# NOTE TO USERS

# The Histogenesis of Cysticercus pisiformis.

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## 1. Introduction.

In order to obtain a true insight into the structure of the Cestodes, which, while apparently complex, is actually simple, and to find the correct answer to those questions concerning their organi-

zation, which have been so warmly disputed in the past, it is necessary, not merely to study in detail many and varied adult forms, but also to make an exhaustive study of the development of the larva in its finer details. Thus far no thoro investigation has been made along this line, tho there are a few papers dealing with the histogenesis of different organs in the adult tapeworm, and some histogenetic details have appeared incidentally in various articles concerned primarily with other lines of investigation, viz.: BUGGE (1902), LUNGWITZ (1895) and SCHMIDT (1888). From my study of a considerable number of larvae in all stages of development I hope to throw some light on this hitherto neglected subject.

It may as well be stated at the outset that, wherever there has been occasion in what follows to dispute the statements of other authors, this is always done with the reservation that such objections apply only to Cysticercus pisiformis, unless the contrary is expressly stated. While it is very probable that the tissues of this bladder worm agree closely with those of other Cysticerci and Taeniae still no such comprehensive statement can be made.

## 2. Materials and Methods.

Cysticercus pisiformis occurs in great numbers in the cottontail rabbit (*Lepus mearnsi*) in the vicinity of Lincoln. Of 50 rabbits examined, 34 were infected. In 21 infected rabbits the total number of larvae present was 227, making an average of 10,8 larvae to each rabbit.[1]) The older larvae occur almost exclusively in the body cavity and chiefly along the rectum, just dorsal to the symphysis pubis, occasionally free, but mainly enclosed in connective tissue cysts, which usually contain only a single larva, but occasionally two or even three, while SCHAAF (1905) states that he has found 18 larvae of Cysticercus pisiformis in one cyst.

In addition to those larvae obtained from market rabbits, all of which were in well advanced stages of development, a large number of larvae in the earlier stages of development were obtained by means of feeding experiments. In each of these the proglottids of *Taenia serrata* were used.

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1) It is possible that in some of these rabbits there were very small larvae present and uncounted in some of the organs. There was no evidence of this, however.

In the first two full grown rabbits were used with entirely unsatisfactory results. One of these rabbits contained no traces of immediate infection with Cysticercus pisiformis. The other rabbit contained 11 well grown Cysticercus pisiformis, but since the liver was healthy at the time of dissection it is highly probable that these larvae were not the result of the experimental feeding.

A second experiment performed on an adult cottontail rabbit was equally unsuccessful. But experiments performed on young Belgian hares (*Lepus cuniculus*) and on a young *Lepus pinetis* were in most cases productive of excellent results.

These results however differed widely in different experiments, conducted under the same conditions and with rabbits of the same age and species. The reason for this must be supposed to lie in the individuality of different rabbits. The failure of the first two experiments may be attributed to the fact that they were performed on full-grown rabbits, which are probably less open to infection than young rabbits. Vogel (1888) believed that a previous infection with *C. fasciolaris* rendered mice to a certain extent immune to successive infection. In my own experiments, however, such an hypothesis is open to grave doubt since I have found hundreds of larvae of different ages in a single specimen. Hofmann (1901) and Bartels (1902) both found difficulty in infecting adult mice with *C. fasciolaris*, but Hofmann showed that the age was a factor of decided importance, young mice being more easily infected than old. My experiments support this view, but they further tend to show that, as just stated, the difference in constitution of different individuals is also an important factor.[1])

A careful study was made by means of sections of the following organs and tissues of two of the infected rabbits: ovary, Fallopian tube, brain, spinal cord, sub-cutaneous connective tissue, voluntary muscles, heart, thymus, lungs, large artery, portal vein, inferior vena cava, pancreas, great omentum, liver, spleen, kidney, testis, gall bladder, urinary bladder, stomach, small and large intestine; and the following were found infected: liver, omentum, lungs, lymph glands in the mesentery and pancreas. In the latter organ no larvae

1) It is possible also that the age of the proglottids employed, and the period intervening between the time at which they were voided by the dog and that at which they were fed to the rabbit, may have had an effect upon the results.

were found, but a few tracks were present similar to those made by the larvae in the liver.

A large number of methods of fixation and staining were tried, but only a few found to give satisfactory results. By far the best fixative employed was FLEMMING's strong chrom-aceto-osmic mixture. The larvae were fixed in this for a period of two to three hours, washed in running water for an hour and carried up thru the alcohols as usual. The next best fixative employed was a concentrated solution of corrosive sublimate in 70 per cent alcohol with an addition of one per cent glacial acetic acid. Use of this fixative for an hour, with subsequent washing out in 70 per cent alcohol to which was added a few drops of iodine solution, gave good results. HEIDENHAIN's iron alum haematoxylin, sometimes employed with no counter-stain, but more often counter-stained with eosin, Bordeaux red, or saturated aqueous solution of wasserblau and picric acid is the stain which gave the best results. VOM RATH's solution followed by pyroligneous acid, as recommended by TOWER (1896) was thoroughly tried and some good preparations of nerves and parenchyma obtained by it, but in general the results from this method are not as satisfactory as those from HEIDENHAIN's haematoxylin. APATHY's foregilding with gold chloride, GOLGI's rapid method and the methylen-blue method were all tried. The first method proved an utter failure, the second gave some good impregnations of the excretory ducts, while with the last some good stains of "myoblasts" and their connected muscle fibres were obtained. None of them, however, gave a satisfactory preparation of the nervous system or sense cells.

## 3. **Histogenesis.**

### a) Earlier Stages of Development.

The larvae occur in the liver usually in groups of three or four enclosed in fibrous cysts composed of modified liver tissue. Tho many small larvae have been examined, some of which did not exceed 40 $\mu$ in diameter, none have yet been found on which the embryonal hooks were present. The larvae are at first usually collected in groups of a few to every cyst. As they leave the cysts to wander out of the liver, they are found singly. In the earliest stages observed in the liver they consist of a simple mass of loose

parenchyma and are without a cuticula, at least the existence thereof is not demonstrable at this time. They are more or less elongated sacs containing a cavity the enlargement of which keeps pace with their growth.

These consist of a syncytium of loose parenchyma strands with a few scattered nuclei. Such a larva is illustrated in Fig. 14. The larva has been cut tangentially at one end, while the plane of section is median at the other; thus at one end the wall of the larva is seen in surface view, and in section at the other. The interior of the sac contains a cavity traversed in all directions by slender, very irregular, anastomosing strands of parenchyma, forming a network in which are supported the few nuclei. These strands are of a looser texture than in the fully developed larva and carry scattered masses of protoplasm which vary considerably in size giving the strands a coarsely granular appearance. The strands themselves, independently of the adherent protoplasmic masses, vary in size and are composed of the finest fibrillae visible under a magnification of 2850 diameters. Here and there may be seen darkly stained granules scattered thru the protoplasm which are developing chromatin granules. A discussion of the origin of the chromatin is given under Cytogenesis. Whether the fibrillae represent the ultimate units of the parenchyma, and whether they are in protoplasmic continuity thruout cannot be determined. It seems probable, however, that this is so. That the fibrillae are primitive and the protoplasmic masses derived is evidenced by the fact that the bladder is originally composed of fibrillae, the granular protoplasm appearing secondarily.

The structure of the nuclei is essentially the same as that of the cytoplasm, but discussion of them will be reserved for a later part of this paper. It may be noted here, however, that they present considerable variation in staining properties, size and number of chromatin granules.

Thus the cavity of the bladder is broken up into many irregular spaces which communicate freely with one another between the parenchyma strands. At this stage, therefore, excretion must take place by direct osmosis between the cells and the bladder cavity; if, indeed, some of the excreta are not passed outward into the liver. A portion of the cavity is not traversed by parenchyma strands. At no stage of development have I found a distinct mem-

brane bounding the latter, the existence of which has been claimed by some authors and denied by others.

It may be well at this point to define the term "cell" as used in this paper. By "cell" is meant a nucleus with the protoplasm immediately surrounding it; since the tissues are syncytial in character, adjoining cells can never be definitely delimited from one another.

Regarding the development of the bladder cavity, I cannot fully agree with LEUCKART (1879—86: 434) who says, ". . . diese Finne . . . erst etwa in der vierten Woche, wenn der Wurm bereits eine Länge von 4 bis 5 mm besitzt, in einen eigentlichen Blasenwurm sich verwandelt. Bis dahin ist dieselbe völlig parenchymatös, im Innern mit einem grossblasigen Schleimgewebe gefüllt, das kaum irgendwo scharf gegen die Rindenschicht sich absetzt und auch gleich dieser von Muskelfasern in verschiedener Richtung durchzogen wird."

Cysticercus pisiformis is a bladder worm from the earliest stages of its development in the liver of the rabbit up to the time of its metamorphosis in the intestinal tract of the dog. A bladder cavity is present from the first; its appearance is not delayed till the fourth week of development; and LEUCKART's „grossblasiges Schleimgewebe" is nothing more nor less than this same bladder cavity traversed by a few sparse strands of parenchyma. The extent to which the bladder cavity is traversed by these strands of parenchyma varies in different larvae and in different parts of the same larva; even in the old bladder-worm a few may be found crossing the cavity. Thus the bladder cavity is not separated by a definite membrane from the parenchyma wall, as is claimed by SCHAAF (1905), but is always in communication with the parenchyma spaces of the latter and these on their part communicate with one another.

To return now to the consideration of Fig. 14: the wall is composed of a close interlacing of parenchyma strands running in diverse directions. At this stage, the presence of a cuticula cannot be demonstrated. It seems most likely that a cuticula has not yet been developed. Studied under a 2850 magnification, the bladder wall shows no continuous membrane surrounding it, but a succession of irregular spaces communicating with the bladder cavity on the one hand and the exterior on the other (Fig. 7). A somewhat older larva enclosed in its cyst is shown in Fig. 1. Here the principal points to notice are the enlargement of the bladder cavity relative

to the thickness of the bladder wall, the increase of nuclei in the latter and the grouping of cells at one end of the larva, representing the rudiment of the future head piece. In Fig. 15, a small part of this rudiment is represented on a larger scale and in Fig. 16 a part of the bladder wall of the same larva. The nuclei are closely grouped in a syncytium of parenchyma strands, whose general alignment is perpendicular to the bladder wall. The structure of the parenchyma and its nuclei is essentially the same as in the preceding stage. There is a very rapid nuclear multiplication taking place especially in the head piece rudiment and a great variation in size of nuclei is apparent. The granular protoplasm, noted in the preceding stage as distributed along the strands of the parenchyma network, is increasing in amount and gathering itself into small irregular masses at scattered intervals (*mcy*, Figs. 15, 16). The formation of a cuticula is not yet apparent altho the fibres of the outer wall appear to be scaling off, which process continues to occur during cuticular development as will be noted later. The outer wall of the larva is differentiated from the underlying parenchyma by the arrangement of its fibres in two sheets which pass at right angles to each other, the rudiments of the sub-cuticular muscle layers (*om* and *im*, Fig. 15). While the anterior end of the larva assumes the histological structure of the adult tapeworm, the bladder wall retains its embryonic character, with increasingly less differentiation toward the posterior, and this is true even in old larvae.

It will aid the reader to appreciate the development of the tissues if he bear in mind thruout the words of Moniez (1881: 212): "Nous pouvons nous expliquer maintenant l'origine et la structure des appareils plongés au sein de ce tissu homogène. Nous croyons qu'ils naissent tous aux depens des cellules (du tissu conjonctif) dont nous venons de parler."

### b) Parenchyma and Chalk Bodies.

The development of the parenchyma beyond the earlier stages presents nothing of striking interest. The fibres become more dense and assume sharper outlines by loss of the granular protoplasm adherent to them in the earlier stages, which is gradually consumed in the manufacture of new tissues. Thus is established a firm, elastic, supporting framework for the body, which interweaves itself among the other tissues in the most intimate manner and forms, as will be seen later, the groundwork of the cuticula itself.

Regarding the question at issue as to the existence of a between- or ground-substance in the parenchyma, I take the side of those who deny it. In both embryonic and adult condition the branching processes of the parenchyma cells may be followed continuously into the finest fibrillae. Any differentiation of cells and ground substance is an untenable view.

Basement Membrane. During the development of the cuticula, this remains as a narrow zone of undifferentiated parenchyma strands between the outer ring muscles and the base of the cuticula. In many places it appears to be absent, the muscles abutting directly against the base of the cuticula. It is probable, however, that there are always a few very fine fibrillae separating the two. It is but feebly developed in the young proglottids of the adult worm, gaining its full development only in the mature proglottids. There is thus a separation established here between the outer ring muscles and the cuticula.

Chalk Bodies. Four different views have been expressed as to the origin of the chalk bodies. By most writers RINDFLEISCH (1865), SOMMER & LANDOIS (1872), SALENSKY (1874), SCHIEFFERDECKER (1874), MONIEZ (1881) and others, they have been considered parenchyma cells metamorphosed by the deposition of calcium carbonate and other salts in the cytoplasm around the nucleus. SALENSKY (1874), however, thinks that they may occasionally burst the enclosing cell wall and thus come to lie in an inter-cellular space in the parenchyma. A similar tho somewhat modified view is that of BLOCHMANN (1896) and SCHNEIDER (1902), who conceived a similar relation to exist in these bodies as exists in fat cells, the calcareous concretion being deposited in the cell, but the nucleus being forced to the periphery and there forming a slight protrusion beyond the outline of the concretion itself (see BLOCHMANN's tab. 1 and tab. 2, fig. 2 and 5); while the third view is that of LEUCKART (1879—86) who, basing his opinion on the work of HARTING (1873) in the experimental formation of similar bodies with calcium carbonate and egg albumen, reached the conclusion that they developed as an inter-cellular deposit. VIRCHOW (1857) considered their formation analogous to that of bone by the deposition of $CaCO_3$ in a connective tissue stroma. The exact relation between the cell and the calcareous concretion is not made clear by him however.

In my own preparations, owing to the presence of acid in all my fixatives, I have not obtained the chalk bodies in normal condition at any stage. The places formerly occupied by them appear as oval spaces varying from those which are greatly flattened to those which are round or nearly so and usually — perhaps always, but this cannot be determined with certainty — containing a remnant of the concretion.[1]) These spaces vary considerably in size and form due partly to the stage of development of the concretion and partly to the state of contraction of the worm when killed. They appear during the early development of the head piece and soon become very numerous in the neck region of the larva, while in the bladder wall they are scarce or wanting.

In places where the tissue is compact and the concretions numerous, it is difficult to find any relation between the latter and the nuclei of the parenchyma; the existence of a definite nucleus to each concretion is impossible to determine. But where the parenchyma is loose and the concretions few, it is frequently possible to find a nucleus in relation with each concretion cavity. As is clearly shown by BLOCHMANN (1896, fig. 5, tab. 2), the cell body is in no way in connection with the concretion remnant, but surrounds the latter like a sac. My own observations, however, have shown that, as is suggested by BLOCHMANN's fig. 2, tab. 2, the cell of the chalk body is nothing more nor less than an ordinary parenchyma cell belonging to the parenchyma network immediately surrounding the space occupied by the concretion. This is confirmed by some sections of *Taenia serrata*, in which the concretions were comparatively little injured by the fixative, where the same relation has been found (see Fig. 3). I therefore incline strongly to the view of LEUCKART (1879—86), that the chalk body is formed as an intercellular deposit, having no direct connection with any cell. As further evidence in support of this view, the reader is referred to Fig. 2 in which is shown a chalk body with two parenchyma cells in close apposition therewith. Now, if the concretion has been deposited within one of these, what is there to show to which one it belongs?

1) The precise nature of this remnant is not known. GRIESBACH (1883) assumed it to be of an organic nature, perhaps a double salt of calcium carbonate and albuminate.

### c) Cuticula and Hooks.

The formation of the cuticula occurs simultaneously over the entire bladder before the invagination of the head cavity commences. In accordance with the development of the larva, the cuticula is soon found in a more advanced stage in the head and neck, while in the bladder it occurs in an embryonic state. In a larva in which the invagination of the scolex cavity has just commenced, the cuticula is already well differentiated as a layer of nearly uniform thickness and structure over the entire surface of the worm, except in the head cavity where it is thicker and of a much looser structure. Beneath the cuticula lie the transverse and longitudinal fibres of the sub-cuticular muscles, as well as parenchyma fibres passing outward perpendicular to the surface. The latter are the rudiments of the outer processes of the future sub-cuticular cells and the parenchyma fibres lying between them. Careful observation with high magnification shows that these fibres extend into and form the groundwork of the cuticula, in which they divide into fibrillae which run in diverse directions, as described by SCHNEIDER (1902). Between these fibrillae is a homogeneous, translucent cement substance laid down by them. From the visible continuity evident at this early stage between the processes of the future sub-cuticular cells and the groundwork of the cuticula, a ready explanation is found for this connection as it exists in the adult. Soon after the first appearance of the cuticula, it begins to scale off on the surface and is continually renewed at its base. There are no marked periods of ecdysis, but rather a continual shedding of the cuticula, which may be seen partly filling the head cavity at about the time the hooks begin to appear.[1])

The pieces of shed cuticula present a granular and vacuolated appearance and this appearance is often visible in the cuticula when still attached to the larva. The second stage in the development of the cuticula is marked by the differentiation of an outer and an inner layer. The relative thickness of these two layers varies at first, but in later stages the outer becomes much thinner and may even be entirely lost. The inner layer alone contains the cement substance of the cuticula. The fibrillae which make up the ground work of the inner layer are continuous with the fibrillae or "hairs"

1) An active ecdysis is not seen anywhere except in the head cavity.

of the outer layer. Thus it is highly probable that a continuity exists between the fibres of the parenchyma and those of the outer or "hair" layer of the cuticula. This stage is represented in Fig. 17, where the two layers and the continuity of their fibres are clearly shown between the developing hooks. The continuity of the parenchyma fibres with the cuticular groundwork is also shown. A further discussion of the "hair" layer will be taken up in a study of the hooks. The further development of the cuticula consists in the increase in thickness of the inner layer and decrease, or in some places complete loss, of the outer layer. The former assumes a more homogeneous appearance due, not to the loss of its fibrillar nature, since this may be demonstrated in favorable places in the head and neck region of adult larvae, but rather to the more intimate binding together of the fibrillae by the inlaid cement substance. The cuticula in the head and neck region of the larva is markedly thicker than in the same regions of the adult, due probably to the contraction of these parts in the former.

From the preceding study the following conclusions may be drawn: The cuticula of Cysticercus pisiformis is developed from a groundwork of simple parenchyma fibrillae by a deposition among them of a cement substance. There are no specialized fibrillae or cellular processes concerned in its development. The fact that in its development the processes of the sub-cuticular cells take part, does not in any way detract from the above statement, since primitively the sub-cuticular cells themselves are undifferentiated parenchyma cells.

A detailed discussion of the various theories relative to the origin and homology of the cuticula will not be undertaken in this paper. Hitherto, the actual development of the cuticula seems to have escaped observation. The views regarding its structure are as varied as those regarding its origin. Two to four layers have been assigned to it by various authors, some of whom have included the basement membrane in the cuticula. By most authors it has been described as perforated by numerous fine pores, the pore canals, which have been supposed to contain processes from the sub-cuticular cells. By others, the existence of these pores has been denied. Some authors have seen the sub-cuticlar cell processes terminating in the basal portion of the cuticula, which they have described as a differentiated layer for this reason. A number of interesting

structures different from those mentioned elsewhere have been described by PINTNER (1903) in the Tetrarhynchidae but a discussion of these lies outside the scope of the present work. ZSCHOKKE (1888) in his studies of several species of the Taeniadae and Tetrabothridae has described the cuticula as varying in different species in the same family.

I wish to consider briefly the description of the cuticula given recently by MINCKERT (1905) in the larvae of *Ligula intestinalis*, *Schistocephalus nodosus* and the adult *Moniezia expansa* and *Triaenophorus nodulosus*. This author in the figure accompanying his article depicts the cuticula as consisting of three layers, an outer or "Comidienschicht", a middle or "homogene Schicht" and a basal layer of "direkt über den Insertionsstellen der Epithelzellfortsätze stehenden Grundstreifen". The outer layer is figured as equaling in thickness the middle, while the third layer is very thin. He says (p. 405) that "Ein besonderer Zusammenhang der Comidien mit den Epithelzellen . . . existiert nicht". In the "homogene Schicht" he describes two kinds of canals, "Trophoporen", which at base divide to form several "Trophoporellen", and "Neuroporen" connecting basally with "Neurophysen", which are synonymous with the endings of the sense cells of other authors. The former he considers synonymous with the pore canals of former writers.

In my own preparations I have frequently encountered cavities of varying shape and size in the cuticula [1]), but the fact that they have no regular arrangement whatsoever, and that in favorable places in the cuticula of adult Cysticercus pisiformis I find, the same as in younger stages, a continuous network of fibrillae, inclines me strongly to the belief that these are merely artifacts and that in this larva at least there are normally no openings in the cuticula whatever. The erratic nature of the GOLGI stain should make us wary of accepting results obtained by its use, until the same have been verified by many observers.

While I am unable to demonstrate an indisputable connection between the elements of the "hair" layer and the sub-cuticular cell processes, the developmental history of the cuticula as described above makes such a connection highly probable. In many preparations there is a more deeply stained layer at the base of the cuticula. I find, however, that this layer varies in thickness in

1) Especially is this true of the cuticula of the adult worm.