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Chelonian Brain-Membranes, Brain-Bladder, Metapore and Metaplexus

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CHELONIAN BRAIN-MEMBRANES, BRAIN-BLADDER, METAPORE AND METAPLEXUS[1]

J. P. MUNSON

NINE FIGURES

The brain of turtles is thought to have been first described in 1687, by Caldesi. Since then the following have made contributions of more or less value: Cuvier, 1809; C. G. Carus, '14; Tiedeman, '16; Bojanus, '19; Swan, '35; Grant, '42; Stannius, '56; Agassiz, '57; Owen, '66; Gegenbaur, '70: Stieda, '75; Herrick, '91; A. Meyer, '92; Sorensen, '94; Humphrey, '94; Gage, '95; Voeltzkow, 1903; Banchi, '03.

The literature will be considered when my completed results are published. The items contained in the present paper, and in the two or three on neuro-cytological subjects, that are to follow, have little in common with the results already published by the above authors. None of them have given any attention to speak of, first, to the minute surface details; second, to the cell structure.[2]

Contrary to opinions expressed by some, the chelonian brain is not too small to be studied macroscopically. It is easily removed from the skull. The method used by Gage, of decalcifying the skull, and sectioning the head entire, is not necessary; and for histological and cytological purposes, would seem impracticable.

THE BRAIN MEMBRANES

The dura is easily removed from the skull bones; especially on the dorsum, where it is most essential, if one wishes to study the epiphysis.

[1] A contribution under Grant No. 154 of the Elizabeth Thompson Science Fund.

[2] *Material.* The following species of turtles have been examined in these studies: Chrysemys picta, Clemmys marmorata, Clemmys guttatus, Terrapena Carolina, and the snapping turtle.

If the brain, after removal be preserved for several weeks in Erlicki's solution:

Bichromate potash	2.5	parts
Sulphate of cu	1	part
Water	100	parts

the membranes are excellently preserved. They do not shrink; and they are tough, allowing necessary manipulation.

The dura is a thick and tough membrane, apparently serving as the periosteum of the skull. It has an outer and an inner smooth surface, consisting of closely packed parallel connective tissue fibers. There is no endothelial lining between it and the arachnoid. The central portion of the dura consists of less closely packed, parallel fibers; and it contains numerous lacunae, which give the appearance, in cross-section, of a tendency to split into two membranes, an outer and an inner (fig. 5, *d*).

The dura peels off readily from the arachnoid beneath; and, when removed, the brain presents a smooth appearance, as if there were no connecting trabeculae between these two outer membranes (fig. 5, *s*).

The arachnoid membrane, when preserved as indicated above, can also be removed, as it is very loosely applied over most of the surface (fig. 5, *a*). It peels off as a comparatively thick, tough membrane. The entire capillary system external to the brain is then revealed (figs. 1, 4, *c*).

A few thin connective tissue strands connect the arachnoid with the pia, which closely invests the substance of the cord and brain (fig. 9, *t*).

These trabeculae are most abundant over the optic lobes. Hence their woolly appearance, after removal of the arachnoid (fig. 4, *t*).

The capillaries never come off with the arachnoid, but remain entirely undisturbed. Even in uninjected specimens, the capillary system covering the entire brain can be seen in all its details (fig. 1, 4).

The pia is closely applied to the brain substance; but with this method of preservation, it, too, can be peeled off and examined in toto. On its inner surface (fig. 9, *u*) it consists of parallel

connective tissue fibers, arranged transversely in the form of a membrane.

Scattered over the inner surface of the pia, peculiar cells are sometimes found, which do not appear to be organically connected with the membrane proper. They are distinctly cyanophilous and they multiply by budding.

In sections, the outer layer of the pia is more open, being made of fine fibers forming a close areolar tissue, continuous with the outer layer of the capillaries (fig. 6, *u*). This outer layer of the pia, can be removed in patches together with the capillaries, but it cannot be called a membrane. Under the microscope, it proves to be a very loose network of connective tissue fibers. The latter have a wavy appearance, like the cell outlines in endothelium; and may easily be mistaken for such a membrane, in some places.

The outer layer of the pia resembles the inner layer of the arachnoid so closely, that one is strongly tempted to infer that the pia and arachnoid are originally one membrane, with two compact surfaces, and a loose areolar tissue between, in which the capillaries develop (fig. 9, *v*). Later the original membrane splits; so that the outer lamina becomes practically separated from the inner (fig. 9, *a*, *u*), with which the capillaries are more intimately associated, because of their extension into the brain substance (fig. 5, *g*).

The space between the pia and the arachnoid is, therefore, not a closed serous cavity, lined with endothelium; but, rather, a large open split, between the inner and the outer fibrous surfaces.

Neither in the subarachnoid nor in the subdural space is there any indication of a serous membrane that can account for the arachnoid fluid, as Huxley maintained in regard to the human brain and cord. This becomes of importance in the interpretation of the choroidplexus and the metatelus.

METAPORE

The metapore (fig. 1, *p*) seems to be an object of contention among investigators. It is generally assumed to be an opening through the dorsal wall of the neural tube, communicating with the subarachnoid space. Strangely enough, neither Humphrey

(16) nor Gage (17) found any positive evidence of it in turtles, and have only conjectures to offer, excellent as their work is in all respects. Their method of study, solely by means of sections, was not well adapted to the subject. It is an interesting problem readily solved by the simple method which I have employed.[3]

The substance of the brain has been developed between two membranes—the original ectodermal epithelium of the neural tube, the ependyma, on the inside; and the pia outside (fig. 5, *e*, *r*). In the region of the hypophysis, on the ventral side, and in the roof of the diencephalon, these two membranes remain intimately associated, there being no nerve matter developed between them. As I shall show in my completed work, both the epiphysis and paraphysis, including the dorsal sac, of the diencephalon, consist of these two membranes, as do the epiplexus, the paraplexus and the plexuses of the lateral ventricles.

The roof of the metencephalon is similarly composed of these two layers (fig. 6) with the single exception of an oval area at the posterior angle of the fourth ventricle, just in front of the dorsal fissure of the cord, where this opens up to form the fourth ventricle (fig. 1, *p*). Here the metacoel is covered by one membrane only, the metatelus, an extension of the endyma cells lining the ventricles and the central canal (fig. 5, *e*, *b*).

This oval opening is the metapore (fig. 1, 3, 4, *p*). But there is no direct communication through it, between the central neural canal, ventricle, and the subarachnoid space.

BRAIN-BLADDER

The brain-bladder is a closed sac (fig. 2, *b*), projecting through the metapore in the pia. It is a single layer of cells derived from and continuous with the endyma lining the brain cavities (fig. 5, *b*). The metapore, consequently, corresponds to the duct leading from the neural canal into the brain-bladder (fig. 4, *p*, *b*).

With the exception of this connection with the ventricles and neural canal, the brain-bladder is a closed sac, similar to a real

[3] Mrs. Gage (20) says: "Wilder (21) has demonstrated that in the adult man and certain apes, in the caudal region of the metaplexus, there is a lack of continuity

serous sac, which, as is well known, with one exception—the peritoneum of females—is always a closed sac.

The brain-bladder seems in fact to be the only serous sac connected with the brain, unless the epiphysis, dorsal sac, and the hypophysis with its corresponding sac, saccus vasculosus of teleosts, be so regarded. These, however, are accompanied by the pia, and cannot be said to lie in the arachnoid space.

The brain-bladder lies in the subarachnoid space without adhering to the walls of that cavity as is usual with serous membranes (fig. 5, *b*).

The arachnoid membrane is attached firmly to the pia along a line corresponding to the superficial origin of the cranial nerves, (figs. 2, 5). Above this line it forms a loose bag in which the brain-bladder lies (fig. 5, *a*). Even the cranial cavity itself is enlarged in this region, as if to accommodate the brain-bladder when inflated.

Removing the arachnoid over the anterior part of the cord, and the medulla, the brain-bladder appears as an oval membrane slightly lighter in color than the surrounding tissues (fig. 2, *b*). It extends back over the cord, out on both sides and forward over the metaplexus.

Occasionally it is partly inflated but usually collapsed. The wide space between the arachnoid and the capillaries on the pia affords ample room for it to expand when inflated. When not inflated, it lies flat, not wrinkled or folded. Being so delicate, one is apt to mistake it for a piece of the arachnoid or some other membrane.

If a hypodermic syringe, filled with water (the entire brain being under water), be inserted through the side of the medulla, below the bladder, and water be thus injected into the ventricle, the brain-bladder becomes inflated through the metapore. It then appears as a spherical or oval body standing up well from the brain like a toy balloon or perhaps rather say, a good sized soap

in the endyma and pia, thus placing the cavities of the brain in communication with subarachnoid spaces?" She says of diemyctylus, that "the conclusion that a true metapore exists is unavoidable." There is of course a metapore in the pia but it does not lead directly into the subarachnoid space. What she calls 'endolymphatic sac' in figure 34 is very probably the brain-bladder.

bubble (fig. 4, *b*). It has the glittering appearance of a soap bubble. It is inelastic and does not collapse after inflation.

I have inflated this brain-bladder through the epiphysis, in the perfectly fresh brain. The removal of the arachnoid, so necessary to get a good view of it, is more difficult in the fresh brain. But if the arachnoid is only ruptured, the bladder is sometimes forced through the rupture when inflated in the fresh state.

If pricked with a needle after inflation, a fine stream issues; in some instances, carrying halfway across the table. It is much more easily studied when preserved as directed. Both its transparency and toughness is preserved; and further staining and preparation for the microscope, is easy

There are absolutely no pores in this bladder, leading into the subarachnoid space. That is very evident when it is inflated. If such a pore is what is meant by metapore, the answer must be, there is none.[4] Neither are there any indications of stomata. The pore is only in the pia (fig. 4, *p*).

The appearance of this brain-bladder is not due to the pressure of the injected fluid. Not only can it be seen in the arachnoid space, but it can be removed entire by means of fine scissors. This may be done either before or after inflation. In the latter case, it retains its expanded shape on the slide, where it can be studied microscopically. It is composed of a single layer of very flat hexagonal cells.

The metapore of the pia forms an oval, with the pia forming a thickened rim around the pore (fig. 1, *p*).

In preserved material, the metaplexus can be removed and with it the brain-bladder (fig. 3, *b*).

If the brain is first stained in toto in hematoxylin, before inflation of the bladder, fine permanent preparations can be made.

When removed with the plexus, the thickened rim of the metapore comes away with the brain-bladder (fig. 3, *p*).

The latter thus retains its connection with the metaplexus by a narrow, tough connective tissue band.

[4]The foramen of Magendie is a similar object in the human brain, defined as "an interval in the piamater that roofs the fourth venticle of the brain, affording communication between the subarachnoid space and the ventricular cavities."

In removing the metaplexus, and the brain-bladder, in this way, the pia tears most readily along the line which extends obliquely from the line of attachment of the arachnoid to the anterior border of the metapore (fig. 1, *m*). Hence a part of the roof of the fourth ventricle remains after the removal of the plexus and the bladder. There appears to be a thickened band of connective tissue along this line, which joins the thickened border around the metapore.

The brain-bladder remains inflated after removal (fig. 3). If it be ruptured and spread out on the slide, it cannot be made to lie flat. To get a good view of its cell structure, it must be torn radically. Stained on the slide with hematoxylin, mounted in glycerine or dehydrated, cleared and mounted in balsam, its cell outlines are very clear, when the hardening has been done by means of Erlicki's fluid. Hermann's and Flemming's fluids give poor results, but are better for some purposes.

The cells of the brain-bladder have the outlines of endothelial cells of the serous membranes with straight edges (fig. 8). The cytoplasm is remarkably clear and free from stainable granules. Indeed no other part of the brain shows cells like these; though sections show very distinctly that they are continuous with the endyma cells lining the ventricle and central canal (fig 5, *b*, *e*).

The entire brain-bladder consists of a single layer of flattened endyma cells. Gage (17) figures the corresponding part in birds (sparrow) as being covered with a layer of the dura. A similar mistake accounts for the failure of previous observers to understand it, as the thing has evidently not been properly studied.

METAPLEXUS

The metaplexus forms the roof of the fourth ventricle, anterior to the brain-bladder (fig. 1, *k*). It consists of endyma cells covered externally by the pia and capillary network. It is so folded that the pia and capillaries come to lie between the folds of the endyma epithelium (fig. 6).

Removed entire and viewed from below, the folds are seen to resemble a series of ruffles, converging to the central line, corresponding to the dorsal fissure of the cord (fig. 3, *f*); and, like it,

composed of connective tissue (fig. 3, *j*). The effect could be imitated by a square, arranged in longitudinal, parallel folds; but compressed at one end, corresponding to the handle of a palm-leaf fan. As the folds are wavy, sections rarely show the typical conditions. It can best be understood when viewed as a whole from the underside.

There are good reasons for comparing this with the dorsal sac of the paraphysis, the brain-bladder being compared to the epiphysis. The band passing transversely between the brain-bladder and the metaplexus would correspond, in position, to the supra-commissure in the diencephalon. But unlike this, the band seems to be wholly connective tissue, belonging to the pia; and no real commissure of nerve fibers exists here. When this band is cut, the walls of the fourth ventricle separate, revealing the fissures and ridges in the floor of the ventricle, arising from the fiber tracts of the cord continued in the medulla (fig. 1, *k*).

GENERAL CONSIDERATIONS

There may possibly be some objectors to the term, brain-bladder, as here used. The term 'sac is certainly overcharged as a name for brain structures. 'Dorsal sac' has been used by writers on the chelonian brain, following Humphrey, to designate the thin walled expansion of the roof of the diencephalon in front of the supra-commissure, and supposed to be associated with the paraphysis, but really the paraphysis itself. To Latinize the word 'sac' would not help matters. 'Tela' is used to designate the thin roof of the neural tube, and does not denote a sac necessarily.

From the morphological point of view, and possibly from the functional side, the name bladder seems appropriate, when its derivation is considered. It is also specific.

Some idea of the function of organs can be gained from their structure, and their relation to other parts. Why not in this case also?

The haemal tube is lined with serous membrane, which lessens friction, by the smoothness of opposing surfaces; and by the fluid produced, keeping them moist.

It is known, also, that the fluid secreted by such membranes have a phagocytic or toxic effect on foreign cells. There are many such cells in the brain of turtles.

From the arrangement of the capillaries, and their relation to the endyma cells, inside the plexus, it is suggested that lymph from the blood oozes through the endyma cells into the ventricles. The brain-bladder is admirably arranged to allow the pressure between the ventricular and the subarachnoid fluid to be equalized (fig. 7, fig. 5). It may also allow lymph to filter through from either space as the pressure becomes unequal.

The nuclei of the bladder cells certainly show that they are not idle; while no indications of solid deposits in the cytoplasm appear. The nuclei may produce substances, antibodies, which when passed out with the lymph, passing through the cytoplasm, has a phagocytic action like that of the serum from the body cavity.

As already stated, the brain-bladder is the only closed sac in the neural tube that can be directly compared to that lining the body cavity, or the synovial sacs between joints.

That it varies with the pressure of the fluids within the ventricles and the central canal is sufficiently demonstrated by its inflation through the epiphysis as well as through the hypophysis.

It would be strange if such an apparatus as the plexus, so admirably arranged for exposing as much surface as possible, like so many gills (fig. 3, *j*) or lungs, were of no physiological importance.

The brain bladder appears to be prominent even in the human brain in its earlier stages. It remains to be seen whether it is not fully as prominent in the developed brain. It may be obscured by the crowding of nerve elements; so that, with the methods of study employed, it has been overlooked; as it certainly has, in the adult turtle's brain. It is prominent in birds, though it has been misinterpreted; and has not received the attention it deserves. Such a thing must be seen entire, not merely in sections.

If the method of preserving, exposing, and inflating, described above, be adopted, any one can see the brain-bladder (fig. 4, *b*). He will doubtless feel amply repaid for his trouble. A good view of this thing will, perhaps, make it as difficult for him as for me, to believe that it can be a mere useless embryonic vestige.

At any rate, it is to be hoped that those engaged in similar studies, on more developed brains than those of reptiles, will give more attention to this than has hitherto been the case.

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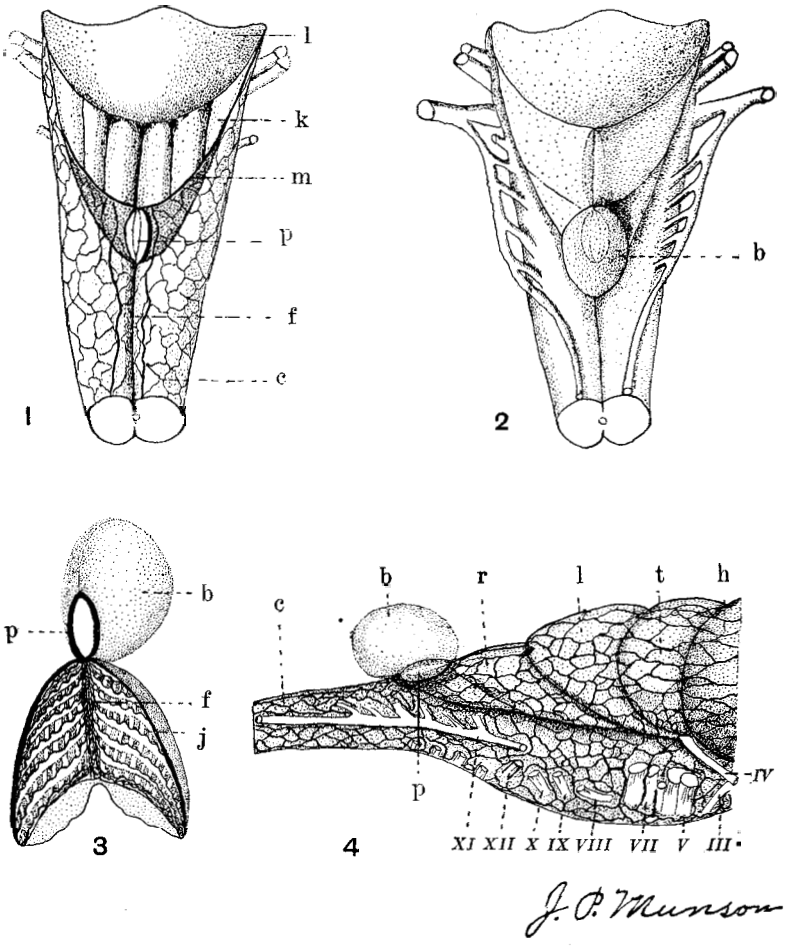


Fig. 1 Dorsal view of cord and metencephalon of the tortoise, Clemmys marmorata, with the dura and arachnoid removed, showing capillaries, *c*; dorsal fissure, *f*; metapore, *p*, after removal of the brain-bladder; the line along which the metatelus tears in removing the metaplexus, *m*; the sulci and ridges in the floor of the fourth ventricle, *k*; the cerebellum *l*.

Fig. 2 Dorsal view of metencephalon after removal of the two outer membranes, showing roof of fourth ventricle with the brain-bladder, *b*.

Fig. 3 Ventral view of the roof of fourth ventricle, showing the inflated brain-bladder, *b*; the thickened rim surrounding the metapore, *p*; the central septum corresponding to the dorsal fissure of the cord, *f*; the ruffle-like folds on under side of the metaplexus, *j*. Imagine this turned over and fitted onto figure 1 as the roof of the fourth ventricle.

Fig. 4. Side view of brain of clemmys, showing capillaries after removal of the dura and arachnoid, *c*; the cranial nerves III–XII; cerebral hemisphere, *h*; optic lobe, *t*; cerebellum, *l*; the roof of fourth ventricle, *r*, and the inflated brain-bladder, *b*, above the metapore, *p*.

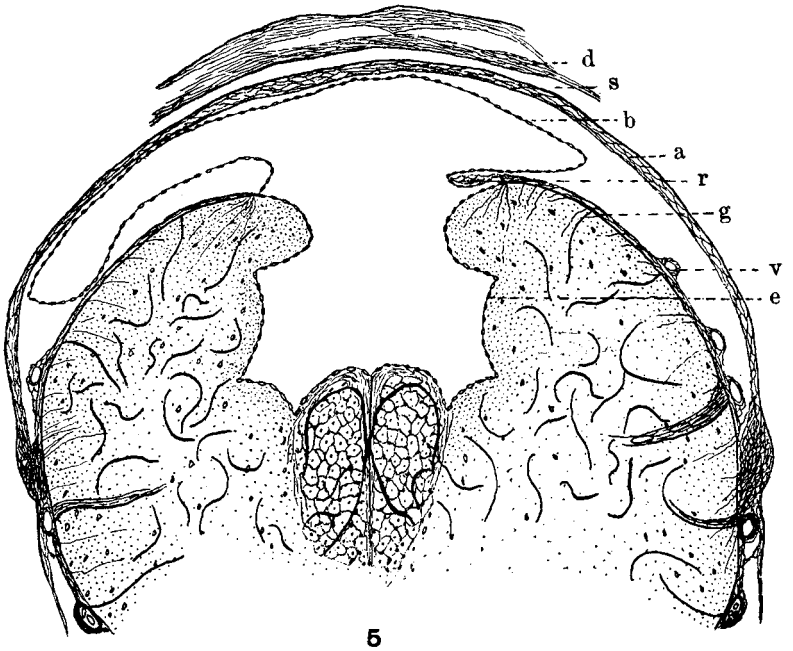
Fig. 5. Section through the metencephalon, in region of metapore, and showing section of brain-bladder, *b*, and its continuation with the endymal lining of ventricle, *e*; the subarachnoid space in which the brain-bladder lies and the connection of the arachnoid on either side with the pia; the connection of the capillaries, *v*, with the pia and their extension into the nerve substance, *c*; the subdural space, *s*, and the two smooth surfaces of the dura, *d*; the sulci and ridges seen in figure 1, *k*, also shown in section with the large ventral motor tracts on either side of the ventral fissure, showing the ventricle to be merely the expanded central canal.

Fig. 6 Transverse section of three folds of the metaplexus, showing the process of folding, *j*; the ependymal membrane, *e*; the pia, *u*; and the capillary network, *v*. Compare *e* and *g*, to note the difference in cells when compressed by lateral pressure in the plexus, or by lateral pull in the brain-bladder, *g*.

Fig. 7 Longitudinal section, showing substance of medulla below; and above it, the cavity of the fourth ventricle; covered above by cerebellum, showing Purkinge cells, *w*; metaplexus, *m*; brain-bladder, *b*; dorsal fissure, *i*; optic lobe, *o*.

Fig. 8 Surface of view of brain-bladder, with high magnifying powers, showing cell outlines, and their different kinds of nuclei.

Fig. 9 Brain membranes, showing tendency to split. Inner and outer layer of dura, *o*, *i*; subdural space; and below the two layers of connective tissue membranes, the outer or arachnoid, *a*; the inner pia, *u*; the connection between them by trabeculae, *t*, traversing the subarachnoid space, *x*; suggesting their original union into a single membrane; the more intimate connection of the capillary network with the deeper layer or pia, *v*; subpial surface of transverse connective tissue fibers, *u*.



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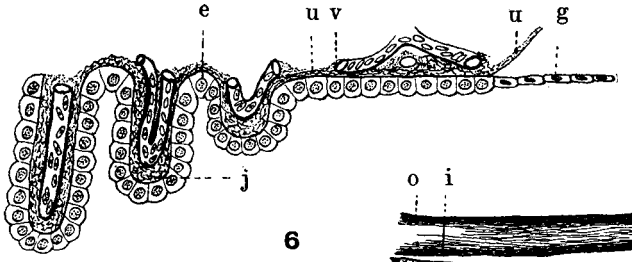

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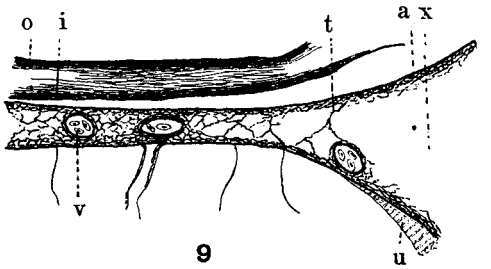

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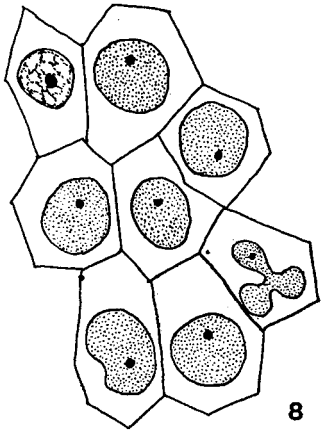

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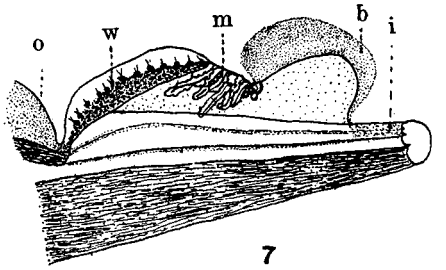

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