IDENTIFICATION OF ESTRADIOL-17β FROM DOGFISH OVA
(SQUALUS SUCKLEYI)*

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Although estrogenic activity of ovarian extracts has been reported for
all classes of vertebrates and certain invertebrates, the chemical nature of
the active compounds has been identified only in mammals. The hormone
of the graafian follicle was isolated by MacCorquodale, Thayer, and Doisy
(1) from liquor folliculi of sow ovaries and named dihydrotheelin, a syno-
ym of estradiol-17β according to present nomenclature. When whole
ovaries were extracted, both estradiol-17β and estrone (theelin) were ob-
tained (2) in an approximate ratio of 0.014:0.010 mg. per kilo. It seems
probable, as suggested by these authors and later by others, that estradiol-
17β is the estrogenic hormone secreted by the graafian follicle and that
estrone is a metabolite. While this may be true for mammals, such informa-
tion is lacking for other vertebrates. In the present investigation an
attempt was made to identify the estrogenic hormone in the mature ova of
the dogfish, Squalus suckleyi.

RESULTS AND DISCUSSION

Graafian follicles containing ova that were approaching maturity and
weighing 20 to 27 gm. were “shelled out” of the ovaries of both pregnant
and non-pregnant dogfish. Each ovum was enclosed in a thin membrane
of ovarian tissue composed chiefly of granulosa and theca interna. The
follicles were usually weighed individually, ruptured, and drained into a
flask containing 95 per cent ethanol; the residual solid tissue was also added.
A total of 836 gm., wet weight, was collected over a period of several days.
The flask was shaken after each addition of material. The contents of the
flask were filtered, and both filtrate and residue were evaporated to dryness
on a water bath. The dried residues were stored in the cold until used for
extraction.

The waxy ethanolic extracts and dry solids were combined and refluxed
for 48 hours in 3 liters of 95 per cent ethanol which contained 2 per cent by

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volume of hydrochloric acid. The solution was decanted, and the residue was washed several times with more ethanol. The combined extracts and washings were neutralized with sodium bicarbonate and dried over sodium sulfate. The filtered solution was then reduced in volume with a Rinco rotating still in vacuo. This crude material was assayed and found to contain estrogenic activity equivalent to approximately 818 Allen-Doisy units.

This activity was determined by a modification of the Allen and Doisy method (3). Female rats 100 days old, and weighing 160 to 170 gm., were castrated and used for assay 4 days later. The unit determined was the minimal amount which, when given in three equal doses over a period of 48 hours, would produce full vaginal cornification in approximately 50 per cent of twelve animals 72 hours after the first injection.

A minimal amount of the substance (0.017 ml.) was injected in a single dose into at least ten 24 day-old rats (54 to 62 gm.) for each determination. Assays were performed at 6 and 30 hours. This amount (0.017 ml.) induced a uterine response at 6 hours equivalent to an Astwood unit (4) of estradiol-17β (0.025 γ) and approximately the same growth of the uterus by the 30th hour. The physiological action of the substance was like that of estradiol-17β rather than that of estrone (Fig. 1). An Astwood unit (5) of estrone (0.45 γ) causes no obvious growth of the uterus by the 30th hour.

The remaining material was taken up in 50 ml. of chloroform and ex-

![Fig. 1. Comparison of the physiological effects of small doses of estradiol and extract of dogfish ova on the water imbibition and growth of the rat uterus. A minimum of ten 22 day-old animals, weighing 54 to 62 gm., was used in each assay group.](http://www.jbc.org/)

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tracted ten times with 20 ml. of 1 N sodium hydroxide. The alkaline solution was neutralized with glacial acetic acid and extracted ten times with 100 ml. of chloroform. The chloroform solution was evaporated to dryness in vacuo. This material was subjected to a twenty-nine transfer partition in a Craig countercurrent distributor in the following solvent system: upper phase, 70 per cent methanol; lower phase, 50 per cent chloroform-50 per cent carbon tetrachloride, as described by Engel et al. (6). In this system estradiol-17β has a partition coefficient of 0.90 and according to

![Figure 2](http://www.jbc.org/)

**Fig. 2.** Paper chromatograms of extract from dogfish ova in a toluene-propylene glycol system and following staining with ferric chloride-ferrocyanide reagent. A, authentic estradiol-17β and estrone (20 γ each); B, estradiol-17β and estrone (20 γ each) and one-third of extract; C, extract from dogfish ova.

calculation can be expected to form a peak in tubes 14 to 15. Repeated partitions in this system of authentic estradiol-17β showed that this substance is found in tubes 10 to 19 after twenty-nine transfers. Partitioning of authentic estrone showed that in this system, in which estrone has a $K$ value of 0.14, this steroid is found in tubes 0 to 9, with a peak in tubes 3 to 4.

Estrogenic activity was found following partitioning of the tissue extract in the fractions representing the combined contents of tubes 3 to 9 and tubes 10 to 19, the latter containing about 10 times the activity of the former. These two fractions were again partitioned in the same system with twenty-nine transfers, and in each of them estrogenic activity appeared in the combined contents of tubes 10 to 19.
One-third of the combined residues left after the bioassay procedure was applied to a 1 cm. paper strip which had previously been treated with a 50 per cent mixture of methanolic propylene glycol and blotted. Another one-third aliquot was applied to a second strip, together with 20 γ of authentic estradiol-17β and 20 γ of estrone. To a third 1 cm. strip 20 γ of estradiol-17β and 20 γ of estrone only were applied. The three strips, joined by a common base, were hung in a cylindrical tank and developed in a descending system with toluene which had been equilibrated with propylene glycol for a period of 18 hours. The strips were then removed from the tank, dried in a warm current of air, and stained with a 1 per cent mixture of ferric chloride-ferrocyanide. Fig. 2 shows the results obtained after staining the chromatographic strips. A blue stain which did not separate from authentic estradiol-17β on the mixed chromatogram was found, but no such staining could be detected in the area corresponding to estrone.

Sufficient material was not available to carry out any further procedures for a more positive identification of the estrogenic compound isolated. However, the results obtained strongly indicate that it was estradiol-17β. This hormone should have been expected in tubes 10 to 19 of the twenty-nine transfer countercurrent partition, while estrone should have been in tubes 3 to 9. The appearance of estrogenic activity in the material from tubes 3 to 9 was most likely caused by the tailing of estradiol, leaving some material in the lower numbered tubes. It is significant that these observations were corroborated by the mixed chromatograms.

SUMMARY

An estrogenic material was obtained from 836 gm. of ovarian eggs removed from the ovaries of the dogfish (Squalus suckleyi) which by countercurrent distribution, paper chromatography, and comparative estrogenic action was identified as estradiol-17β. Therefore, these studies indicate that the graafian follicle of the dogfish, like that of mammals, secretes estradiol-17β.

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