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PRACE ZOOLOGICZNE
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ANNALES ZOOLOGICI MUSEI POLONICI HISTORIAE NATURALIS.

Dr. WACŁAW ROSZKOWSKI.

Materjały do poznania rodziny *Lymnaeidae*. VII.

Budowa gruczołu przyprątnego u błotniarek.

Contributions to the study of the Family *Lymnaeidae*. VII [1]).

The structure of the prostate of the *Lymnaeidae*.

[Pl. I].

This paper is a continuation of a research conducted jointly with Dr. A. Żebrowska (18), published in 1916 in the „Prace Tow. Nauk. Warszawskiego" (Travaux de la Soc. Sc. de Varsovie). The object of these morphological researches on the *Lymnaeidae* was fully discussed in the introduction to the above paper. The present research was undertaken with a view to find whether and to what extent the various genera and species of the *Lymnaeidae* differ among themselves, insofar as the character or arrangement of the histological elements of the prostate gland are concerned.

Materials.

As material for investigation, the following genera and species were taken: *Lymnaea stagnalis* L., *Radix auricularia* (L.), *Radix ovata* forma *B* Roszk., *Amphipeplea glutinosa* (Müll.), and *Galba palustris* (Müll.), all from the environs of

[1]) Part IV of this series was in 1916 submitted for publication to the editorial board of the publications of the Caucasian Museum, but up till present time I do not know whether it actually appeared in print or not. Parts V and VI were published in the „Disciplinarum Biologicarum Archivum Societatis Scientiarum Varsoviensis", vol. I, (1922) 1923.

Warsaw and Petersburg (Leningrad), as well as *Lymnaea stagnalis* L., *Radix auricularia* (L.), *Radix ovata* forma *A* Roszk., together with morpha *yungi* Piag. and *Galba palustris* (Müll.) from the neighbourhood of Lausanne [1]).

The gland were fixed either in 8% formalin, in sublimate with acetic acid, or more usually in Zenker's fluid, which gave the best results. For staining, Heidenhein's ferrons haematoxylin or Delafield's haematoxylin were used, together with orange or eosin.

Bibliography.

Comparatively numerous descriptions and drawings of the external appearance of the prostate gland exist, viz., that of *Lymnaea stagnalis* [Prévost (12), Baudelot (3), Lehmann (9), Baker (1, 2), and Roszkowski (13, 17)], of *Radix auricularia* [Moquin-Tandon (10), Baker (2), Roszkowski (13, 14, 15, 17), Soós (21)], of *Radix ovata* forma *A*, and morpha *yungi* (Roszkowski, 13), *Radix ovata* forma *B* [Eisig (6), Klotz (7), Roszkowski (14, 15), and probably Lehmann (9), the drawings of the latter being, however, too inaccurate to enable one to state with certainty what species they represent]. Whether the *Radix ovata* described by Soós belongs to form A or B it is also difficult to decide from his description, although in my opinion it shall be rather the former. *Amphipeplea glutinosa* has been described by van Beneden (4) and Roszkowski (16), and *Galba palustris* by Lehmann (9), Baker (2), and Roszkowski (13).

The internal anatomical structure of the prostate has not been described up till present, although certain authors, in discussing the histological structure or development of this gland, have mentioned the existence of certain internal folds, without, however, describing them more closely, or giving drawings.

The histological structure of the prostate of *Lymnaea stagnalis* has been described by Semper (19) and recently by

[1]) P. Hesse (Arch. f. Mollusk, 1923) objects to the introduction of the generic name *Galba*, but until this question is not finally decided, I shall retain this term, since it has been accepted in the most complete monograph on the *Lymnaeidae* (Baker).

Kopystyńska (8), and of *Radix ovata* forma *B* by Semper (19), Eisig (6) and to a certain extent by Klotz (7), the latter author dealing, however, only with the development of this organ.

Anatomical Structure.

The external appearance of the prostate gland of those species here examined has been so often described that I shall not here deal further with it; in this paper only the internal structure will be more closely dealt with.

The prostate of the *Lymnaeidae* comprises the posterior portion of the sperm-duct, commencing immediately after the bifurcation of the hermaphrodite duct into its male and female branches. Anteriorly, it extends to the point where the narrow vas deferens, free from glandular cells, issues from the greatly widened front end of the gland. The lumen of the prostate is therefore at the same time that of the male duct, through which the spermatozoa must pass on the way to the external orifice. The secretion of the glandular cells, lining the walls of the prostate, mixes with the spermatozoa, forming a liquid medium in which they can move freely, and serving, possibly, as a nutrient medium. The prostate of the *Lymnaeidae* is thus an organ analogous to that of mammals, and for this reason to this gland alone in the mollusc organism can the name of prostate be assigned.

Thus the group of glandular cells located on the sheath of the penis of *Physa acuta*, named by Sługocka (20), following the example of Moquin-Tandon (11), the prostate gland, is neither analogous nor homologous to that here described under this name. We have previously showed (18) that the group of cells found on the sheath of the penis of *Physa acuta* has ist homologue in the group of glandular cells on the head of the first sheath of the penis of the *Lymnaeidae*. The same name cannot therefore be applied to two entirely different formations, neither homologous nor analogous, in two such closely related families as the *Physidae* and the *Lymnaeidae*. The appellation of prostate gland belongs, therefore, not to the group of glandular cells of the sheath of the penis, but to that portion of the sperm-duct which Sługocka calls the glandular portion of the vas deferens.

The internal secretory surface of the prostate gland is greatly enlarged by the formation of folds whose number and arrangements varies with different groups of the *Lymnaeidae*. In those species which I have investigated, the three following types may be found.

Type I, characterised by the presence of only one fold in the widened frontal end of the prostate. This fold is of considerable size, and disappears in the narrowed posterior end of the gland. To this type belong all the examined species of *Radix* and *Amphipeplea*.

Fig. 1 represents a series of schematic cross-sections through the prostate of *Amphipeplea glutinosa* [1]). Sections A and B are

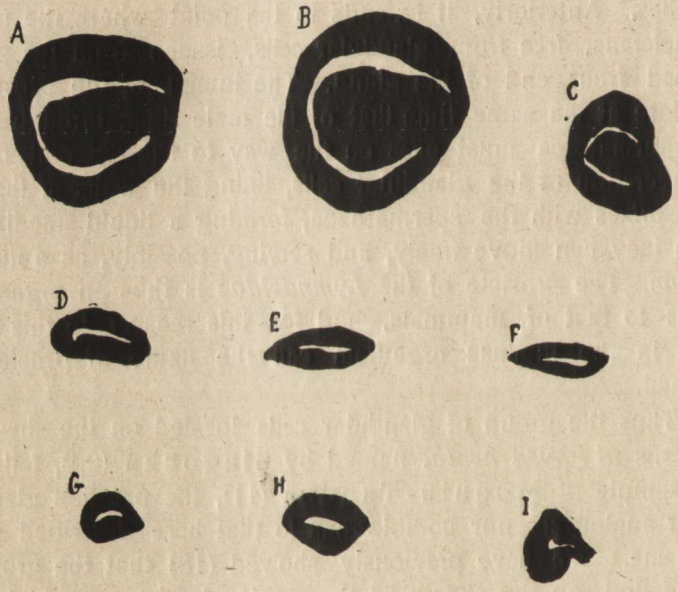

Fig. 1. Scheme of cross-sections through the prostate of *Amphipeplea glutinosa*.

through the anterior part of the gland, where it attains its maximum width. As we see, the lumen of the gland at this part has a crescentic cross-section, caused by the fold, which enters from the periphery. Section C, fig. 1, is of the same portion,

[1]) All figures are drawn by the aid of a camera lucida, and are thus comparatively accurate. Figs. 1, 2 and 3 are enlarged to the same extent, and their dimensions are therefore directly comparable. The long axis of section A, fig. 3, is in reality 4 mm. in length.

but further towards the narrowed end than the previous section— the cross section is here diminished in size, but the fold is still distinct. The remaining sections are of the hind constricted and flattened portion of the gland. Here, the internal fold disappears, although in section C traces still remain — furthes on, the walls are smooth and unwrinkled. Towards the origin of the prostate, i. e., the point at which the hermaphrodite duct divides into its male and female branches, the cross-sections become more and more nearly circular (sections G and H). Section F shows the end of the hermaphrodite duct and the commencement of the prostate.

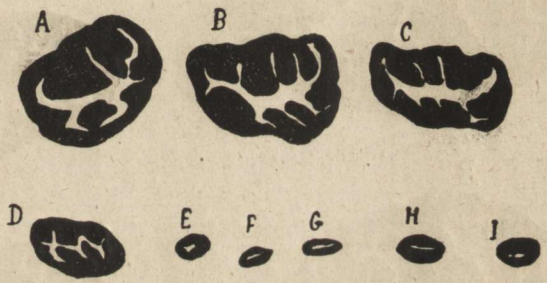

Fig. 2. Scheme of cross - sections through the prostate of *Galba palustris*.

Prostate glands of type II may be found in *Galba palustris*, and differ from type I in that the anterior widened end of the gland possesses not one large fold, as in *Radix* or *Amphipeplea*, but several smaller ones, as can be seen in fig. 2, cross-sections A to D, these folds being distributed over all the walls of fhe gland. In the posterior portion of the prostate, as for type I, the folds disappear (sections E to I).

Finally in type III, exemplified by *Lymnaea stagnalis*, the development of the internal folds attains its maximum. These folds appear not only in the anterior widened partion, as in the two previous cases, but throughout the whole gland, and the number and complexity of their arrangement is much greater [fig. 3]. Section A is of the anterior portion of the gland at its greatest width, and shows numerous internal folds, from which again secondary folds develop, thus greatly increasing the internal sufrace of the prostate. This type differs from the two preceding, by the complexity of his anterior part; in the middle portion the secondary branches disappear [fig. 3, sections C — E]

the sections assuming here the apperence of those of the anterior widened portions of Type II, viz., the same, perhaps slightly more numerons, unbranched folds. Further towards the back of the gland the sections lose their marked flatness, their diameters

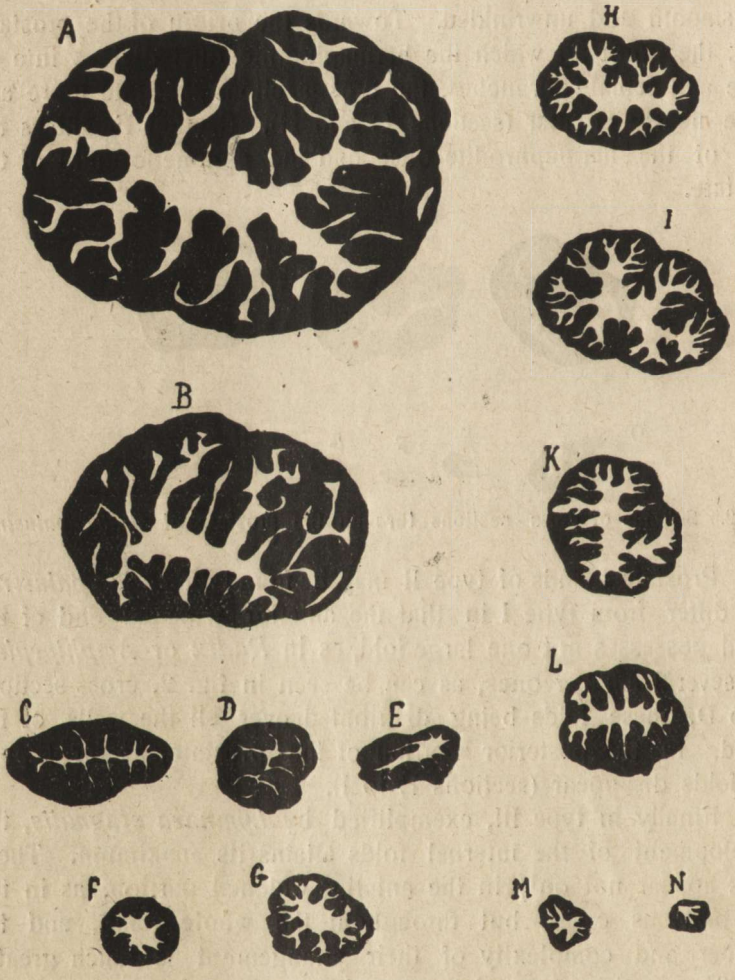

Fig. 3. Scheme of cross-sections through the prostate of *Lymnaea stagnalis*.

regularly increasing, and at the same time the secondary folds again appear (sections G to L), finally disappearing in the vicinity of the hermaphrodite duct (sections M and N).

Histological structure.

The above observations, indicating the presence of hitherto unknown generic differences in the arrangement, character and number of the internal folds of the prostate gland, are for systematic purposes extremely interesting and important, allowing of a more fundamental and exact delimitation between the different genera of *Lymnaeidae*. The anatomical investigation of this gland has thus yielded positive results, whilst from its histological examination no generic or specific differences have been observed, either in the character or in the arrangement of its histological elements [1]). In view of this, it would seem that a description of the histological structure is superfluous, the more so as several papers have already been published on this subject. Since, however, the researches of Semper and Eisig, published in 1857 and 1869 respectively, are somewhat antiquated, and that of Klotz (1889) is dealing only with the development of the prostate gland, and since the more recent research of Kopystyńska (1914) presents the structure of this gland in an entirely mistaken way, it would not, perhaps, be out of place to state here briefly my observations on this subject.

The exterior of the gland is covered by a comparatively thin membrane of connective tissue [plate I fig. 2], whose cells possess elongated nuclei, the cytoplasm forming a thin membraneous envelope, beneath which, apart from fibrous connective tissue elements, may be found here and there larger oval mucous cells, with large nuclei, and numerous pigment cells, scattered plentifully throughout the membrane, containing pigment in the form of small black granules. Among the above elements may be found some smooth muscle fibres, distributed scarsely over the whole membrane, not forming anywhere any greater aggregation or layer, and being in general disposed circularly. From this membrane develop bands of connective tissue, for-

[1]) Here, perhaps, an exception is afforded by the size of the glandular elements, which appear to vary with different representatives of this family. I have not, however, more closely investigated this question in view of the variability of the dimensions of the glandular cells, depending upon their content of secretion, and upon the condition of the latter; finally, the magnitude of the cells varies in different parts of the same gland, thus rendering it difficult to form any exact estimate of their average size.

med of the above elements, and entering into the interior of the folds, forming their axial basis. The thickness of these bands, or more exactly, partitions (in view of the direction and form of the folds themselves), varies greatly with their location and with the species in question, and is sometimes fairly great, especially in those cases where the number of folds is small, as in *Radix* or *Amphipeplea*. Fig. 5 [plate I] shows a portion of such an axial basis of a fold in *R. auricularia*. The centre is occupied by a band of smooth muscle fibres, much more numerous here than in the envelope of the gland, the fibres running from the periphery of the latter to the free internal edge of the fold. On both sides, closer to the base of the glandular cells, are scattered numerous pigment cells, containing large numbers of pigment granules.

Fig. 4. Scheme of a glandular „nest" in the prostate of the Lymnaeidae. The black space at the bottom of the drawing represents the connective-tissue base of the „nest", from which develop two thick black lines, representing the walls of the „nest"; towards the interior, we see the elongated glandular cells, and above them the ciliated epithelium, directed towards the lumen of the cell.

Passing further from the periphery towards the lumen of the gland we meet the dominating layer of glandular cells. Their arrangement is extremely characteristic, and since neither the

description nor the drawing given by Kopystyńska (1914) corresponds with reality, I shall describe this peculiarity more fully.

The glandular cells are not distributed evenly over the internal wall of the gland, but in groups [fig. 4 and plate I fig. 1], separated from each other by partitions of connective tissue, which develop, on the internal walls of the gland, from the envelope, and on the folds from the axial band. In fig. 2 (plate I), we see the envelope, with a thin partition developing from it, and a similar partition may be seen on the left of fig. 5 (plate I), developing on a broad base from the axial connective tissue of the fold. These partitions divide into a great number of „nests", or cells, producing the impression of honeycomb. In these „nests", whose walls consist of thin layers of connective tissue, are located the glandular cells. This „nest" — arrangement of the glandular cells is so obvious, even from the most cursory examination of a section, that it is difficult to understand how Kopystyńska could have failed to remark so striking a feature: neither her description nor her drawings indicate the slightest trace of such an arrangement, and this is the more strange that long previously Klotz and Eisig had shown it, in perhaps rather a schematic manner, in their drawings.

The individual glandular cells, which together make up a „nest", are invariably elongated; apart from this, their shape varies with their position in the „nest". The central cells, whose base rests on the floor of the „nest", and whose upper end opens on the internal surface of the gland (all cells debouch on the internal surface on the same level) are obviously the longest. They are usually pear-shaped, since their lower end, invariably containing a large nucleus, in the vicinity of the connective tissue walls of the nest, is widened, whilst the upper end narrows into a long neck, whose opening into the lumen of the gland is situated between the ciliated epithelial cells. Those cells whose lower end rests on the sides, and not on the floor of the nest, are for this reason shorter, their length depending upon the distance between the support and the ciliated epithelial surface of the lumen of the prostate. These cells are retort-shaped, their broadened base, containing the nucleus, resting on the partition, whilst the neck is prevented by the bases of the adjacent cells

from going directly to the surface, being forced to run towards the centre of the nest, and then to bend, in order for their ends to reach the lumen of the gland.

The appearence of the glandular cells is entirely dependent upon their physiological state. Most often they are, with, of course, the exception of the base, containing the nucleus, filled with secretion, in the form of spheres of varying size, staining strongly with eosin [plate I fig. 2 and 3]. It appears that the secretion may remain at this stage for a long time, and only immediately before its expulsion the granules undergo liquefaction, the secretion being then coagulable into a more or less fibrous mass under the influence of fixing solution. The expelled secretion, or that ready for expulsion stains an intense blue Delafield's haemotoxylin. As a rule, all the glandular cells of the entire gland are in the same physiological stage, i. e., their contents are either granular, staining with eosin, or are fluid, and ready for expulsion. Very rarely the cells of one part of the gland contain the former, and of another part, the latter type of secretion; occasionally the character of the contents of some cells is indefinite, possessing neither the characteristic granulation of the one type nor the traits of the other; whether this is a transition stage between the two types of secretion, or whether the cells in question are youug, or regenerating cells, as yet incapable of producing normal secretion, I have not been able to decide.

The granular secretion is absent from the widened base of the cells, which is occupied by undifferentiated cytoplasm, in which is placed a large nucleus, possessing a well-defined nucleolus. With the moment when tha granular secretion changes to a liquid or fibrous (after fixing) mass, ready for expulsion, the nucleus assumes a less normal appearence. It then stains very intensely, as though the scattered chromatin had been concentrated, and the entire nucleus appears to have shrunk.

As to the fate of the glandular cells after expulsion of secretion—whether they are regenerated, or whether they are replaced by fresh cells, differentiated from the epithelium, it is difficult to judge — in any case, this question does not lie within the scope of this research. However, sections fixed at the mo-

ment of expulsion of secretion appear to indicate comparatively far-reaching destruction of the glandular cells.

The internal layer of cells, lining the lumen of the prostate, is made up of epithelial ciliated cells, cylindrical in shape fig. 4 and [plate I, fig. 3], which possess light-colored cytoplasm, with a fairly large nucleus, situated either at or slightly above the centre of the cell. From the free surface of these cells develop comparatively long cilia, at whose origin the basal bodies are clearly visible under the microscope. The lower end of these cells rests upon a very well developed basal membrane; I have never observed them to pass through and below this membrane, as in Kopystyńska's drawings. Kopystyńska, further, does not even mention the existence of the basal membrane, or of the cilia, which were described and drown by Semper in 1857 for the same species as she describes *(Lymnaea stagnalis)*.

The connective tissue partitions forming the walls of the nests reach only to the basal membrane of the ciliated epithelial cell layer, never going higher among these cells. The end of such a partition may be seen on the righthand side of fig. 3 [plate I].

Between the ciliated cells appear the free ends of the glandular cells, and upon the physiological state of the latter depends to a certain extent the shape of the former cells, which are fairly broad when the inactive stage, and form a more or less uniform layer lining the lumen of the gland, the necks of the glandular cells being then narrow, and scarcely visible. When, however, the gland is in a state of activity, and the glandular cells are filled with secretion ready for expulsion, the necks of these cells dilate considerably, exerting a pressure on the adjacent ciliated cells, as a result of which they extend, becoming slender and elongated, as I have described in a previous paper for similar cells.

In the region where the vas deferens leaves the prostate, the area occupied in a compact mass by the ciliated epithelial cells increases; the openings of the glandular cells disappear, and the lining passes directly into that of the vas deferens. The same may be observed on the free edges of those folds directed towards the lumen of the vas deferens, as is shown schematically in fig. 5, where those portions lined exclusively with ciliated

epithelium are staded. It is obvious that in such places nests of glandular cells cannot lie beneath the epithelium, since there are no openings into the lumen of the gland, and in this region their place is taken by layers, of varions thicknesses, of large,

Fig. 5. Scheme of a cross-section through the prostate of *Radix auricularia*, at the point where the vas deferens issues from the gland.

light-colored plasmatic cells, among which may be found numerous fibres [plate I, fig. 4], similar to those found in molluscs by Brock in his researches on interstitial connective tissue. The question of the origin of these cells would be interesting, as it appears very possible that they are not connective tissue cells, but are homologous to the glandular cells, i. e., that they are of epithelial origin.

CONCLUSIONS.

1) Considerable differences exist in the *Lymnaeidae* family in the formation of the interior folds of the prostate gland. In *Radix* and *Amphipeplea* one single fold exists, whilst in *Galba* several straight untranched folds may be found; in both cases the folds exist only in the widened portion of the gland. Finally in *Lymnaea* the whole interior surface is covered with folds, from which develop numerous secondary folds.

2) The above observatious, amongst others, point to a close relationship between *Radix* and *Amphipeplea*.

3) Generic and specific differences are absent in the arrangement or character of the histological elements.

4) The glandular cells are arranged in groups or „nests", separated from each other by thin layers of connective tissue.

5) The epithelium, lining the lumen of the gland, is ciliated, and rests upon a well-defined basal membrane.

BIBLIOGRAPHY.

1. Baker F. C. Notes on the genitalia of Lymnaea. Amer. Natur. Vol. 39, 1905.
2. — The Lymnaeidae of North and Middle America. Chicago Ac. Sc. Spec. Public, № 3, 1911.
3. Baudelot. Recherches sur l'appareil génital des Mollusques gastérodes. Ann. Sc. Nat. Zool t. 19, 1863.
4. van Baneden. Mémoire sur le Limnaeus glutinosus. Mém. Ac. R Belg.
5. Brock. Untursuchungen über die interstitiellen Bindesubstanzen der Mollusken. Zeitschr. f. wiss. Zool. Bd. 39, 1883.
6. Eisig. Beiträge sur Anatomie und Entwicklungsgeschichte der Geschlechtsorgane von Lymnaeus. Zeitschr. f. wiss. Zool. Bd. 19, 1869.
7. Klotz. Beitrag zur Entwicklungsgeschichte und Anatomie des Geschlechtsapparates von Lymnaeus. Jen. Zeitschr. f. Natur. w. XXIII (N. F. XVI), 1889.
8. Kopystyńska. Beiträge zur Kenntnis der Histologie der Geschlechtsausführungsgänge der Mollusken. Bull. Ac. Sc. Cracovie Ser. B, 1914.
9. Lehmann. Die lebenden Schnecken und Muscheln der Umgegend Stettins. Cassel 1873.
10. Moquin-Tandon. Histoire naturelle des Mollusques terrestres et fluviatiles de France etc. Paris, 1855.
11. — Observations sur les prostates des Gastéropodes androgynes. Journ. d. Conchyl. T. 9, 1861.
12. Prévost. De la génération chez la Limnée: Helix palustris. Mém. Soc. Phys.-Hist. nat. Genève. T. 4, 1828.
13. Roszkowski. Contribution à l'étude des Limnées du lac Léman. Rev. suisse de Zool. Vol. 22, 1914.
14. — Note sur l'appareil génital de Limnaea auricularia et ovata. Zool. Anz. 44, 1914.
15. — Przyczynek do znajomości anatomji narządów płciowych u błotniarek podrodzaju Gulnaria. Spraw. Tow. Nauk. Warsz. VII, 1914.
16. — Z badań nad otułką (Amphipeplea Nills.). Rozprawy Uniwers. Warsz. № 1, 1925.
17. — Contributions to the Study of the Family Lymnaeidae II, III. Annales Zool. Mus. Polon. Hist. Natur. IV, 1926.
18. Roszkowski i Żebrowska. O budowie pochewek prącia u błotniarek. Prace Tow. Nauk. Warsz. Wydz. III, № 9, 1925.

19. Semper C. Beiträge zur Anatomie und Physiologie der Pulmonaten. Zeitschr. f. wiss. Zool. Bd. 8, 1857.
20. Sługocka. Recherches sur l'appareil génital des Gestéropodes pulmonés du genre Physa. Rev. suis. de Zool. 21, 1913.
21. Soós Z. Vizsgálatok a Magyarországi pulmonáták rendszertani anatomiája köréből. (Zur systematischen Anatomie der ungarischen Pulmonaten). Ann. Mus. Nation. Hungar. XV, 1917.

EXPLANATION OF PLATE.

Plate I. All drawings are of *Radix auricularia.*

Fig. 1. Microphotograph, illustrating the „nest" distribution of the glandular cells.

„ 2. Lower, basal portion of the glandular cells, resting on the external wall of the prostate, from which develops a partition, on the left, constituting the wall of the „nest".

„ 3. Upper part of the glandular cells, with the dilated external orifice between epithelial cells.

„ 4. Ciliated epithelium on the free edge of an internal fold, directed towards the point of issue of the vas deferens. Beneath the epithelium are plasmatic cells.

„ 5. Axial basis of a fold, with the wall of a „nest" issueing from it on the left.

STRESZCZENIE.

1) Pomiędzy badanemi rodzajami rodziny *Lymnaeidae* istnieją duże różnice w wykształceniu fałd wewnętrznych gruczołu przyprątnego. U *Radix* i *Amphipeplea* fałda jest pojedyńczą; u *Galba* występuje kilka fałd prostych, pozbawionych sfałdowań wtórnych; u obydwóch grup powyższych fałdy występują tylko w rozszerzonej części końcowej; u *Lymnaea* fałdy istnieją wzdłuż całego gruczołu, przyczem posiadają liczne fałdy wtórne.

2) Fakty powyższe zgodnie z innemi wskazują na bliskie pokrewieństwo między *Radix* i *Amphipeplea.*

3) Brak różnic gatunkowych lub rodzajowych w rozmieszczeniu lub charakterze elementów histologicznych.

4) Komórki gruczołowe są rozmieszczone grupami, „gniazdami", przyczem każde „gniazdo" jest otoczone i oddzielone od sąsiednich przez cienką warstwę tkanki łącznej.

5) Nabłonek wyścielający światło gruczołu jest nabłonkiem migawkowym, opartym podstawą o wyraźną błonę podstawową.

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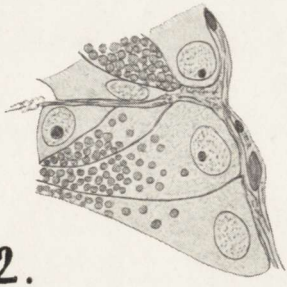

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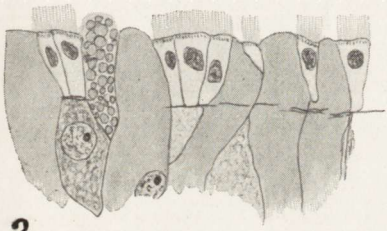

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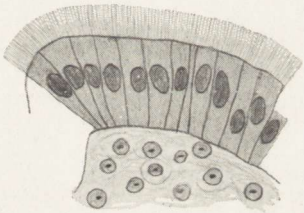

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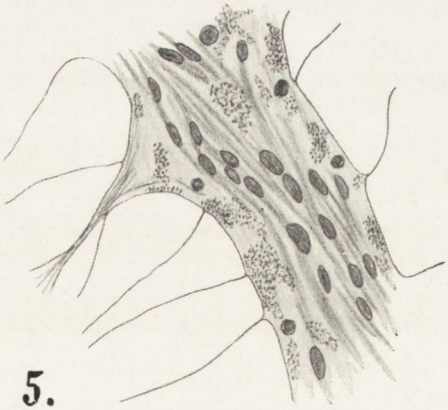

5.

W. Roszkowski.