what similar with the exception of the euglobulin prepared with hydrochloric acid, which had a higher per cent of hexose to protein. All showed marked increase in content when compared with euglobulins isolated from a pooled *"latex negative"* normal serum. 3. Paper electrophoretic examination reveals proteins that migrate as gamma globulins and are highly PAS positive. 4. A uronic acid-containing mucopolysaccharide was isolated from the cold agglutinating euglobulin.

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Estrogens and Progesterone in the Sea Urchin (*Strongylocentrotus franciscanus*) and Pecten (*Pecten hericius*).* (26511)

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Estradiol-17β, estrone, and progesterone have been obtained, in vertebrates other than mammals, from the ovaries of dogfish(1), while estradiol, estrone, and estriol have been found in the ovaries of laying hens(2) and lungfish(3). Extracts of gonads from many invertebrates have produced estrogenic effects in mammals(4,5), but the chemical identity of the active substances is mostly unknown. Only recently estradiol and progesterone have been identified in the ovaries of the starfish(6). This report presents the results of an effort to isolate and identify possible estrogenic steroids and progesterone from ovarian tissue of 2 other representative invertebrates: the sea urchin, *Strongylocentrotus franciscanus*, and the mollusk, *Pecten hericius*.

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Methods and results. The ripe ovaries (3-5 kg) of each species were collected and stored in approximately twice their volume of acetone. The acetone extract was evaporated to an oily residue, taken up by 70% ethanol and extracted 3 times with petroleum ether. Next, the ethanolic fraction was diluted with water to contain 35% of alcohol and washed with petroleum ether 3 times. These 35% ethanolic fractions were tested for estrogenic action by the Astwood method, as previously described(7,8), and all gave positive results, although it was evident that only low concentrations of hormone were present. 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 only in material obtained

TABLE I. Results of Bioassay from Countercurrent Separation.

Fractions	Sea urchin		Pecten	
	Uterine wet wt, mg	% increase over controls	Uterine wet wt, mg	% increase over controls
Controls*	22.8		22.8	
0- 2	22.3	0	21.6	0
3- 9	21.9	0	24.8	9.2
10-19	24.9	9.2	25.7	13.0
20-26	22.2	0	20.3	0
27-29	21.7	0	21.4	0

* Avg uterine wet wt for 20 untreated animals.

from tubes 10-19 for the sea urchin, and tubes 3-9 (estrone) and 10-19 (estradiol) for the pecten. The estrone and estradiol fractions from each species were divided into 3 aliquot portions, and each portion was subjected to the following 3 paper chromatographic systems, at room temperature: (1) benzene-formamide (1:1) for 22-24 hours; (2) toluene-propylene glycol (1:1) for 6 hours; and (3) ligroin-propylene glycol (1:1) for 64 hours. In each of these 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 authentic estrogens were stained with a ferric chloride-ferricyanide 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 sea urchin, like the previously tested starfish(6), upon bioassay indicated the presence of estradiol-17β, while the test fractions for the pecten corresponding to both estradiol and estrone gave positive results (Table III). Assuming that estradiol can be converted to estrone in the echinoderms, it seems quite probable that estrone also might have been present, but in a quantity insufficient for detection by our methods. It has been estimated for purposes of comparison that the ripe ovaries of starfish and sea urchin contain a minimum of 0.04 to 0.1 μg of estradiol-17β per kilo of fresh tissue, while in the pecten there is a minimum of 10.0 μg.

The brown oily residue obtained on evaporation of the original petroleum ether fractions was used for isolation of progesterone by procedures previously described(1). In brief, the experimental material was subjected to column chromatography. Those fractions that corresponded to authentic progesterone on a control column gave positive results by the Hooker-Forbes method. Other progestational substances were also present, one of which was identified as probably being Δ^4-3-keto-pregnen-20β-ol. The evidence for progesterone, though not as convincing as that for estradiol and estrone, is nevertheless significant. The amount of active material obtained was too small for a more thorough characterization, and absolute chemical identification will require further study.

Conclusions. It has become well established that estradiol-17β, estrone, and progesterone are present in the ovaries of vertebrates from mammals to fishes, and the present report extends these observations to include certain of the invertebrates. Therefore,

TABLE II. Sea Urchin—Bioassay of Eluted Paper Strips from 3 Different Chromatographic Systems.

Chromatographic systems	CC fractions 10-19 chromatography*			
	Estrone		Estradiol	
	Uterine wet wt, mg	% increase over control	Uterine wet wt, mg	% increase over control
Benzene-formamide	21.7	0	27.2	19
Toluene-propylene glycol	22.2	0	25.3	13
Ligroin-propylene glycol	23.6	0	41.5	82

* 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 22.8 $\pm$ 0.3 mg. Tests for tubes 3-9 gave uniform negative results, so were not recorded.

TABLE III. Pecten—Bioassay of Eluted Paper Strips from 3 Different Chromatographic Systems.

Chromatographic systems	CC fractions 3-9 chromatography*				CC fractions 10-19 chromatography*			
	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	27.5	20.6	21.3	0	25.8	13.1	35.2	54.8
Toluene-propylene glycol	29.2	28.0	21.5	0	22.2	0	43.8	92.1
Ligroin-propylene glycol	34.1	49.5	22.3	0	21.6	0	45.3	98.6

* 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 22.8 $\pm$ 0.3 mg.

it seems a distinct possibility that they may be of common occurrence in the animal kingdom. It is known, of course, that these compounds have important endocrine functions in the vertebrates, but whether this is also true of the invertebrates is at present unknown. However, there is now considerable evidence indicating that these steroids may take an important part in growth and development of the vertebrate Graafian follicle(9). It seems possible that this may represent the original and hence most primitive function of these substances, and that their action as hormones affecting structures beyond the confines of the ovary may represent later specializations.

Summary. The ovaries of the sea urchin, *Strongylocentrotus franciscanus*, and the pecten, *Pecten hericius*, were extracted for steroids. Estrogenic activity equivalent to 10 μg or less per kilo of wet tissue was found in each species by bioassay. The most active estrogenic substance was identified as estradiol-17β. Separations were made by counter-current distribution (29 transfers: upper phase—70% methanol; lower phase—50% chloroformic, 50% carbon tetrachloride), paper chromatography (3 systems: toluene-propylene glycol; ligroin-propylene glycol; benzene-formamide), and bioassay (Astwood method). Progesterone was also identified tentatively by column chromatography and the Hooker-Forbes assay. Other progestational substances were present, one of which may be Δ^4-3-keto-pregnen-20β-ol. The occurrence of these steroids in 2 distinctly different phyla indicates a wide distribution and may be of some significance in the evolution of sex hormones.

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