Note on the structure and relations of the kidney in Aplysia.

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With plate 30.

The notices which I have been able to find in Zoological literature on the kidney of Aplysia are scanty and somewhat obscure. Delle Chiaje[1] describes the »triangular gland« as large and pink in color; he says that its anterior border forms the boundary of the pericardium, that its left margin is reflected at the wall of the abdomen, while its right circumscribes the bottom of the branchial cavity, and that its inferior face is in contact with the intestinal tube. This description of the general relations of the organ is to a great extent correct, though somewhat incomplete. He then proceeds to discuss the function of the gland; he says it was the opinion of Cuvier that in A. fasciata the triangular gland secreted the purple fluid, which the animal gives out and which in his own opinion is derived from superficial small glands of the mantle: the latter are violet in color, while the triangular gland, when pressed or cut, gives no trace of purple fluid and is itself of a pinkish white tint. He mentions the fact, that in A. leporina, Cuvierana and Poliana the fluid secreted by the superficial glands of the mantle, is white and viscous, and then suggests that the function of the triangular gland is to secrete the »bony molecules« of the shell, both gland and shell being absent in Notarchus. The interesting hypothesis of the great Neapolitan Naturalist would derive some support from the latter fact, if it were true; but although Notarchus does not possess a shell, its renal organ is a gland

[1] Delle Chiaje, Animali senza vertebre del Regno di Napoli. Napoli 1841. Vol. II p. 64.

corresponding in almost every respect with the »triangular gland« of Aplysia. Notarchus may be described as an Aplysia, in which the lateral lobes have fused together along their free edges above the mantle from behind forwards, a small aperture being left anteriorly, by which the chamber so formed communicates with the exterior. When these fused lobes are cut away, the upper surface of the mantle is seen quite bare with the pericardium in front and behind it the kidney, which though relatively smaller than in Aplysia, is still of considerable size.

I have not been able to consult Cuvier's Memoirs on Mollusca, as the library of the Zoological Station does not possess a copy of them, but the figures of the anatomy of Aplysia in Bronn's Thierreich [1], which are taken from that work, are not clear as to the relations of the renal organ. There is a reference letter to the opening of the renal organ in the description of the plate, which cannot be found anywhere in the plate itself. In the text all that is said of the kidney, is that its external opening is situated beneath the branchia, a statement which is taken from an incidental remark of Lacaze-Duthiers in his Memoir on Pleurobranchus aurantiacus [2].

The nomenclature of the species of Aplysia is somewhat uncertain. The examples which I have used in the Zoological Station belong to two large species which are both very plentiful: they are known by the names Aplysia leporina and Aplysia depilans. The size of these is usually about 6 inches in length and 3 or 4 in breadth and height. There is a 3rd species common at the Station which is much smaller, not exceeding $1\frac{1}{2}$ inches in length. The first of the three mentioned is black in color, with white flecks here and there, which are caused by groups of small opaque white spherules in the skin: it is usually the largest in size, it is flaccid when handled, and possesses very little muscular power: its lateral lobes are easily spread open and are only slightly fused together behind. It gives out from the cutaneous glands on the under surface of the free lobe of the mantle a large quantity of purple fluid, and its poison gland has a single external opening, which is small and circular in shape and is situated behind and to the right of the genital aperture. In giving out a purple fluid and in having a common aperture for the poison glands, this form agrees with the A. fasciata, camelus and Neapolitana of Delle Chiaje, and therefore it will be convenient to call it as Dr. Blochmann does, A. camelus Cuv.

[1] Keferstein, Weichthiere. 1862—1866. Taf. LXV.

[2] Ann. Sci. Nat. 4th Ser. Vol. XI p. 257.

The second form mentioned varies in color from yellowish brown to light reddish brown, sometimes having lighter colored spots; it has strong powers of contraction; its lateral lobes are fused together posteriorly to the level of the mantle-siphon and are difficult to separate; its mantle glands exude a white viscous fluid, and its poison glands open by many openings grouped over an elliptical area behind the genital opening. In these characters it agrees with the A. leporina, Cuvierana and Poliana of Delle Chiaje; but it is best to retain for it the name A. depilans L. with Dr. Blochmann. The third form I have not used for my preparations; it exudes a purple fluid and has the opening of the poison glands as in A. depilans: Dr. Blochmann calls it A. punctata. The first form has no opening in the duplicature of the mantle which covers the shell; the other two have a circular opening in the centre of the shell-covering of considerable size. Both A. camelus and A. depilans have a somewhat strong characteristic smell which is stronger in the latter.

In order to see the kidney, which is the triangular gland of Delle Chiaje, the lateral lobes of one of the larger forms are separated or cut away; the covering of the shell is then removed with the scissors and the shell taken away, then in the area which was covered by the shell, are seen the pericardium anteriorly and the surface of the kidney; the latter is of a light yellow color and its front edge runs along the posterior border of the pericardium, from which it extends backwards growing narrower towards its posterior rounded termination in the left corner of the area covered by the shell. To the right of the kidney is the free mantle fold which forms the roof of the gill-cavity. The shell extends nearly but not quite to the edge of this fold. To the left the upper surface of the kidney extends beneath the skin, which lies between the shell area and the left lateral lobe or epipodium, for some distance. When the pericardium is cut open, the anterior face of the kidney is seen forming the posterior wall of the pericardium, and at the lower edge of this face to the right close, to the auricle is seen the depression leading to the canal which forms the communication between the pericardium and the cavity of the kidney. The relations now described are seen in fig. 1, which represents a view of the dorsal surface of A. depilans: the epipodia have been cut away along the line *ep*; the shell has been taken out from its chamber and part of the mantle fold which covered the shell is seen turned aside at *mf*; the pericardium is laid open and the position of the reno-pericardial opening is seen at *sp*. The floor of the shell chamber is formed on the left by the upper surface of the kidney (*k*, fig. 1) and on the right by the free part of the mantle which forms a thin trans-

parent membrane. To examine the surface of the kidney which lies in the wall of the branchial cavity, the animal is turned slightly on its left side and the mantle fold drawn to the left as far as possible so as to expose the cavity completely. The branchial cavity is divided into two compartments by the gill which is in the form of a triangle with the apex curved towards the posterior end of the animal and attached continuously along the base. This line of attachment runs across the surface of the kidney, a small part of which is seen above the gill and a larger part below. The external opening of the kidney is a small aperture below the line of attachment of the gill, close to the latter and near its posterior extremity. In the living animal the opening is seen to expand and contract. A better view of the branchial cavity is seen, when the gill is cut away near its base as in fig. 2, where *br* represents the remains of the gill so removed. In the same figure the so called siphon or semitubular prolongation of the mantle is seen at *si*; on the inner surface of the siphon, near the angle where its lower border joins the body is the anus, *an*. The lower side of the anterior end of the gill-attachment carries the olfactory organ of SPENGEL, *ol*: the opening of the kidney is shown at *eo*: the area occupied by the »poison glands« *pg* is on a level with the anterior end of the gill, inside the line of origin of the right epipodium and in front and to the left of these are the genital opening, *go*, and the commencement of the seminal groove *sg*.

All the surfaces of the kidney have now been described except that which is turned towards the internal organs of the body: this is quite flat and lies upon the rounded mass formed by the large liver and the convolutions of the intestine to which it is attached. This internal surface extends from the lower boundary line of the surface belonging to the branchial cavity, to the outer boundary line of the dorsal surface, and as the branchial cavity is very deep, the internal surface of the kidney is of slight extent.

To determine the position and nature of the renopericardial opening, which cannot be done by mere dissection, I injected the pericardium with a cold solution of Berlin blue in weak oxalic acid, in the fresh animal. The injection ran down into the kidney along a line parallel and close to the attachment of the gill, and its course could be seen when the branchial cavity was examined. I then dissected out the kidney hardened it and cut a series of sections perpendicular to the surface bordering the pericardium and parallel to the direction taken by the injection. This method is excellent for tracing similar passages, as the injection remains attached to the surfaces it has reached, and does not

interfere with the processes of preparing or cutting. In this case it was not necessary to trace the injection through a number of successive sections, because the sections being thin, the passage not very minute and the direction of the sections the same as that of the passage, two or three of the series showed the whole canal from the cavity of the pericardium to that of the kidney. One of these sections is given in fig. 3, where the dorsal part of the kidney is to the left and the ventral to the right. The pericardial cavity, *pc*, is seen at the top of the section and from it runs down a narrow canal containing the blue injection and opening below into the cavity of the kidney, *kc*. In the middle of its length the canal is divided into two by a band of connective tissue, *x*, which appears to be chiefly cellular. On the right of the figure is shown the wall of the branchial cavity consisting of muscular fibres and connective tissue intertwined in all directions, *ct*, and containing irregular blood-spaces, *bl*. The epithelium covering the wall of the branchial cavity is composed of very small cells, like the external epithelium of the rest of the surface of the body, and is represented as seen under low power by a dark line. No epithelium can be seen lining the wall of the pericardium, but the canal of communication is lined by an epithelium composed of cells of considerable size; whether this epithelium is ciliated or not I have not determined; cilia cannot be detected in the sections.

The cavity of the kidney is divided by flat trabeculae of connective tissue which start from the wall of the cavity, into a number of communicating chambers and passages of various sizes; and the cavity communicates with the exterior by the small circular opening whose external position and appearance has already been described, fig. 2 *eo*. In order to examine more minutely the nature of this opening I cut a series of sections through the part of the kidney containing it. For this purpose the posterior end of the kidney was taken and cut into sections, whose plane is perpendicular to the wall of the branchial cavity and to the dorsal surface of the kidney. One of these sections passing through the external opening is represented in fig. 4; where the wall of the branchial cavity and the opening of the kidney are seen on the right side. The opening is seen to be lined by a columnar epithelium continuous with that of the branchial cavity, on one side and passing into the renal epithelium on the other. The contractions and expansions of the opening are due to the ordinary musculature of the body wall.

The appearance presented by the renal tissue under a low power is seen in figs. 3 and 4. The trabeculae in section are seen as thin

deeply stained lines forming a network without any regular arrangement and on each side of these lines is seen the renal epithelium. In the upper part of fig. 3 the renal epithelium is not drawn. The smaller spaces enclosed by the trabeculae in the sections are filled completely by the outlines of the vesicular renal cells. This seems to be due to the continual proliferation of the cells of the renal epithelium, the escape of the mature cells being impeded by the narrowness of the intertrabecular spaces. The trabeculae are in many places extremely thin and no blood lacunae can be detected in these, the epithelium covering them deriving its nourishment from the blood, which they absorb from neighbouring lacunae: probably some lacunae, wich exist in the living animal, are obliterated by the contraction, which takes place during the process of preparation. But a great many of the trabeculae are hollow consisting of two membranes enclosing a blood lacuna between them; in the sections such blood lacunae may be seen extending along the trabeculae for some distance and containing blood corpuscles; in some places, especially where the trabeculae take their origin from the side of the renal cavity towards the wall of the branchial chamber, there are blood lacunae of considerable size traversed by delicate strands of connective tissue (fig. 3 *bl*[1]). In fig. 5 a portion of a trabecula with a blood lacuna running along it axially is figured as seen under high power; it is taken from a section from the posterior part of the organ. Muscular tissue is present in the trabeculae here and there, but not in any great quantity. An example is given in fig. 6, where the central part of the trabecula is occupied by a band of parallel muscular fibres; the renal epithelium in this particular part of the section was not well preserved.

The renal epithelium of Aplysia seems somewhat different at first sight to that which is characteristic of most Gasteropoda: but it has the same fundamental structure. When a portion of the kidney in the fresh state is teased up on a slide and examined under the microscope, the field of view is seen covered with vesicular cells of various sizes containing concretions. Most of the cells are quite free, and spherical in shape; in some of them a layer of transparent protoplasm extends round part of the circumference, but no nucleus can as a rule be distinguished even after the addition of acetic acid; sometimes as in *f* fig. 7 an elliptical body is seen in the protoplasm, which may be a nucleus, but the whole is so transparent, that its nature cannot be distinctly made out. In sections large nuclei are seen belonging to the cells of the renal epithelium as in fig. 5. In a teased preparation the upper surface of the epithelium is often seen, slightly pressed by the cover glass, and the appearance it

presents is shown in fig. 7*a*. The cells here have a somewhat polygonal shape due to mutual pressure; inside them are seen the brown or black concretions, and occasionally the outline of part of one cell is seen through another which lies over it. The shape and quantity of the concretions varies in different cells: the most common form is that of an aggregation of minute spherical molecules, which often show a trembling motion, the Brownian movement; but occasionally a cell contains a single large crystal, whose shape is a regular pyramid with a square base (fig. 7 *b* and *c*). When a little of the contents of the kidney is pressed by gentle pressure from the external opening of the organ and examined the material so obtained is found to consist of cells similar to those in teased preparations of the renal tissue, and of débris derived from such cells. The entire cells are often still connected together in masses and groups as seen in fig. 8.

I have not been able to find any ciliated cells in either sections or teased preparations from the kidney of Aplysia, although the vesicular renal cells containing concretions are ciliated in many Gasteropods e. g. Patella: in this respect Aplysia agrees with Pleurobranchus according to Lacaze-Duthiers[1], with Tethys according to von Ihering[2], and with Tritonia according to my own observations.

The process of renal secretion evidently takes place as in other Gasteropods, e. g. Patella and Tethys, by the development of a vesicle in each epithelial cell as it matures and the escape of the cell, when the vesicle is fully developed. The proper excretion of the organ consists in the concretions and fluid contained in the vesicles. The escape of the vesicular cells from the external opening, their loose attachment in teased preparations, and the appearances to be seen in sections all point clearly to this as the method of secretion; but I have not followed out completely either in sections or teased preparations the various stages of development of the renal cells.

Morphologically the kidney of Aplysia corresponds in its relations to other organs, with the kidney of those Prosobranchia in which the organ is single and with the left renal organ of Zeugobranchia and of Patella: the external opening lies in front of the anus on the right side, as would be the case in a typical Prosobranchiate e. g. Tritonium, if we suppose the twisting, which has carried the gill, rectum and renal organ to the anterior part of the dorsal surface, to be reversed to a certain

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[1] Ann. Sci. Nat. 4th Ser. Vol. XI.

[2] Morph. Jahrbuch. Vol. 2.

extent, till these organs lie on the right side. What is known of the development of Nudibranchs indicates that they are to be derived from forms similar to the Anisobranchia (or Ctenobranchia in Claus' Zoologie) among the Prosobranchiata. Unfortunately little is known of the position of kidney, gill and rectum in the young stages of Aplysia or other forms among the Tectibranchiata; but from the appearance of the embryo it seems probable, that the relations of these parts in the latter are similar to those which obtain in the adult Anisobranchia, and that in the course of growth an actual untwisting takes place, which carries the organs from the anterior dorsal surface to the right side.

Explanation of Figures.

Plate 30.

Fig. 1. Dorsal surface of Aplysia depilans; the epipodia have been cut away along the line *ep*, the shell cavity has been opened and the shell removed, and the pericardium laid open.

k kidney.
mf fold of the mantle which lies above the shell.
si tubular prolongation of mantle, or siphon.
m free lobe of the mantle.
sg seminal groove.
rp depression containing the opening from pericardium into kidney.

Fig. 2. View of the branchial chamber of A. depilans. The free fold of the mantle has been drawn as far as possible to the right side so as to expose the chamber completely, and the branchia has been cut away.

k kidney.
eo external opening of kidney.
an anus.
pg area covered by openings of poison glands.
go genital opening.
sg seminal grove.
si siphon.
ol olfactory organ of Spengel.

Fig. 3. Section of kidney perpendicular to the dorsal surface and to the face bordering the pericardium. The section passes through the canal leading from the pericardium to the cavity of the kidney. Zeiss aa.

pc pericardial cavity.
kc cavity of kidney.
cl connective and muscular tissue.
bl blood lacuna.
ke kidney epithelium.
rpc renopericardial canal.

Fig. 4. Section passing through external opening of kidney. Zeiss aa.
eo external opening of kidney.
bl blood lacunae.
br origin of branchia.
f^1 right hand fold to cover the shell.
f^2 left hand ditto.
ep^1 epithelium lining shell cavity.
ep^2 epithelium of the body surface between the left epipodium and the mantle.
mu musculature.

Fig. 5. Sections of a trabecula containing a blood lacuna. Zeiss E. Oc. 4.
rep renal epithelium.
bl blood lacuna.
blc blood corpuscles.

Fig. 6. Section of a trabecula containing muscular fibres. Zeiss E. Oc. 4.
rep renal epithelium.
mu muscular fibres.

Fig. 7. From a fresh teased preparation of the renal tissue. Zeiss E. Oc. 4.
a surface of the renal epithelium.
b and *c* cells containing a crystal.
d cell containing crystal and irregular concretion.
e concretions escaped from cells.
f cell with protoplasm and nucleus (?).

Fig. 8. Cells pressed from external opening of the renal organ.

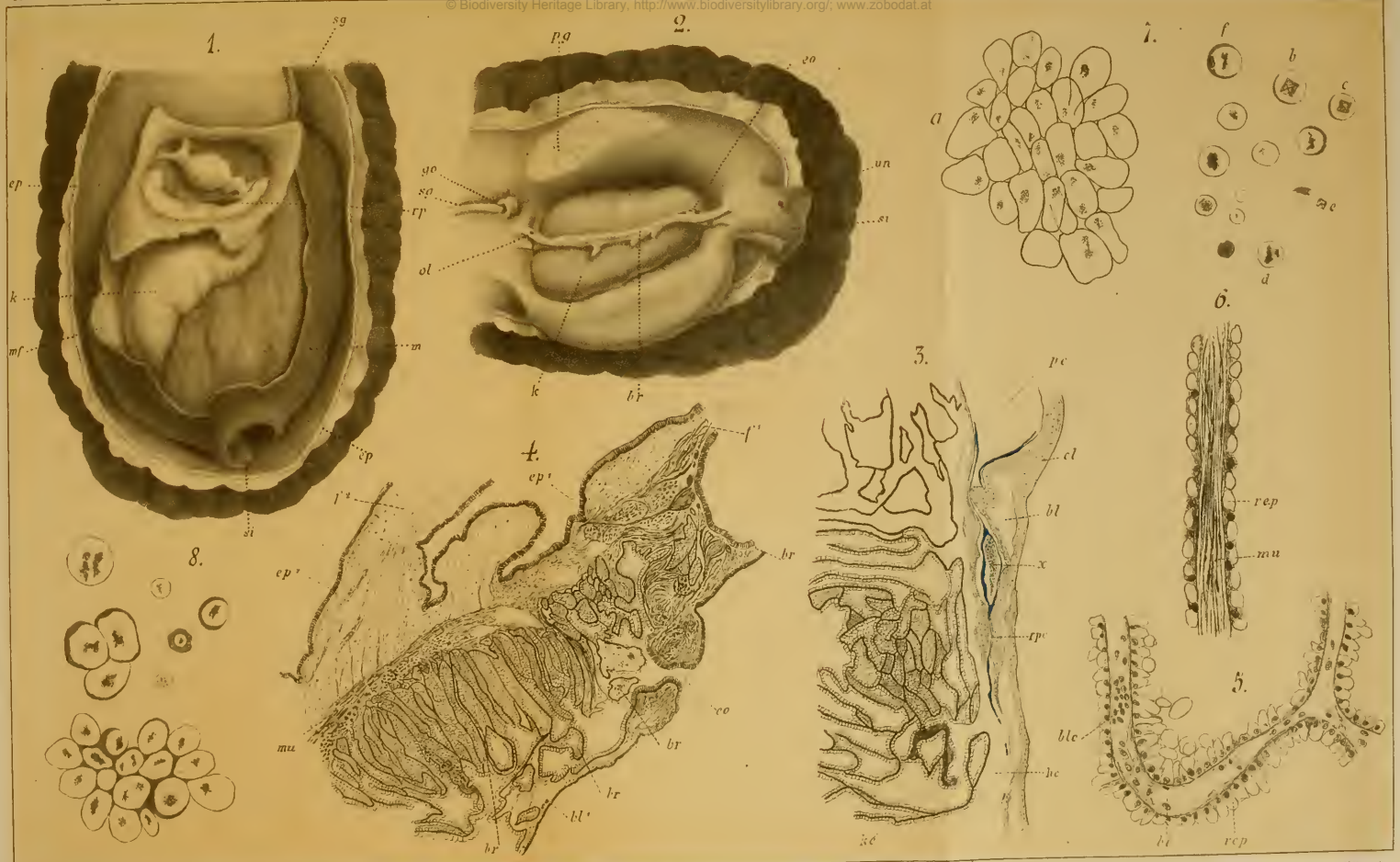

Fig. 1, 2. Marcellusne 3:4 d. T. Cunningham del.
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