Estrogen Induces Lipophosphoprotein in Serum of Male Xenopus laevis

Abstract. *Administration of estradiol-17β to male* Xenopus laevis *induces the appearance in serum of large amounts of a serum lipophosphoprotein which is not readily removed from the circulation and which can be resolved from other serum components by chromatography on triethylaminoethyl-cellulose. The initial rate of production of serum lipophosphoprotein is independent of the amount of estrogen administered, but the maximum rate of production and the time taken to attain this rate are dose-dependent.*

Administration of estrogens to male oviparous vertebrates induces a marked increase in certain serum components such as phospholipid, calcium, and phosphoprotein (*1*). These components also occur normally in corresponding females during vitellogenic periods (*2*) and presumably are precursors to yolk (*3*). Along with the evolution of viviparity, mammals appear to have lost the capacity to respond similarly to estrogens; although serum proteins are transferred to the developing mouse oocyte (*4*), there is no evidence that yolk phosphoproteins, homologous to those of lower vertebrates, are present in mammalian eggs or that serum phosphoproteins appear in estrogen-treated mammals (*1*).

We have examined the advantages that the estrogenic response by oviparous vertebrates may have for studies concerning the normal regulation of protein synthesis, and we have chosen the South African clawed toad *Xenopus laevis* for experiments; the required large numbers can be readily maintained in the laboratory, and, unlike the situation in birds and some reptiles, its serum profile during estrogenic response is not complicated by the presence of low-density lipoproteins or the calcium complexes needed for production of eggshell.

In normal males of *X. laevis* the amount of phosphoprotein present in the serum is very small, but phosphoprotein appears concomitantly with an increase in the concentration of total protein in the serum after administration of various amounts of estradiol-17β (*5*). Thus serums from normal males, when chromatographed on triethylaminoethyl-cellulose, display only trace amounts of phosphoprotein at an elution position of about 0.66 (Fig. 1a). The serums from males given a single injection of 1.0 mg of estradiol-17β 14 days previously, however, contain large amounts of a single phosphoprotein component that can be well resolved chromatographically from the other serum components (elution position, 0.58) and that accounts for more than 50 percent of the total serum protein as indicated by the absorbance trace (Fig. 1b). This component rechromatographs reproducibly (Fig. 1c; elution position, 0.59), and preliminary studies have shown it to contain about 13 percent lipid and 1.4 percent protein phosphorus, and that it has a K_D value (*6*) on a calibrated 6-percent agarose column (r, 25.8 mμ) of 0.206; an effective Stokes radius of 7.8 mμ and an approximate molecular weight of 7×10^5 are suggested. We have designated this component serum lipophosphoprotein (SLPP).

When injected into normal males, $[^{32}P]$–NaH_2PO_4 labels none of the serum protein components (Fig. 1a); whereas, if an equivalent amount is injected into estrogen-treated males during the period of production of SLPP, it appears covalently bound (acid stable, alkali labile) only to SLPP (Fig. 1b). Therefore, using this labeling method (*7*), we examined the rate of production and the accumulation in the serum of SLPP as functions of both time and dose by injection of $[^{32}P]$–NaH_2PO_4 and subsequent bleeding of the toads at various times after a single administration of estrogen (*5*).

The results for three different amounts of estradiol-17β (0.1, 0.25, and 1.0 mg) indicated that most likely any amount of estrogen induces some production of SLPP, since the initial rate of response was the same for all three doses. The time of maximum response and the level of maximum rate

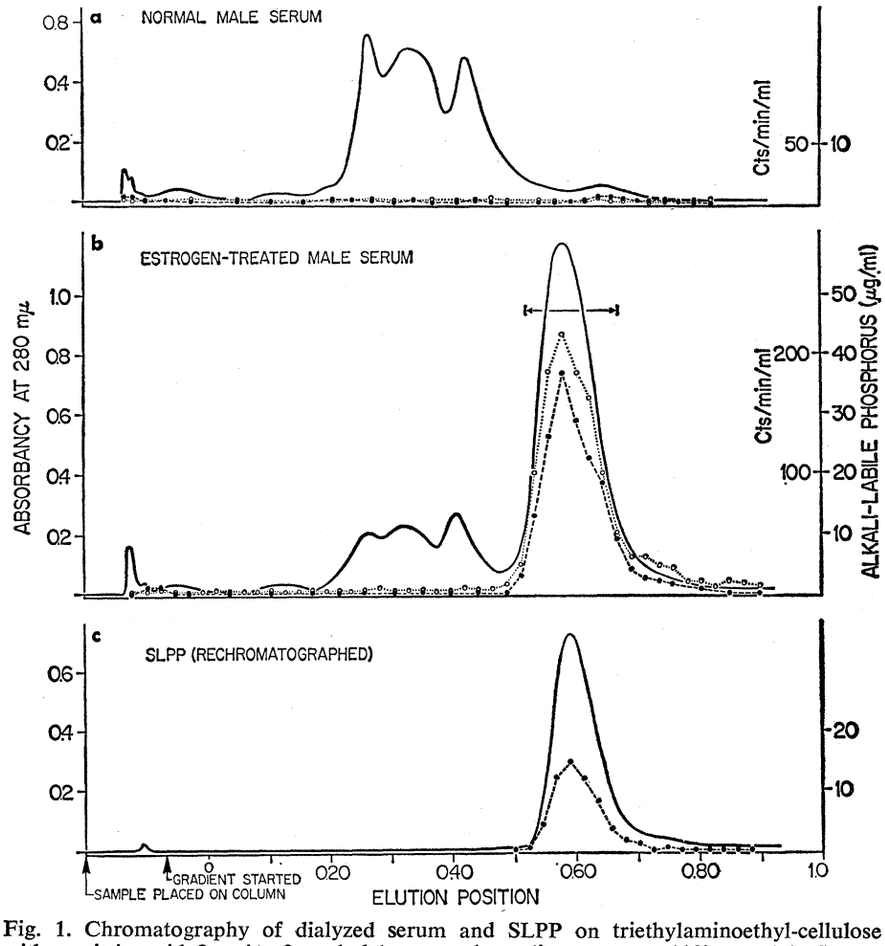

Fig. 1. Chromatography of dialyzed serum and SLPP on triethylaminoethyl-cellulose with a citric acid–2-amino-2-methyl-1-propanol gradient system (*13*). (a) Serum from normal males (190 mg protein). (b) Serum from estrogen-treated males (300 mg protein). (c) Sample (80 mg protein) of a SLPP solution obtained by concentration of that portion of the effluent indicated by an arrow in (b). Absorbancy values are indicated by solid lines. Alkali-labile phosphorus values (●——●) for effluent fractions were determined in a manner described (*14*). Labeling in phosphoprotein (○——○) was determined by injection of toads with $[^{32}P]$–NaH_2PO_4 20 hours before bleeding, placing samples of the effluent chromatograph fractions on filter-paper disks, processing the disks to remove lipid and nucleic acids (*15*), and counting in a planchet counter.

of response were dose-dependent, however, so that rate studies at a given time as a function of dose proved to be meaningless. Since SLPP has a long physiological half-life in the serum of male toads (*5*), meaningful dose-response data could be obtained nevertheless after a sufficient time lapse by determination of serum phosphoprotein levels.

On the basis of studies of the chicken (*7–9*), SLPP probably represents several yolk proteins complexed together in a manner not yet understood. One of the components that can be derived from the complex is the yolk protein phosvitin (*9, 10*), and it has been shown in both the chicken (*11*) and *X. laevis* (*12*) that the liver is the site of synthesis of phosvitin found in the serum of estrogen-treated males or vitellogenic females.

These preliminary results indicate that male *X. laevis* are excellent animals with which to study the production of a given protein as a response to administration of a hormone. Since there are many steps between the synthesis of SLPP and its appearance in the circulation, however, other approaches will be needed for examination of the mechanisms involved in the hepatic response to estrogens.

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Amoebic Meningoencephalitis: Sixteen Fatalities

Abstract. *The parasitologic examination of pathologic brain tissue from 16 cases of acute purulent meningoencephalitis occurring between 1962 and 1965 in northern Bohemia disclosed massive infection of the central nervous system by amoebas of the limax type. The common source was an indoor swimming pool.*

Within 4 years between 1962 and 1965, 16 otherwise healthy persons between 8 and 25 years of age died in a district in northern Bohemia, showing the symptoms of peracute purulent meningitis. The disease occurred in small outbreaks during the summer and autumn and completely resisted sulfonamide and antibiotics. As a rule death came 2 to 7 days after the onset of illness, which was manifested mainly by severe headaches. Epidemiologic investigations revealed that the only possible source of the infectious agent was a modern indoor swimming pool in which the water was kept at about 24°C. The deceased all bathed in this pool and the average time between exposure and onset of illness was 7 days.

The clinical picture of the disease, results of the clinical and laboratory tests, and pathologic and anatomic findings supported the theory of a bacterial etiology. Attempts to isolate a bacterial agent by culture *intra vitam* were unsuccessful. Postmortem examination absolved the three most probable agents: meningococcus, pneumococcus, and hemophilus; the true etiologic agent remained unknown.

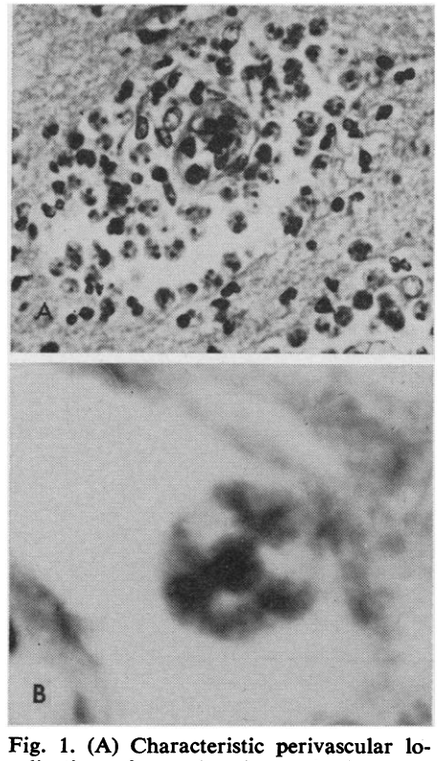

Fig. 1. (A) Characteristic perivascular localization of amoebas in cerebral cortex; trichrom stain. (B) Detail of an amoeba; typical morphology; trichrom stain. The average diameter of each is about 8 μ.

At the end of 1967 we obtained the histologic materials from the deceased for parasitologic examination. In all brain tissues we identified large numbers of amoebas (Fig. 1) which in morphology very closely resembled the organisms described as *Acanthamoeba* or *Hartmannella* in the eight cases of amoebic meningoencephalitis found in Australia and the United States (*1*). In dimensions the amoebas in our histologic preparations were much smaller than the formerly known pathogenic strains of *Acanthamoeba (Hartmannella) castellanii* (*2*). The exact taxonomic position of this meningoencephalitis-causing amoeba remains unclear because it could not be isolated in culture. The number of amoebas, their localization in tissue, the extent of lesions, and the cellular reaction provoked by the presence of parasites prove the decisive role of amoebas in the development of illness and in death.

These cases of amoebic (limax type) meningoencephalitis are the first reported from Europe and are the first having a common source of infection. The fact that an indoor swimming pool was identified as the source of the infectious agent is very important; the possible risk of more-frequent occurrence of brain amebiasis is demonstrated. A more detailed report will follow.

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