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# ESTROGEN CAUSES A RAPID, LARGE AND PROLONGED RISE IN THE LEVEL OF NUCLEAR ESTROGEN RECEPTOR IN XENOPUS LAEVIS LIVER

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SUMMARY: In male Xenopus laevis, a single large injection of estradiol causes a large rise in the level of estradiol receptor in liver nuclei. The rise is almost certainly due to synthesis, and the newly-synthesized receptor is indistinguishable from pre-existing receptor. The high level of receptor induced by estradiol persists for over 30 days, well after the vitellogenin synthesis that is also induced has disappeared.

The induction of vitellogenin synthesis by estrogen in Xenopus laevis liver provides a good system for studying the regulation of gene expression by steroid hormones because vitellogenin is not synthesized at all unless estrogen is supplied, when it may constitute 70% of total protein synthesis (1), and because induction can be obtained both in vivo (2) and in vitro (3,4) without cytodifferentiation (5). If one injection of estradiol into an adult male frog is followed by a second well after the vitellogenin synthesis induced by the first injection has ceased, the secondary induction of vitellogenin synthesis is faster than the primary induction (6), but the reasons for the faster secondary response are not known. However, a receptor protein which specifically binds estradiol with high affinity (Kd = $0.5 \times 10^{-9}$ M) is present in adult male Xenopus liver (7), but at low concentrations (about 200 binding sites per cell) compared to other estrogen target tissues (about 10,000 sites per cell in the chick oviduct). It also has an unusual intracellular distribution in that at least 50% is found in the nucleus (7). Because overall receptor levels may be affected by estradiol, and because the amount of receptor in the

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nucleus may affect the response of the vitellogenin gene to estradiol, we have measured nuclear receptor levels in male frogs at increasing times after a single injection of estradiol.

## METHODS

The nuclear extracts used for the measurements prepared using liver from animals recently injected with estradiol contain unknown amounts of free estradiol. This does not affect the measurement of the number of binding sites in Scatchard plots, but does affect the calculation of the dissociation constant unless it is first removed. Figure 1A shows two Scatchard plots (8) of [3H]estradiol binding in nuclear extracts prepared from liver two days after the frogs had been injected with estradiol. When the extract was first passed through Sephadex LH-20 to remove the endogenous free estradiol, the data subsequently obtained (closed circles) revealed a single class of binding sites with a dissociation constant of $0.5 \times 10^{-9}$ M, identical to the value previously obtained using liver nuclei from untreated males (7). However, if the passage through LH-20 was omitted, the results (open circles) indicated an apparent dissociation constant of $2.5 \times 10^{-9}$M, although the estimate of the number of sites per nucleus was the same. Figure 1B shows that 16 days after injecting the animals, Scatchard plots obtained either before or after passing extracts through LH-20 were identical, showing that there was no longer any significant contamination of the nuclei with free estradiol. Using gel-filtered extracts, dissociation constants close to $0.5 \times 10^{-9}$M were consistently found in all samples examined, and we therefore calculated the number of binding sites in different samples from the amount of 3H-estradiol that was bound by LH-20-filtered extracts at a single, saturating concentration ($5 \times 10^{-9}$M). The values obtained in this way were checked in some cases by complete Scatchard analyses, to confirm the reliability of the simpler assay.

## RESULTS

Figure 2 shows that when a male frog is injected with 1 mg of estradiol, the amount of nuclear estradiol receptor in the liver rises within six hours, reaching a maximum of 1000 sites per nucleus, ten times the starting level, within 12 hours. The level remains high and steady for about eight days and then gradually declines, although at 32 days it is still at least three times the original level. Two findings suggest that the increased level results from new synthesis. First, the cytoplasmic pool of receptor present in untreated frogs is small (7) (about 100 binding sites per cell), so that even complete translocation of the cytoplasmic receptor to the nucleus cannot account for the highest level of nuclear receptor found in treated frogs.

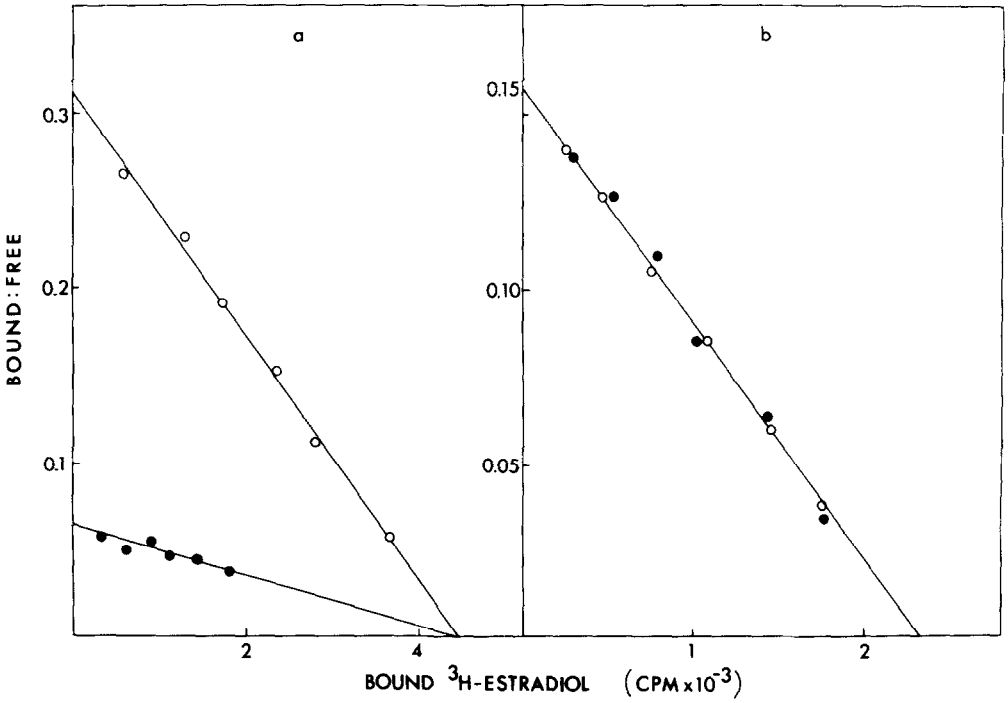


Fig. 1. Effect of endogenous estradiol on measurement of estradiol receptor levels in liver nuclei.
Homogenates of liver were prepared (7) using 10 volumes of a modified buffer (0.25 M sucrose, 1 mM $MgCl_2$, 2 mM Tris, 150µg/ml of phenylmethylsulphonyl fluoride (pH 7.3), filtered through two layers of gauze and centrifuged. The pellets were washed twice with homogenisation buffer and extracted with buffer (0.5 M KCl, 10% glycerol, 10 mM Tris, pH 7.3) containing 150 µg/ml of phenylmethylsulphonyl fluoride (7) which markedly stabilised the receptor at elevated temperatures and during sucrose gradient centrifugation. When extract was passed through LH-20, the void volume was collected and diluted with extraction buffer to twice the starting volume, while untreated extracts were adjusted to the same volume. Receptor levels were then assayed (7), except that samples were incubated with [3H]estradiol for one hour at 20°C to allow complete equilibration with the endogenous, bound estradiol.

A. Scatchard plots of [3]estradiol binding in extracts of liver nuclei prepared either including passage through LH-20 (o—o), or omitting it (•—•), from frogs injected with estradiol two days earlier.

B. Similar plots obtained with LH-20-treated extracts (o—o) and untreated extracts (•—•) 16 days after injecting the frogs.

Second, about 80% of the rise in nuclear receptor found during the first 12 hours is eliminated if cycloheximide is injected (100 µg per 50 gm body weight in 0.3 ml of propylene glycol) with the estradiol.

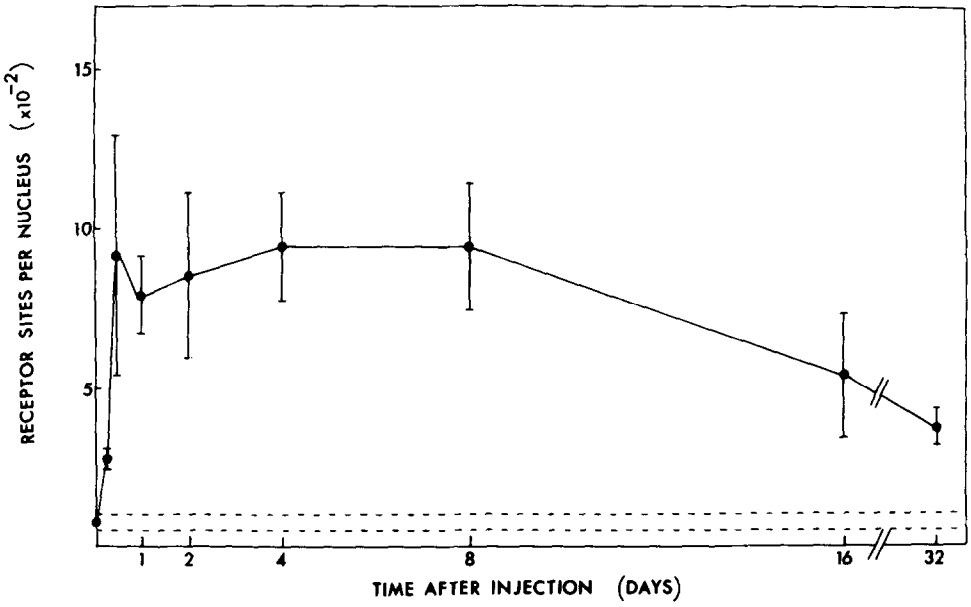


Fig. 2. Time course of effect of estradiol injection on levels of nuclear receptor in Xenopus liver.
Male frogs (~50 gm) were injected once with estradiol (1 mg/50 gm body weight), and nuclear estradiol receptor levels were measured in the liver at various times afterwards. The vertical bars represent the standard error of the mean (SEM) for receptor levels in injected animals, and the horizontal dotted lines the SEM of those in untreated animals. At least three measurements were made for every point. All receptor levels in animals injected with estradiol were raised significantly ($p<0.01$ by Student's t-test) compared with controls.

The newly-synthesized receptor is very similar if not identical to that present in untreated frogs. Figure 1 shows that both have the same affinity for estradiol; Figure 3 that their sedimentation and gel filtration behaviour are also the same. In 0.5 M KCl, the molecular weight of both species, calculated from the sedimentation coefficient (3.5 s) and the Stokes' radius (2.6 nm), is 40,000.

DISCUSSION.

The overall rate of vitellogenin synthesis is probably affected by a number of factors, such as the temperature during induction and whether it is performed in vivo or in vitro (9), but seems to be most intimately connected with the level of nuclear receptor. In male frogs nuclear receptor levels have more than doubled six hours after an in-

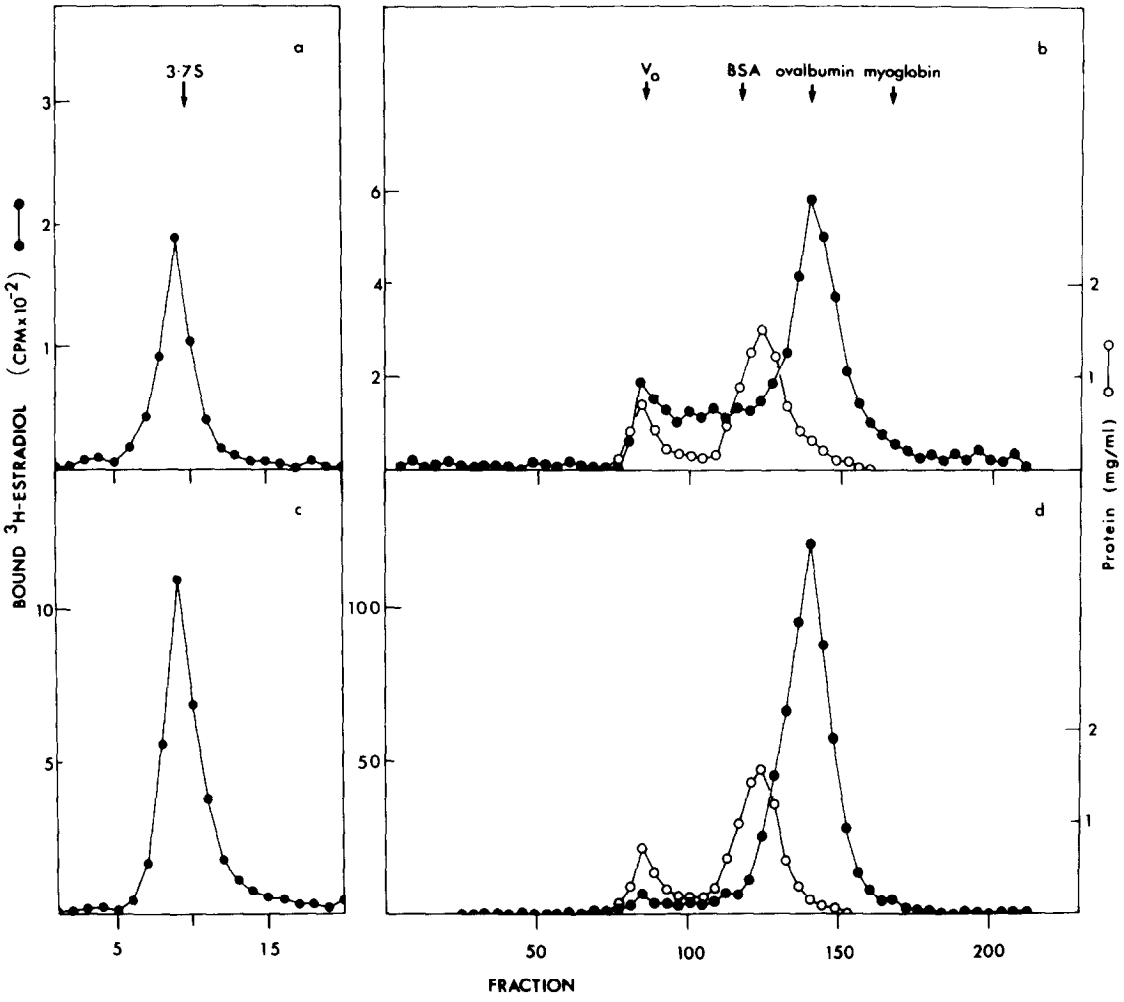


Fig. 3. Sucrose gradient and gel filtration behaviour of receptor from untreated and estradiol-injected male frogs.
Nuclear estradiol receptor was analysed by sedimentation on 5-20% sucrose gradients containing 0.5 M KCl and by gel filtration in the presence of 0.5 M KCl on Sephadex G-100 using liver from frogs before treatment (a and b) and eight days after a single injection of estradiol (c and d). On the sucrose gradients (a and c) the arrow marks the position of ovalbumin (3.7 s); on the G-100 columns (b and d) the positions of BSA, ovalbumin and myoblobin (Stokes' radii 3.63, 2.80 and 2.02 respectively) are marked.

jection of estradiol, but, using previously described methods (3), vitellogenin synthesis cannot be detected in their livers until about 12 hours after the injection. At 24 hours or eight days after the injection, both the rate of vitellogenin synthesis and the level of receptor are very high. Because the level of nuclear receptor rises soon

after the injection, it is not clear whether the low level of receptor present in untreated frogs is itself sufficient to initiate vitellogenin synthesis, or what the minimum amount of receptor needed for rapid vitellogenin synthesis may be. However, it seems likely that an increased level of nuclear receptor is a prerequisite for the highest rates of vitellogenin synthesis that can be obtained. By contrast to the synthesis of receptor that seems to be required in the frog, the rise in nuclear receptor when the rat uterus is treated with estrogen is due to translocation from an extra-nuclear pool (10). In both cases, however, the effect is the same; to increase the concentration of a presumptive regulator of gene expression in the nucleus.

Finally, it is clear from Figure 2 that the high level of nuclear estradiol receptor persists for over 30 days, well after vitellogenin synthesis has ceased. This shows that the increased level of receptor, once established, is stable for some time, even after the concentration of estradiol has fallen to below the minimum that will induce vitellogenin synthesis; and suggests that the increased receptor level is at least partly responsible for the higher rate of vitellogenin synthesis found during secondary stimulation.

Acknowledgements

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