GENERAL AND COMPARATIVE ENDOCRINOLOGY 67, 119–125 (1987)

Identification of an Estrogen Receptor in the Testis of the Sea Lamprey, *Petromyzon marinus*[1]

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Accepted March 17, 1987

Employing a hydroxylapatite batch assay, estrogen-binding activities (EBAs) were demonstrated in the cytosol and nuclear extract of the testis of the anadromous sea lamprey, *Petromyzon marinus*. The lamprey testicular EBAs are sensitive to trypsin digestion and bind [^{3}H]estradiol-17β with high affinities (cytosolic K_d = 0.52 n*M*; nuclear K_d = 0.39 n*M*) and limited capacities (cytosolic: 56.2 fmol/g tissue; nuclear: 68.2 fmol/g tissue). Androgens, progesterone, and corticosterone displayed little affinities for lamprey EBAs. Thus, lamprey testicular EBA possessed many definitive properties of an estrogen receptor as described in amphibian, reptilian, and mammalian studies. No specific binding to androgens was detected in either testicular subcellular fraction. The presence of a putative estrogen receptor in lamprey testis suggests a functional role of estrogen in testicular regulation in this ancient vertebrate.

The sea lamprey, *Petromyzon marinus*, is a member of the most ancient group of living vertebrates, the Agnathans, which have changed little since their appearance 400–500 million years ago. Therefore, the lamprey provides an excellent animal model for studies of biochemical and physiological evolution. The sex steroids progesterone, testosterone, and estradiol have been found in blood (Weisbart *et al.*, 1980; Katz *et al.*, 1982) as well as in gonadal tissues of the lamprey (Botticelli *et al.*, 1963; Callard *et al.*, 1980). The physiological role of these steroids in lamprey reproduction is unclear, although earlier studies indicate some degree of dependence of secondary sex characteristics and gametogenesis on sex steroids (Larsen, 1969, 1974). Recently Sower *et al.* (1985) and Fukayama and Takahashi (1985) have reexamined the role of androgens and estrogens in lamprey reproduction. These authors have carefully established the plasma androgen and estrogen profiles in sea lampreys undergoing the terminal spawning migration. During this final breeding phase, estrogen is the principal circulating steroid, peaking several times and covarying in both males and females. Testosterone levels in (male) lampreys in this phase are significantly lower and lack variation (Sower *et al.*, 1985) or are undetectable (Fukayama and Takahashi, 1985). Moreover, in male lampreys spermiation is accompanied by a final surge in plasma estradiol. These findings suggest a role of estrogen in male lamprey reproduction. In this study, we demonstrate the presence of a putative estrogen receptor in the testis of the sea lamprey, in support of the notion that estrogen is physiologically significant to testicular function (Kelch *et al.*, 1972; Leinonen, 1980) in this ancient vertebrate group.

[1] This study was supported partially by grants from National Science Foundation PCM 8310200 (to S. M. Ho) and from the Great Lakes Fishery Commission (to S. Sower).

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MATERIALS AND METHODS

Animals and tissues. Landlocked adult sea lampreys were captured by trap in the Cheboygan River in May 1984 and 1985 during their anadromous

0016-6480/87 $1.50

spawning migration, which follows completion of their parasitic lake phase. They were transferred to the Hammond Bay Biological Station (Millersburg, MI) where they were retained in raceways supplied with flowing lake water at ambient temperatures ranging from 5.5 to 20°.

Steroids. [^{3}H]Estradiol-17β ([^{3}H]E_2, 93 Ci/mmol), [17α-*methyl*-^{3}H]methyltrienolone ([^{3}H]R1881; 17β-hydroxy-17-methylestra-4,9,11-trien-3-one); 87 Ci/mmol), and unlabeled R1881 were purchased from New England Nuclear Corp. (Boston, MA). All other unlabeled steroids were obtained from Steraloids, Inc. (Wilton, NH), except triamcinolone acetonide (9α-fluoro-11β,16α,17α, 21-tetrahydroxy-1,4-pregnadiene-3,20-dione-16,17-acetonide), which was obtained from Sigma (St. Louis, MO). Stock solutions were prepared in absolute ethanol and stored in brown bottles at −20°.

Buffers and solutions. Buffer A contained 20 m*M* Tris–HCl, pH 7.5 (Schwartz/Mann, Orangeburg, NY), 1 m*M* phenylmethylsulfonyl fluoride, 1.5 m*M* EDTA tetra–sodium salt (Sigma), 1 m*M* dithiothreitol (DTT; Sigma), and 10% (v/v) glycerol (Fisher reagent). Buffer B contained 10 m*M* Tris–HCl, pH 7.5, 0.3 *M* sucrose, and 5 m*M* $MgCl_2$. Buffer C contained 0.6 *M* KCl in buffer A. Buffer D contained 10 m*M* Tris–HCl, pH 7.6, 5 m*M* DTT, 10 m*M* NaH_2PO_4, 10% (v/v) glycerol, and 0.02% (v/v) sodium azide. Buffer E contained 10 m*M* Tris–HCl, pH 7.4, 5 m*M* DTT, 0.25% Triton X-100, 10 m*M* NaH_2PO_4, and 10% (v/v) glycerol. Scintillation fluid was 3:1 (v/v) xylene/Triton X-114 (Rohm & Haas, Philadelphia, PA) with 3 g of 2,5-diphenyloxazole and 250 mg of 1,4-bis-2-(5-phenyl-oxazolyl)benzene (Research Products International Corp., Mount Prospect, IL) per liter for all receptor assays. Trypsin solution was 2.5 mg trypsin (type III; Sigma) per milliliter of buffer A. Trypsin inhibitor solution contained 3 mg soybean trypsin inhibitor (type I-S; Sigma) in 1 ml of buffer A.

Preparation of cytosol and nuclear extract. Testicular tissues were removed from male lampreys, pooled, weighed, washed, and minced in buffer A at ice temperature. All subsequent procedures were performed at 4°. Minced tissues were homogenized in 3 vol (per gram tissue net weight) of buffer A with a Polytron PT-10 (Brinkmann Instruments, Westbury, NY) using 10 5-sec bursts at a rheostat setting of 8. The tissue homogenate was centrifuged at 800*g* for 15 min. The low-speed supernatant was then centrifuged at 105,000*g* for 1 hr at 4° to yield the particle-free cytosolic fraction. The nuclear fraction (pellet of low-speed centrifugation) was resuspended in 10 vol of buffer B (per gram original tissue wet weight), filtered once through double-layered cheesecloth, and washed twice with 10 vol of buffer B by resuspension and centrifugation at 800*g* for 15 min. The washed nuclear pellet was resuspended in 3 vol of buffer C (per gram original tissue wet weight) and incubated for 1 hr with frequent mixing. At the end of incubation, the nuclear extract was obtained by ultracentrifugation at 105,000*g* for 1 hr at 4°. Aliquots of cytosol and nuclear extract were stored at −70° until use. Receptor levels in stored samples remained constant for a period of 3 months, and usually were assayed before the end of the first month of storage.

Determination of cytosolic and nuclear steroid receptor concentrations. For the determination of specific E_2 binding, aliquots of diluted cytosol or nuclear extract (0.4 ml) were incubated with a known concentration of [^{3}H]E_2 ± 100 × molar excess of unlabeled diethylstilbestrol [DES; 4,4′-(1,2-diethyl-1,2-ethenediyl)bisphenol]. After the incubation, bound and free steroids were separated by a hydroxylapatite (HAP) procedure as described in Ho *et al.* (1985). HAP powder (1 g:Biogel–HTP, Bio-Rad, Richmond, CA) was washed in 18 ml of buffer D and resuspended in 3 ml of the above buffer. An aliquot (0.2 ml) of HAP slurry was added to the receptor assay tubes, which were vortexed and incubated for 30 min at 4° with additional vortexing every 5 min. The mixture was then centrifuged at 1000*g* for 10 min and the supernatant was discarded. The precipitate was washed three times with 1 ml of buffer E. After the last wash, receptor-bound steroid was extracted from the hydroxylapatite pellets by two 0.5-ml ethanol extractions. Radioactivity extracted from each sample was counted at 40% efficiency in a Packard Tri-Carb 300C scintillation counter. Specific E_2 binding was calculated by subtracting the radioactivity measured in the presence of unlabeled steroid (nonspecific binding) from that measured in the absence of unlabeled steroid (total binding). Data were expressed as fmoles bound [^{3}H]E_2 per gram wet weight of the testis (fmol/g tissue). Specific androgen binding in lamprey testicular cytosol was analyzed by procedures similar to those described in Ho *et al.* (1985). In short, aliquots of diluted cytosol or nuclear extract (0.4 ml) were incubated with known concentrations of [^{3}H]R1881 ± 100 × molar excess of unlabeled R1881 in the presence of 1 *M* of triamcinolone acetonide. At the end of incubation, bound and free steroids were separated by the HAP procedure.

Saturation analysis and competition assays. Saturation curves were constructed by incubating aliquots of cytosol with increasing concentrations of radioactive steroids (total bound radioactivity). For the determination of specific [^{3}H]E_2 binding, bound radioactivity found in incubations with 100-fold unlabeled DES was taken as nonspecifically bound radioactivity. For the determination of specific [^{3}H]R1881 binding, nonspecific R1881 binding was measured in the presence of 100-fold unlabeled R1881. Corrections for nonspecific binding were calculated as described by Chamness and McQuire (1975). Specifically bound radioactivity was used to compute the apparent dissociation constant (K_d) and concentration of binding sites (capacity)

according to Scatchard (1949). In competition assays, cytosolic samples were incubated with 3 n*M* of [^{3}H]E_2 in the absence or the presence of 100-fold molar excess of unlabeled steroid competitors. Inhibition of [^{3}H]E_2 binding by DES was taken as 100% "maximum binding" and inhibition caused by other steroid competitors was calculated as a percentage of the maximum inhibition.

Effect of trypsin. Aliquots of cytosol and nuclear extract (0.4 ml) were preincubated with 0.1 ml of trypsin solution or 0.1 ml of buffer A for 35 min at 32°. At the end of the preincubation period 0.1 ml of the trypsin inhibitor solution was added to all incubates. The incubates were then cooled to 15° and labeled with 5 n*M* of [^{3}H]E_2 ± 100 × molar excess DES. Incubation with the labeled steroid was carried out for 2 hr at 15°. Specific [^{3}H]E_2 binding was determined by the HAP procedure following incubation. The effect of trypsin on specific [^{3}H]E_2 binding in the testicular subcellular fractions was determined by comparing the results obtained from incubates with and without trypsin exposure.

RESULTS

In preliminary experiments, we found the HAP procedure to be superior to the dextran-coated charcoal assay in measuring specific [^{3}H]E_2 binding activity in testicular cytosols and nuclear extracts. The HAP procedure yielded more consistent data with lower intra- and interassay variations than did the dextran-coated charcoal procedure. The optimal incubation condition that allowed maximal binding to occur in the cytosol and nuclear extract was found to be at 15° for 2 hr. Extention of the incubation time did not result in a further increase in [^{3}H]E_2 binding while prolonged incubations at elevated temperatures (e.g., 2 hr at 35° or 24 hr at 25°) caused a reduction in specific binding. Preincubation of cytosol or nuclear extract samples with trypsin before labeling with [^{3}H]E_2 completely abolished specific [^{3}H]E_2 binding in these samples.

Saturation analysis experiments were performed by incubating aliquots of cytosol or nuclear extract with increasing concentrations of [^{3}H]E_2 (0.5–10.0 n*M*) at 15° for 2 hr. Specific, high-affinity binding activity for [^{3}H]E_2 was demonstrated in both cytosol and nuclear extract of lamprey testis. Specific binding of [^{3}H]E_2 in testicular cytosol saturated between 5 and 6 n*M* (Fig. 1A) and Scatchard plot of the data (Fig. 1B) revealed an apparent dissociation constant (K_d) of 0.52 n*M* and a capacity of 56.2 fmol/g tissue. Likewise, specific binding of [^{3}H]E_2 in testicular nuclear extracts saturated between 4 and 5 n*M* (Fig. 2A) and displayed a K_d of 0.39 n*M*. Specific [^{3}H]E_2 binding capacity in nuclear extracts was 68.2 fmol/g tissue.

To demonstrate specific [^{3}H]R1881 in lamprey testicular cytosols and nuclear extracts, aliquots (0.4 ml) of the testicular subcellular fractions were incubated with 0.5–15.0 n*M* of [^{3}H]R1881 ± 100 × molar excess unlabeled R1881 at 4° for 24 hr. No specific binding [^{3}H]R1881 was detected in either subcellular fraction; binding was mostly nonspecific and displayed no tendency of saturation. Incubations were also performed at 15° for 24 and 48 hr. Again, no specific binding of the labeled steroid was demonstrated under these incubation conditions. In our previous studies on androgen receptors in rat prostrate, incubations at 4° for 24 hr and at 15° for 24 hr were shown to be optimal for allowing exchange between [^{3}H]R1881 and receptor-bound androgens in prostatic cytosols and nuclear extracts, respectively (Ho *et al.*, 1985).

To evaluate the ligand specificity of [^{3}H]E_2 binding activities present in the lamprey testis, competitive binding experiments were employed. Samples were incubated with 3 n*M* of [^{3}H]E_2 with and without a 100-fold molar excess of various nonradioactive competitors (Table 1). In both cytosol and nuclear extract, estrogenic hormones competed most effectively in the order of E_2 = DES > estrone > estriol. Testosterone exhibited minimal competition while progesterone and corticosterone were poor competitors. The androgen 5α-dihydrotestosterone inhibited specific [^{3}H]E_2 binding by approximately 32% in

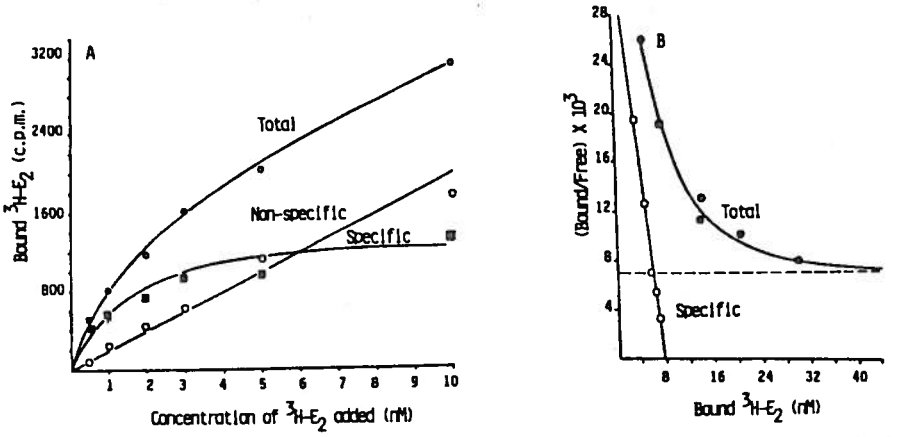

FIG. 1. (A) Saturation analysis of the [^{3}H]E$_2$ binding activity in cytosols obtained from male lamprey testis. Aliquots of lamprey testicular cytosol (1:1 dilution with buffer A) were incubated with increasing concentrations (0.5–10 n*M*) of [^{3}H]E$_2$ in the absence (Total) and presence (Non-specific) of 100-fold excess DES for 2 hr at 15°. At the end of incubation, protein-bound radioactivity in each sample was determined by HAP assay. Specifically bound radioactivity (Specific) was calculated as described under Materials and Methods. Data points are means of two separate experiments. (B) Scatchard plots of the total and specific binding.

cytosols but had little effect on the [^{3}H]E$_2$ binding in nuclear extracts.

DISCUSSION

In this study, we were able to demonstrate specific intracellular estrogen-binding activities (EBAs) in the testis of the lamprey. These EBAs bind estrogen with high affinity, limited capacity, and selectivity. They are sensitive to trypsin digestion and are present in both cytosolic and nuclear compartments of the lamprey testis. The dissociation constants (K_d's) for EBAs in lamprey testicular cytosols and nuclear extracts are 0.52 and 0.39 n*M*, respectively. These K_d values fall well within the range for estrogen receptors (ERs) described in the testis of the rat (Abney, 1976), dogfish (Callard and Mak, 1985), and turtle (Mak *et al.*, 1983), as well as in other estrogen target tissues of nonmammalian

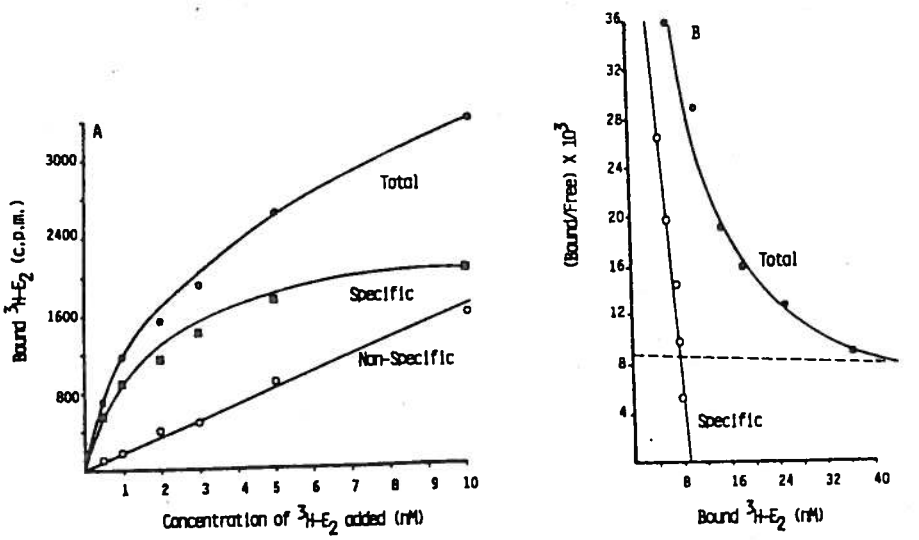

FIG. 2. (A) Saturation analysis of the [^{3}H]E$_2$ binding activity in nuclear extracts obtained from male lamprey testis. Procedures employed were similar to those used for Fig. 1A. (B) Scatchard plots of total and specific binding.

TABLE 1
STEROID BINDING SPECIFICITY OF EBA IN LAMPREY TESTICULAR CYTOSOL AND NUCLEAR EXTRACT AS DETERMINED BY COMPETITION ASSAY[a]

Competitor	% Inhibition	
	Cytosolic	Nuclear
None	0	0
Estradiol-17β	91	91
Diethyl stilbestrol (DES)	100	100
Estrone	93	80
Estriol	53	61
Testosterone	9	0
5α-Dihydrotestosterone	33	0
Progesterone	18	2
Corticosterone	13	0

[a] Data points are means of two separate assays. Unlabeled steroid competitors are present as 100-fold molar excess. Inhibition caused by DES was taken as 100% or "maximum inhibition," and inhibition caused by other steroid competitors was calculated as a percentage of the maximum inhibition.

vertebrates (Salhanick *et al.*, 1979; Mak *et al.*, 1982; Ho *et al.*, in preparation).

Estrogen-binding capacities in lamprey testicular cytosol and nuclear extract were 56.2 and 68.2 fmol/g tissue, respectively. These values are comparable with those reported for other nonmammalian vertebrates. For example, 77 fmol/g tissue of nuclear ER is found in the dogfish testis (Callard and Mak, 1985), and 100–300 fmol/g tissue of cytosolic ER and 70–200 fmol/g tissue of nuclear ER are found in the female turtle liver (Ho *et al.*, in preparation). The fact that EBA was detected in the nuclear fraction as well as in the cytosol of the lamprey testis is physiologically significant, according to current steroid receptor models as outlined by Walters (1985). Such models, drawn from mammalian studies, suggest that the nuclei ERs are "functional" receptors. They express high affinity for chromatin or other nuclear components and thus are able to resist solubilization during homogenization. If these steroid receptor models can be extended to the evolutionarily ancient class of Agnatha, then the presence of nuclear EBA in lamprey testis suggests that estrogen is biologically active at this time in the lamprey testis.

The ligand specificity for lamprey testicular EBAs exhibits the same preference for estrogens and among them (E_2 = DES > E_1 > E_3) as ERs found in the target tissues of other vertebrates (Westley and Knowland, 1978; Mak *et al.*, 1983; Dickson and Eisenfeld, 1979). Although EBAs in both subcellular fractions clearly demonstrated estrogenic selectivity, the specificity appeared to be less marked in the cytosol fraction. The minor differences observed in the steroid specificity of cytosolic and nuclear EBAs may be caused by the presence of other nonreceptor estrogen-binding components in the cytosolic fraction. These non-ER components could be other intracellular steroid binders or contaminants from the plasma. Boffa and co-workers (1972) have reported an estrogen-binding protein in the plasma of *Petromyzon*.

Androgen receptors were not found in the lamprey testis using standard assays for measuring mammalian androgen receptors (Ho *et al.*, 1985). The absence of androgen receptors in lamprey testis and the finding that undetectable or very low circulating levels of androgens are present in the lampreys in this developmental stage (Fukayama and Takahashi, 1985; Sower *et al.*, 1985), lead us to hypothesize that androgens have no physiological role in testicular regulation during the period we examined. This conclusion, however, does not exclude androgens from any major role in regulating male reproductive activities in the sea lamprey. Earlier studies have shown that testosterone implants could induce male secondary sex characteristics in gonadectomized male lampreys (Larsen, 1974). In addition, hypophysectomy of male lampreys in January halted spermiation, whereas hypophysectomy in March of that year allowed normal spermiation (Larsen, 1969). This latter finding suggests a possible role of androgens in early testicular development.

The nature of estrogen's physiological role in testis of the lamprey as well as in

other vertebrates is still unclear. In a model put forth by Moger (1980), testicular estrogen production is inhibitory to androgen output, which may have relevance in the regulation of testicular function (Mak *et al.*, 1983). The high circulating levels of estrogen in male lamprey and the presence of both cytosolic and nuclear ER in the lamprey testis support the hypothesis that estrogen may be of importance in the regulation of testicular function. Sower *et al.* (1985) suggested that sex steroids in the spawning phase of the lamprey may not be sex specific, but rather developmentally programmed. A change in metabolism occurring in the andromous phase may be concomitant with a shift from androgen to estrogen production. This developmentally programmed shift of steroid production may be accompanied by changes in steroid receptor levels in the testis of the animal.

In conclusion, demonstration of a putative estrogen receptor in the testis of the lamprey adds evidence for a regulatory role of estrogen in testicular function which appears to be well conserved throughout vertebrate evolution.

REFERENCES

Abney, T. O. (1976). A cytoplasmic estradiol receptor in rat testicular tissue. *Endocrinology* **99**, 555–556.

Botticelli, C. R., Hisaw, F. L., and Roth, W. D. (1963). Estradiol-17β, estrone and progesterone in the ovaries of the lamprey (*Petromyzon marinus*). *Proc. Soc. Exp. Biol. Med.* **114**, 255–257.

Boffa, G. A., Martin, B., Winchenne, J. J., and Ozon, R. (1972). Interactions steroides–proteines dans le serum de l'Homme, d'un Amphibien et d'un Cyclostome. *Biochimie* **54**, 1137–1145.

Callard, G. W., and Mak, P. (1985). Exclusive nuclear location of estrogen receptors in *Squalus* testis. *Proc. Natl. Acad. Sci. USA* **882**, 1336–1340.

Callard, G. W., Petro, Z., and Ryan, K. J. (1980). Aromatization and 5-reduction in brain and nonneural tissues of a cyclostome, *Petromyzon marinus*. *Gen. Comp. Endocrinol.* **42**, 155–159.

Chamness, G. C., and McQuire, W. L. (1975). Scatchard plots: Common errors in connection and interpretation. *Steroids* **26**, 538–542.

Dickson, R. B., and Eisenfeld, A. J. (1979). Estrogen receptor in liver of male and female rats: Endocrine regulation and molecular properties. *Biol. Reprod.* **21**, 1105–1114.

Fukayama, S., and Takahashi, H. (1985). Changes in serum levels of estradiol-17β and testosterone in the Japanese River Lamprey, *Lampetra japonica*, in the course of sexual maturation. *Bull. Fac. Fish. Hokkaido Univ.* **36**, 163–169.

Ho, S. M., Damassa, D., Kwan, P. W. L., Seto, H. S. K., and Leav, I. (1985). Androgen receptor levels and androgen contents in the prostate lobes of intact and testosterone-treated noble rats. *J. Androl.* **6**, 279–290.

Ho, S. M., Fehrer, S., Yu, M. S., Liang, L. C., Press, D., and Medverd, D. Nuclear and cytosolic estrogen receptor in the liver of the turtle. I. Characterization and sexual differences. In preparation.

Katz, Y., Dashow, L., and Epple, A. (1982). Circulating steroid hormones of anadromous sea lampreys under various experimental conditions. *Gen. Comp. Endocrinol.* **41**, 506–519.

Kelch, R. P., Jenner, M. R., Weinstein, R., Kaplan, S. L., and Grumbach, M. M. (1972). Estradiol and testosterone secretion by human, simian, and canine testes, in males with hypogonadism and in male pseudohermaphrodites with feminizing testes syndrome. *J. Clin. Invest.* **51**, 824–830.

Larsen, L. O. (1969). Effects of hypophysectomy before and during sexual maturation in the cyclostome, *Lampetra fluriatilis* (L.) Gray. *Gen. Comp. Endocrinol.* **12**, 200–208.

Larsen, L. O. (1974). Effects of testosterone and estradiol on gonadectomized and intact male and female river lampreys (*Lampetra fluriatilis* (L.) Gray. Gen. Comp. Endocrinol. **24**, 305–313.

Leinonen, P. (1980). Estrone and estradiol concentrations in the testis and spermatic and peripheral venous blood of elderly men: The influence of estrogen treatment. *J. Steroid Biochem.* **193**, 265–275.

Mak, P., Ho, S. M., and Callard, I. P. (1982). Estrogen receptors in the turtle brain. *Brain Res.* **231**, 63–74.

Mak, P., Ho, S. M., and Callard, I. P. (1983). Characterization of an estrogen receptor in the turtle testis. *Gen. Comp. Endocrinol.* **52**, 182–189.

Moger, W. H. (1980). Temporal changes in testicular estradiol and testosterone concentrations, cytoplasmic estradiol binding, and desensitization after human chorionic gonadotropin administration to the immature rat. *Endocrinology* **106**, 496–503.

Salhanick, A. R., Vito, C. C., Fox, T. O., and Callard, I. P. (1979). Estrogen-binding proteins in oviduct of the turtle, *Chrysemys picta:* Evidence

for a receptor species. *Endocrinology* 105, 1388–1395.

Scatchard, G. (1949). The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* 51, 660–672.

Sower, S. A., Plisetskaya, E., and Gorbman, A. (1985). Changes in plasma steroid and thyroid hormones, and insulin during final maturation and spawning of the sea lamprey, *Petromyzon marinus*. *Gen. Comp. Endocrinol.* 58, 259–269.

Walters, M. (1985). Steroid hormone receptors and the nucleus. *Endocrine Rev.* 6, 512–543.

Weisbart, M., Dickhoff, W. W., Gorbman, A., and Idler, D. R. (1980). The presence of steroids in the sera of pacific hagfish, *Eptatretus stouti*, and the sea lamprey, *Petromyzon marinus*. *Gen. Comp. Endocrinol.* 41, 506–519.

Westley, B., and Knowland, J. (1978). An estrogen receptor from *Xenopus laevis* liver possibly connected with vitellogenin synthesis. *Cell* 15, 367–374.