classical model of temperature- and ligand-dependent activation which has been presumed to occur in cytosol prior to 'translocation' of the hormone-ER complex to the nucleus. Since increased adherence for nuclei/chromatin/DNA is a measure of receptor activation, we compared the relative extractability of occupied and unoccupied ER (1) from nuclei *in situ* and (2) from DNA cellulose affinity columns. Receptor-rich regions of *Squalus* testis containing premeiotic



germ cell stages were pooled and homogenized in 3 vol of homogenization buffer (B_H , 50 mM Tris-HCl, 1 mM EDTA, 12 mM monothioglycerol and 30% glycerol, pH 7.5). In Exp. 1, the homogenate was then divided into aliquots, which were centrifuged at $1,000 \times g$ for 15 min. The crude pellets were washed twice with buffer (B_W , 10 mM Tris-HCl, 3 mM $MgCl_2$, 2 mM monothioglycerol and 0.25 M sucrose, pH 7.5) and then incubated for 1 hr with B_H containing 0 - 1,000 mM KCl. The suspensions were centrifuged at

$100,000 \times g$ for 1 hr to obtain nuclear extracts. In each extract, total and unoccupied ER were measured by incubating samples for 1 hr with 10 nM [3H]estradiol + 100-fold excess radioinert estradiol at $22^\circ C$ (exchange conditions) and $4^\circ C$ (non-exchange conditions), respectively. Bound and free steroid were separated on Sephadex LH-20 columns. Specific binding was calculated as the difference between [3H]estradiol bound in the absence or presence of 100-fold excess radioinert estradiol. Occupied ER ($\bullet-\bullet$) were determined by subtracting unoccupied ($\circ-\circ$) from total ER ($\Delta-\Delta$). As shown in Fig 1, both occupied and unoccupied ER were progressively extracted from crude nuclei by increasing KCl in the extraction buffer. However, the half maximal KCl concentration required (arrows) for extraction of unoccupied ER was lower than for occupied ER (200 vs 300 mM, respectively). In Exp. 2, the homogenate was processed to a nuclear pellet and an extract prepared using B_H containing 700 mM KCl. Samples were labeled with [3H]estradiol under non-exchange ($4^\circ C$) or exchange ($22^\circ C$) conditions as described above and then applied to DNA-cellulose affinity columns. On a linear NaCl gradient (50 - 700 mM), unoccupied ER exhibited an elution maximum of 190 mM. When the same samples were labeled at an elevated temperature, however, the receptors eluting at 190 mM NaCl were absent but were replaced by a new peak of binding activity at 350 mM NaCl. Thus, two methods of analysis confirm the presence in *Squalus* nuclear extracts of different ER forms distinguishable on the basis of their nuclear/DNA affinity. Exp. 1 indicates that activation of ER is a normal *in vivo* occurrence in *Squalus* testicular nuclei, whereas Exp. 2 demonstrates ER activation in nuclear extracts *in vitro*. These studies support and extend the recently revised concept of steroid hormone action which states that all ER are located in nuclei whereas cytosolic ER are artifacts of preparation. (Supported by NICHD-16715)