12. Meites, J., Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.*, 1948, no. 415.

13. Everett, N. B., Baker, B. L., *Endocrinology*, 1945, v37, 83.

14. Barrnett, R. J., Roth, W. D., Salzer, J., *ibid.*, 1961, v69, 1047.

15. Baker, B. L., Everett, N. B., *ibid.*, 1944, v34, 254.

16. ———, *ibid.*, 1947, v41, 144.

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Estradiol-17β, Estrone, and Progesterone in the Ovaries of Lamprey (*Petromyzon marinus*).* (28645)

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Present knowledge of the sex hormones in the lower vertebrates and the invertebrates is limited for the most part to inferences based entirely on what is known about the mammals. This tendency to project such knowledge down through the animal kingdom has led to some rather peculiar conclusions. How far down the phylogenetic series these mammalian endocrine situations actually do extend ultimately depends upon a knowledge of the presence and identity of the gonadal hormones. Recent analyses have confirmed the presence and identity of estrogens and progesterone in a representative series of invertebrates and vertebrates. Such steroids were hitherto assumed on the basis of indirect evidence(1). The series includes echinoderms, mollusks, arthropods, fish and birds(2-10). This report presents results of an effort to isolate and identify estrogens and progesterone from the ovarian tissue of cyclostomes.

Methods and results. Lamprey ovaries (568 g) were collected and stored in approximately twice their volume of acetone. At time of extraction, the acetone was poured off, the ovarian tissue was homogenized and reextracted with acetone. The combined acetone extracts were flash evaporated and finally taken to near dryness under nitrogen. The oily residue was dissolved in sufficient 70% ethanol and extracted 3 times with ligroin (B.P. 30-60°C). The ethanolic fraction was diluted with water to contain 35% alcohol and subsequently washed with ligroin (3 ×). The alcoholic solution, bioassayed by the Astwood method(11), showed the presence of estrogenic material. The ethanolic fraction was then dried *in vacuo* and the residue partitioned countercurrently between 70% methanol and a 50:50 mixture of chloroform and carbon tetrachloride with 29 transfers. In brief, those tubes corresponding to authentic estradiol, estrone, and estriol were pooled and bioassayed.

The results (Table I) indicated estrogenic activity in tubes 3-9 (estrone) and 10-19 (estradiol). The estrone and estradiol fractions were divided into 2 aliquot portions, and each portion was subjected to the following 2 paper chromatographic systems, at room temperature; (1) benzene-formamide 1:1 for 22-24 hours; (2) toluene-propylene glycol 1:1 for 6 hours.

In each of the solvent systems the unknown was applied to one of 4 strips, estrone to another, estradiol to a third, and a mixture of estrone and estradiol to the fourth. The 4 strips in each system were run simultaneously. The 3 strips treated with the au-

TABLE I. Results of Bioassay (Astwood Method) from Countercurrent Separation.

Fraction	Uterine wet wt (mg)	% increase over control
Controls*	21.5	0
0-2	21.13	0
3-9	25.2	17.2
10-19	27.7	28.8
20-29	21.7	0

* Avg uterine wet wt for 20 untreated animals.

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TABLE II. Cyclostome—Bioassay of Eluted Paper Strips from 2 Different Chromatographic Systems.

Chromatographic systems	Countercurrent fractions 3-9* chromatography				Countercurrent fractions 10-19*			
	Estrone		Estradiol		Estrone		Estradiol	
	Uterine wet wt, mg	% increase	Uterine wet wt, mg	% increase	Uterine wet wt, mg	% increase	Uterine wet wt, mg	% increase
Benzene-formamide	27.7	28.8	21.4	0	24.1	12	34.3	59.5
Toluene-propylene glycol	28.8	34.0	21.2	0	24.9	15.8	37.2	73.0

* Areas of test strips corresponding to authentic estrone and estradiol-17β. Uterine weights are avg for 2 animals in each experimental test. Avg uterine wet wt for 20 untreated control animals was 21.5 ± 0.2 mg.

thentic estrogens were stained with a ferric chlorideferricyanide stain. The areas on the experimental strips corresponding to the standards were cut and eluted 5 times with absolute ethanol. Similar elutions also were made of the remaining paper from the experimental strips. An aliquot portion of each eluate was bioassayed. The chromatographic test fractions corresponding to standard estradiol and estrone gave positive results (Table II). It has been estimated for purposes of comparison that the ripe ovaries of lamprey *(Petromyzon marinus)* contain a minimum of 16 μg of estradiol-17β per kilo of fresh tissue.

The brown oily residue obtained on evaporation of the petroleum ether fraction was used for isolation of progesterone by procedures previously described(8). In brief the experimental material was subjected to column chromatography. Those fractions that corresponded to authentic progesterone gave positive results by the Hooker-Forbes method(12).

Discussion. Dodd *et al.*(13), in a review of the reproductive endocrinology of cyclostomes and elasmobranchs, emphasize the importance of establishing the nature of the physiological mechanism which underlies the development and control of the gonads and secondary sexual characteristics as well as those which initiate spawning. It would be expected that the feedback principle, well-known from mammalian studies, should operate in lower vertebrates, so that the ovary by its endocrine activity would control in some manner the "gonadotropic" action of the pituitary. Many experiments definitely establish an ovarian function but say nothing about the chemical nature of the ovarian hormone or hormones. Prerequisite to a clear understanding of the pituitary-gonadal relationships in cyclostomes is an unequivocal analysis of the nature of the hormones involved. This study has established the presence of steroids in cyclostome ovaries, although the exact role of such steroids in feedback mechanisms and control of reproductive processes is as yet not clearly understood.

Summary. Ovaries of cyclostomes *(Petromyzon marinus)* were examined for sex steroids. The ripe ovaries contain a minimum of 16 μg estradiol-17β/kilo of fresh ovarian tissue determined by the Astwood 6 hour assay. The biologically active material was identified as estradiol-17β and estrone by countercurrent distribution, paper chromatography and bioassay. Presence of progesterone was tentatively identified by methods of column chromatography and Hooker-Forbes assay.

1. Hanström, B., *Hormones in Invertebrates,* Oxford Univ. Press, 1939.

2. Botticelli, C. R., Hisaw, F. L., Jr., Wotiz, H. H., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 875.

3. ———, *ibid.,* 1961, v106, 887.

4. Donahue, J. K., State of Maine Dept. of Sea and Shore Fisheries, *Research Bull.* No. 8, 1952.

5. ———, *ibid.,* No. 22, 1957.

6. Lisk, Robert D., *Can. J. Biochem. Physiol.,* 1961, v29.

7. Dean, F. D., Jones, I. C., *J. Endocrin.,* 1959, v18, 336.

8. Wotiz, H. H., Botticelli, C. R., Hisaw, F. L., Jr., Olsen, A. G., *Proc. Nat. Acad. Sci.,* 1960, v46, 580.

9. Hagerman, D. D., Wellington, F. M., Villee, C. A., *Biol. Bull.,* 1957, v112, 180.

10. Layne, D. S., Common, R. H., Maw, W. A., Fraps, R. M., *Nature,* 1958, v181, 351.

11. Astwood, E. B., *Endocrinol.,* 1938, v23, 25.

12. Hooker, C. W., Forbes, T. R., *ibid.*, 1947, v41, 158.
13. Dodd, J. M., Evennett, P. J., Goddard, C. R., *Symp. Zool. Soc. Lond.*, 1960, No. 1, 77.

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Fatal Syndrome Associated with Vitamin E Status of Pregnant Rats.* (28646)

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The syndrome traditionally associated with Vit. E deficiency in the female rat is fetal resorption. Other reproductive disorders apparently related to Vit. E status include mortality of pregnant rats at parturition(1,2,3, 4). Stamler(1) noted that rats fed diets containing peroxidized fat from the 13th day of gestation seemed healthy until near the end of pregnancy, when they exhibited restlessness, pallor, labored breathing, bloody nasal exudate, and sometimes convulsions, followed by death. Hemorrhagic edema, massive pleural effusion, renal or adrenal damage, and varying amounts of intrauterine bleeding, together with well-developed fetuses, were common findings at death. Oral supplements of Vit. E begun as late as the 19th day of gestation or injection of tocopherol by the evening of the 21st day prevented death.

Armstrong and Swanson(2) reported fatal toxemia of pregnancy in rats fed a diet containing 25% dried autoclaved pork. The syndrome, characterized by lethargy, paleness, water retention, hepatic damage, and convulsions, could be prevented with wheat germ oil (unpublished data).

The effect of varying doses of Vit. E early in pregnancy on the outcome of gestation in Vit. E-deficient rats has been investigated.

Methods. Four groups of weanling female Wistar rats were depleted of Vit. E by a diet containing 66.5% cornstarch, 16.5% vitamin test casein,‡ 4% Hawk and Oser salt mix,‡ 1% NaCl, 2% non-nutritive fiber,‡ 5% Torula yeast,§ and 5% cod liver oil. Another group was maintained on a stock ration which had supported reproduction in the stock colony for many generations. Daily vaginal smears were begun when the animals were 9 weeks old, and the rats were mated to littermate males. One group (CClo+0) received no tocopherol. Other depleted animals were given, orally, a total of 2, 6, or 20 mg dl-α-tocopherol‡ in cottonseed oil on the 8th and 9th days after confirmation of positive mating. Vaginal smears were examined daily after the 10th day for appearance of blood as a sign of implantation. Hemoglobin concentration and hemolysis by dialuric acid were measured on the 21st day of gestation. After delivery or resorption, animals were anesthetized with sodium pentobarbital. Blood was removed from the vena cava and refrigerated immediately. The liver, kidneys, adrenals, and any remaining fetuses were removed and weighed, and a weighed portion of liver was frozen for analysis of fat and nitrogen. Uterus, lungs, and other organs were examined.

Hemolysis by a solution of dialuric acid (40 mg/100 ml) was determined by the method of Friedman *et al.*(5). Hemoglobin was measured as oxyhemoglobin in a spectrophotometer calibrated against blood of known oxygen capacity. The ferric chloride-α,α'-dipyridyl microdetermination of Quaife *et al.*(6) was used for estimation of serum tocopherol. The correction for absorption due to carotene was omitted because this substance is absent from rat blood. Non-protein nitrogen in a tungstate protein-free filtrate of serum, digested with sulfuric acid and hydro-

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