SOME OBSERVATIONS ON THE HERMAPHRODITE APPARATUS OF *VALVATA TRICARINATA* (SAY)*

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These observations have been made as a part of a larger study on the problem of development and differentiation of the ovo-testis of the fresh-water prosobranchiate, *Valvata tricarinata* (Say). It has been pointed out in an earlier report [5] that dimorphic spermatozoa are formed during the normal functional activity of the ovo-testis. In studying the development of these dimorphic spermatozoa, many interesting observations on the more general biological aspects of the animal have been made. The material used was collected from Lake Okoboji in northwestern Iowa. The preliminary observations and early stages of the work were carried on in the Iowa Lakeside Laboratory.

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This problem involves the use of living and fixed material. Dissections were used for the study of the anatomical elements. Other material was preserved for the study of the histological and cytological aspects of the problem. Some smears were prepared but they have not contributed to the analysis of the problem, largely due to the presence of abundant large yolk spheres and granules which were liberated from the developing eggs and to pigment bodies from the capsular covering of the ovo-testis which obscured the details of the smears. Satisfactory results have been obtained from material prepared in Zenker's Solution, Bouin's picro-formol-acetic mixture, Allen's modification of Bouin's Fluid, Gilson's fixation modified according to Petrunkewitsch, Hermann's Fluid, Helly's Solution, and Burckhardt's Fluid.

Flemming has been the least satisfactory as a fixation. Sections were stained in Heidenhain's iron-haematoxylin, with and without

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eosin as a counter stain; some were stained in Delafield's haematoxylin and counterstained with Congo Red and Orange G. Mallory's "Triple" stain was used with good results for the supportive elements. Iron-haematoxylin followed by a mixture of gentian violet and erythrosin has proved useful in many instances.

Valvata tricarinata is a small fresh-water streptoneuran prosobranchiate gastropod with a sharply carinated spiral shell about four millimeters in diameter. The animal possesses a large foot which is bluntly rounded posteriorly and bears two anterior filament-like lobes. Sessile eyes are located at the base of long cylindrical tentacles. Valvata possesses external gills. The gills are external; the left is plumose and extends backward over the shell as the snail moves through the water—the right is a rudimentary structure forming a slender appendage which protrudes from the shell when the animal is extended [3].

The animal is hermaphroditic. The reproductive system is composed of a single ovo-testis with a single hermaphrodite duct leading to a seminal vesicle; a continuation of the duct after it is joined by the duct from the prostata becomes the vas deferens and finally terminates in the pyriform penis. The female aperture is situated on the right side between the right gill and the anus. A short vestibule which bears the female aperture receives the oviduct from the spermatheca or seminal vesicle (also called Bursa copulatrix), the duct from the albumen gland, and the duct from the large crescent-shaped accessory shell gland [4].

The breeding season in Lake Okoboji begins usually in March and continues until late in August. The eggs are small oval structures (fig. 2) sharply pointed at each end and are about 0.25 by 0.37 millimeter in size. Four to eighteen (Table I) elliptical eggs are deposited at one time in small globose albuminous capsules (fig. 1) which are greenish in color. In the natural environment the egg capsules were observed attached to aquatic plants (*Potamageton sp.*, and *Sagittaria* sp.), to floating objects, and to the shells of other Valvatae. In the laboratory the egg capsules were found attached to the sides of the glass aquaria. Development is completed under conditions of the laboratory in twelve to fifteen days. The young Valvatae are very active and were observed moving rapidly over the sides of the aquaria and were often found oriented to the surface film of water with their shells hanging downward. Some of the young animals were removed from the stock aquaria and transferred to smaller aquaria jars. Small leaves of fresh lettuce and occasionally small amounts of spirogyra were added to the aquaria as food. Water was added to compensate for

evaporation. Under conditions of laboratory environment young Valvatae which emerged from the egg capsules on July fifteenth attained their maximum adult shell-size of about four millimeters about the first of the following November. At the present stage of the study nothing is known concerning the sexual cycle or the duration of the life span of *Valvata tricarinata.*

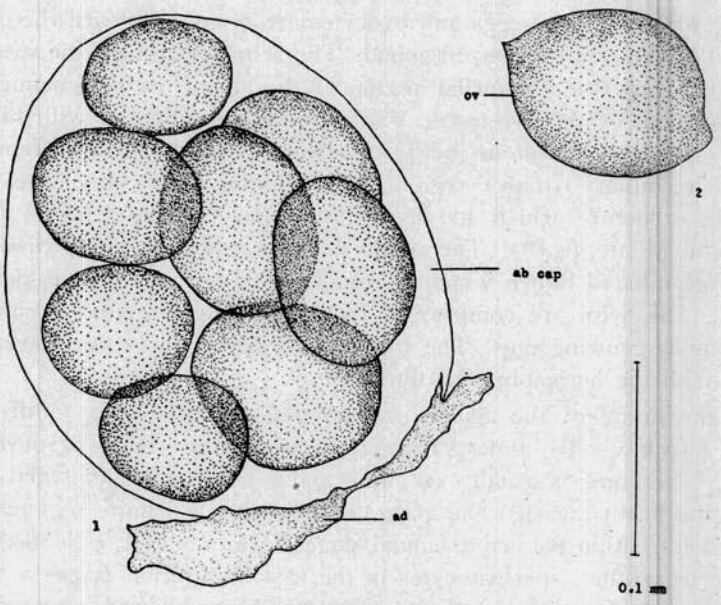

Fig. 1. A camera lucida sketch of an albuminous egg capsule containing eight eggs. Magnification x 80.

Fig. 2. Sketch of one of the eggs from the above capsule. Magnification x 80.

In Valvata the hermaphrodite gland, the ovo-testis, occupies the last whorl of the shell. The gland is smooth, non-lobated, conforms to the cavity of the shell, and is made conspicuous by its dense black color due to the pigmentation of the thin outer capsule. The cells composing the covering of the ovo-testis are heavily laden with large dense black pigment bodies which impart a black color to the gland. The proximal portion of the ovo-testis is embedded in the liver tissue. The distal portion terminates freely in the cavity of the apical whorl of the shell. The hermaphrodite duct lies on the columellar side of the gland within the capsular covering of the ovo-testis where it receives the outlets of numerous atria of the acini. The hermaphrodite duct leads finally to the seminal vesicle.

The ovo-testis of the sexually mature Valvata is a compact structure embedded in the liver. A thin, darkly pigmented layer (Plate, fig. 1) covers the entire gland. This supportive layer which is three to four cells thick is made up of flattened cells with prominent nuclei. Dark pigment granules fill the cytoplasm of the cells of this capsular layer.

In the adult ovo-testis the female and male elements are quite loosly separated—the eggs and ovocytes are oriented toward the outer region of the crescent-shaped gonad. The acini which bear the spermic tissues occupy the columellar region of the gland. In this manner of arrangement of the ovo-testis, Valvata may be compared with higher forms which exhibit in the indifferent stages a definite cortical (female) and a medullary (male) region. Parenchyma forms the loose supportive structure which loosely delimits the female elements from the acini (Plate, fig. 3). The acinus is made especially conspicuous by its large inflated lumen which is usually filled with fluid and spermatozoa. The acini are compressed frequently into irregular forms by the rapidly growing eggs. The atria (Plate, fig. 3) serve to connect the acini with the hermaphrodite duct.

The lining of the acinus is composed of epithelium, indifferent cells, nutritive cells, and spermatogonia in various stages of development. The lining is usually very thin and is made up of flattened cells with prominent nuclei. The germ cells showing a definite regional arrangement within the acinus-gonial stages form one cyst, cells in division form another, spermatocytes in the first maturation stages a third cyst, spermatids another, and ripe spermatozoa are likewise contained in another separate cyst.

EXPLANATION OF PLATE

Fig. 1. Photomicrograph of a section of the ovo-testis cut parallel to the columellar axis. It shows the peripheral arrangement of the eggs and female elements. Magnification x 111.

Fig. 2. Section through the hermaphrodite duct during the discharge phase. The duct contains ripe spermatozoa. Magnification x 170.

Fig. 3. Section of the apical end of the ovo-testis cut at right angles to the columellar axis. The apical end shows the differentiating female components. Magnification x 112.

LEGEND

ab cap = albuminous capsule
ac=acinus
ad=adhesive disc of capsule
at=atrium
go=young egg cell in growth stage
hd=hermaphrodite duct
la=lumen of acinus
lv=liver
oc=young ovocyte
ov=egg cell
pc=pigmented capsule of ovo-testis
sp=cyst of spermatoza
st=supportive (parenchyma) tissue

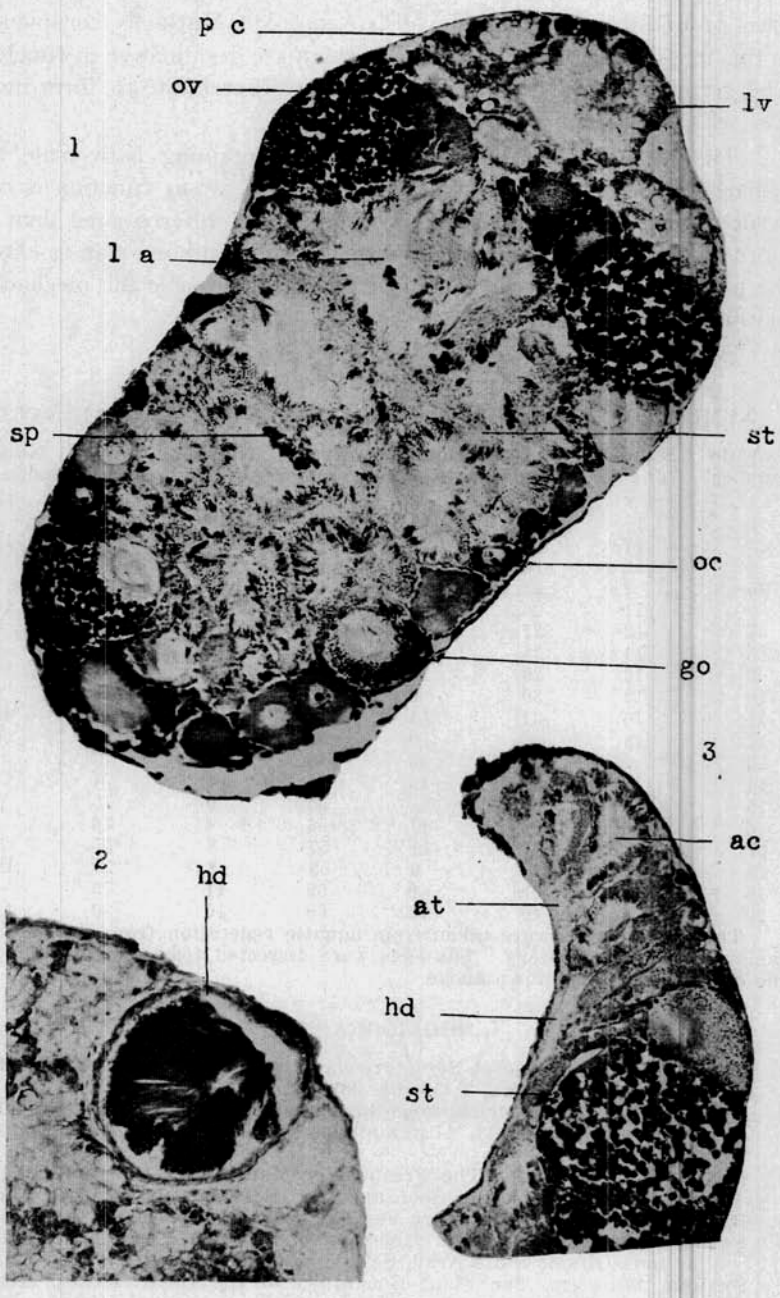

PLATE I

The apical or terminal portion of the ovo-testis is made up of a mass of undifferentiated tissue (Plate, fig. 3). Markedly conspicuous in this mass of parenchyma are cells which are germinative in function. The germinative cells multiply and finally differentiate to form nutritive cells and primary ovogonia.

The ovo-testis is, although a gland containing both male and female sexual elements, a dual structure in-so-far as function is concerned. The female components seem to be less differentiated than the more highly specialized male elements. It is the author's plan to extend the problem in this direction and determine if possible the mechanism of this differentiation.

TABLE I

NUMBER OF EGGS PER CAPSULE IN VALVATA TRICARINATA

Capsule number	Number of eggs	Capsule number	Number of eggs	Capsule number	Number of eggs	Capsule number	Number of eggs
1	8	21	8	41	7	61	12
2	17	22	9	42	13	62	9
3	17	23	9	43	6	63	13
4	12	24	7	44	8	64	8
5	9	25	16	45	10	65	9
6	11	26	7	46	8	66	10
7	12	27	12	47	7	67	9
8	11	28	8	48	7	68	6
9	12	29	14	49	4	69	11
10	12	30	6	50	8	70	8
11	16	31	14	51	10	71	14
12	18	32	10	52	12	72	8
13	9	33	13	53	7	73	8
14	12	34	15	54	8	74	6
15	6	35	12	55	9	75	7
16	10	36	11	56	4	76	8
17	8	37	11	57	8	77	9
18	12	38	9	58	8	78	10
19	11	39	9	59	11	79	8
20	10	40	13	60	10	80	12

The egg capsules were taken from aquatic vegetation from the lake and removed to the laboratory. The eggs were dissected from the capsules and the data recorded as shown above.

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