On the Rectal Tracheal Gills of a Libellulid-Nymph and Their Fate during the Course of Metamorphosis.

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With Plate III, IV and a Textfigure.

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I. Introduction.

After the publication of Palmen's work [1]) on the tracheal system in dragonfly-nymphs, Hagen has come to an opinion adverse to that of Palmen concerning the fate of the rectal tracheal gills of the insects in question. Prof. Hagen says: „Dr. Palmen's Angabe, daß das Gewebe der

[1]) Palmen: Zur Morphologie des Tracheensystems. 1877.

Tracheenkiemen des Afterdarms nicht abgeworfen wird, sondern in der Imago verbleibt, ist in dieser Allgemeinheit unrichtig. Bei *Epitheca bimaculata* aus Europa und bei *E. princeps* aus Amerika bleibt stets der ganze Apparat in der Exuvienhaut und geht nicht in die Imago über. Ich meine mit „stets" natürlich nur die Zahl der mir vorliegenden Häute, bei der ersten Art 24, bei der zweiten über 100. Further: „Durch Aufweichen habe ich deutlich nachweisen können, daß die ganzen Kiemen nebst der sie innen bedeckenden Membran völlig erhalten in der Exuvie zurückbleiben, und ein im Ausschlüpfen begriffenes Stück zeigt, daß sie aus dem Leibe der Imago herausgezogen werden und der Darm darüber getrennt wird". [1])

Observing the exuviae of a libellulid-nymph with special attention, one will always find the remnant of a semitransparent body attached to the anus at its one end. Further if the exuviae be that of the last moult, with the aid of a microscope, one will be able to detect without difficulty groups of large cells which one could readily suppose to be the hypodermic epithelial tissue building up the underlining wall of cuticle of the gills. Thus Hagen's view is appearently verified. Shortly after his publication concerning this problem he abandoned his former view that the gills in dragonfly-nymphs are never transferred into the adult, but remain in the exuviae, since he had met with an exception in *Euphaea*, observing the nymphal side gills that still exist in the stage of imago [2]). But the side gills of this dragonfly are very different in nature from the rectal gills of other libellulids, so that we must exclude the fact of continuance of the side gills from our consideration of the rectal gills in general. More recently, in 1905, Scott ascertained again that the nymphal rectal gills are not present in imago of *Plathemis lydia*. [3])

Now the question is: whether the rectal tracheal gills are transferred into the adult body or are completely free from the imago, remaining in the exuviae when the last moult ends. If Palmen's view be correct what is the fate of these useless organs for aerial life? Or if we take Hagen's opinion as correct in general, there arises a question; what are the changes undergone during the ecdysis in the alimentary canal of this kind of insect? The following study is undertaken to determine the truth of the matter.

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[1]) Hagen: Zool. Anz. III, 1880, p. 159.

[2]) Hagen: Zool. Anz. III. 1880, p. 304; Korschelt und Heider: Vergl. Embryol. I. 1890, p. 850; Deegener: Handb. Entom. von Schröder 1913, p. 348.

[3]) Deegener: loc. cit. 1913, p. 347; the original paper of Scott was not accessible to me.

II. Material and Methods.

I took *Libellula 4-maculata* as the material of the present study, as it is easily obtainable in the pools around Sapporo and it has the rectal gills well developed. To fix the material the following mixture[1]) was most successful.

1. Alcohol absolutus 6 vol.
   Chloroform 1 vol.
   40% Formol 1 vol.
   Glacial acetic acid 0.5 vol.

2. Picric acid, 1% against the total mixture 1. After immersion for 24 hours or more, the samples were taken out and washed with 90% alcohol, then preserved in alcohol of the same grade. Beside this fluid the following mixture[2]) was also found good.

Alcohol absolutus	1 vol.
Aqua destillata	30 vol.
40% Formol	6 vol.
Glacial acetic acid	3 vol.

The samples fixed with this fluid were washed several times with 70% alcohol and preserved in 90% alcohol.

Both paraffin and celloidin were used in imbedding; and the double staining was done either with Delafield's hämatoxylin and eosin or with the former and Lichtgrün, both of which brought good results.

III. The Rectal Tracheal Gills in the Last Nymphal Stage.

Relation between the Alimentary Canal and the Tracheal System.

Concerning the anatomy of the alimentary canal in the nymphal stage of dragonflies extensive studies have been made by several authors, of these I regard the work by Sadones[3]) as the most painstaking. His material was *Libellula depressa* from Europe as well as an undetermined species of Aeschna. The material used by Sadones and by me show, as a whole, a similar structure, although the both are a little different from each other in the minute points in the tracheal gills of rectum as mentioned below.

In this stage of nymphal life are to be seen three principal divisions of the whole canal wich are subdivided distinctly as follows.

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[1]) W. Docters van Leeuwen regarded this mixture as the best for fixation of insect-larvae (Zool. Anz. Bd. 32. 1908. p. 316-318).

[2]) This mixture is recommended by Bedau in his research on the compound eyes of water bugs (Zeitschr. f. Wiss. Zool. Bd. XLIX. 1911 p. 418).

[3]) Sadones: La Cellule, Tome XI. 1896. pp. 274-324.

1. Stomodaeum (Fore-intestine) { Buccal intestine. Oesophagus. Crop. Proventriculus.
2. Mesenteron (Mid-intestine) — Stomach or Ventriculus.
3. Proctodaeum (Hind-intestine) { Small intestine or Ileum. Rectum. Anal piece.

The rectum is converted into so-called rectal tracheal gills in almost its whole length, except the anal piece, the hind most short portion, just before the anus, and swells throughout the canal to the stoutest appearance. There is on the dorsal surface of the stomach a pair of trachii of remarkable size, called dorsal trunks; their peripheral cell contain rich pigment granules of brownish color. The diameter of each trunk varies according to portion: at the anterior half of the rectum it attains the largest size and therefrom gives off, in both sides, many parallel branchlets to the rectum. They enter into the rectal papillae or evaginations of rectall wall and form the rectal gills. The hinder part of this trunk again becomes slender, and its extremity extends into the last abdominal blind spiracle on eighth segment. Besides these dorsal trunks another pair of finer trunks, containing no pigments in the wall, is found along the sides of the stomach, in very close contact with it and gives off minute branches to the stomach, while the posterior ends are splited into tufts which also enter the rectum from its ventral side. This second pair of trachii are called visceral trunks; at the anterior part of the stomach they bend upwards, and after crossing each other like the letter x over the stomach, each of them connects with the dorsal trunk on the opposite side.

Structure of the Rectal Gills.

The rectum, swelling enormously, is more or less hexagonal in outline on a cross section. On the inner surface the rectum has six double rows of triangular lamellae or gills which run parallel to the long axis of it, and the hexagonal outline is merely due to this arrangement of gill rows. In a double row of gills every single gill stands at an angle less than the right angle against the median line of the row. Therefore, on a cross section through the rectum one finds that either half of a double row is composed of several cut pieces of gills which have been bisected in various positions **(fig. 2)**. A single gill, or lamella is merely a strong evagination or folding of the rectum wall, in which larger and finer tracheal branches of considerable number are imbedded. One may distinguish near the median line of the double row the basal whitish part from the other larger part which is thinner and highly pigmented. They are called respectively the basal cushion and gill lamella **(figs. 3 und 4)**. In addition to Sadones' detailed investigation

on the structure of the gill of *Libellula depressa*, here are to be mentioned some diverse results of my present study on *L. 4-maculata*. The whole surface of the gill is covered by a thick cuticula, under which the single layer of hypodermal cells is found, and the structure and arrangement of the cells vary according to various parts of the gill. Those in the lamella are much flattened, each cell containing a small, oblong nucleus and many pigment granules; the space between two layers of opposite side is generally so narrow that it is scarecely able to contain any thing more than the finest trachii, which often penetrate into the cytoplasma of the cells. At the basal cushion, two opposite layers are not only widely separated, but also show a different structure of cells, of which they are composed; the layer on the farther lateral from the median line of the double row is composed of cells similar to those which compose the lamella, while the layer near the median line is chiefly made up by a set of larger cells containing larger nuclei **(fig. 3)**. These peculiar larger cells are grouped in a roundish patch, whence the name „Bourrelets epitheliaux" was given by Sadones **(fig. 2, EC)**.

The Cells of the Epithelial Cushion.

The cells forming the epithelial cushion of a gill are not other than the cells of the hypodermal epithelium arranged in a unicellular layer, but peculiarly modified. They vary in number according to the individual gills. All of these cells take their arrangement of several concentric rings around the imaginal centre of the cushion, forming a roundish or frequently elliptical disc. The size of a single cell as well as of its nucleus also varies according to the position they occur. At the central part of the cushion the cells are the tallest, and the nuclei are generally roundish in shape, while at the periphery the cells are much flattened and then nuclei are rather like a slender rod **(fig. 7.)** Those cells and nuclei which lie intervening between these two zones show an intermediate form, the latter being in this zones largest in size. The elongated nucleus lies near the periphery and its long axis is parallel to the cushion surface **(fig. 7)**, a fact just contrast to the case of *L. depressa* investigated by Sadones. Therefore on any transverse section of a cushion, there may be found the nuclei which are roundish or elongated on section, as they are at the right angles cut trough their long axesparallel to the surface, and on a tangential section of a cushion, all of them show an elongated shape except those in the centre. In regard to contents of a cell one is able to recognize without difficulty three distinct parts of the protoplasm; the part nearest to the periphery is finely striated; the next zone which surrounds the nucleus, is more or less granulated, while the third or the innermost part is occupied by a fibrous structure of protoplasm. The first of these three is doubtless homologous to the intima of the epithelium of mid-intestine, but in this cell is the thin and transparent

cuticular covering distinctly visible at the outermost surface where the water of the exterior comes in direct contact, and it is probable that, when the earlier ecdysis takes place, this covering is cast off from the cell and remains in the exuviae. Such being the structure, the cells of the cushion remind us of those of the glandular epithelum in the other part of the alimentary canal. The boundaries of each cell are not so indistinct as Sadones says, especially on a tangential section **(fig. 6)**. Besides the normal series of nuclei, near the basement membrane, there may often be observed a few different nuclei in the cushion a few nuclei of smaller size which were called the accessory cells by Sadones, who did not give accurate demonstration of their destination or nature. Lastly, a thin membrane **(fig. 4 bm)** lining the inner surface of the cushion is, I as think, not other than the basement membrane which was overloked by Sadones. This membrane has a peculiar function in the next period of growth, as mentioned elsewhere. To summarize the obove accounts adverse to those Sadones says on the structure of cushion cells: In his material, *L. depressa*, (1) the elongated nuclei are laid without exception, perpendicular to the cushion surface, while in my material - *L. 4-maculata* — they otherwise lie as alreay noted; (2) the concentric arrangement of the cells is not the case, while it is obvious in my materials; (3) the trabecular structure of the cell contents which is emphasized by him is in fact the feature which is common to all glandular epithelium of alimentary canal; and (4) the presence of the basement membrane, on which says nothing.

It is also noteworthy that the cuticula of the cushion is thin and is in very close contact with cells, while in other parts of the gill its attachment is so loose as to be seen separated entirely from the gills in prepared specimens.

Muscles and Tracheal Branches in the Rectum.

The rectum is provided with two kinds of muscle layers in its wall. One of these, the circular muscle layer, consists of striped fibres running around the rectum, and the other situated superficially to the last layer is separated into six distinct bundles of muscle fibres, which are distributed on six lines where every two of the double gill rows come in contact to each other.

Each three of six double rows of gills stands separated by the dorsoventral sagittal plane of the rectum. The tracheal branches enter into each half in such a way that those from the dorsal trunk enter the upper and middle rows, while those from the visceral trunk go into the lamellae which constitute the ventral row of gills. These tracheal branches, after entering the lamellae, ramify into smaller branches successively, until they are divided at last into the finest anastomose.

IV. The Rectal Tracheal Gills of Imago ready to emerge.[1])

Surface Observation.

First of all there comes to our notice a considerable change in the digestive canal. The oesophagus is followed by a part instead of two, which is regarded either as the crop or the proventriculus; the growth of the stomach and ileum is comparatively slight, but one can not overlook the great changes which take place in the rectum. In this stage the rectum shows two distinct divisions: the anterior portion where the gills develop is highly reduced in its lenght and size by the constriction of intermediate parts of two double gill rows, which stand distinct from each other owing to the constriction **(fig. 8 RG).** Followed this reduced rectal portion, one finds a newly grown posterior piece of rectum, which, in the preceding stage, was an inconspicuous part between the rectal gills and the anus. This part will be called the postbranchial rectum and it is this new formation which appeared during the metamorphosis by active multiplication of cells in this anal piece. Even in such constricted rectal gills, the tracheal branches are still recognizable, so that it reminds us of the feature of the former stage. Only the dorsal trunk is less modified than the visceral trunk which grows stouter.

That the rectal tracheal gills are transferred, either modified or unmodified, to the imago, as Palmen says correct. The continuance of that organ now is so conspicuous that the superficial observation above mentioned enables us to note.

Exuviae of the Gills.

At this stage, in the imago's body taken out from the nymphal skin, we shall find a tranparent matter coming off the rectum. This body is the exuviated mass of rectal gills or cuticular integment once attached to the surface of the gills. In the fixed material, leaving these gill exuviae and the rectum are found the following facts: on the longitudinal sections through the rectum are clearly recognizable the exuviated gills filling up the rectum throughout its length and up to the ileum **(fig. 9).** Among the foldings of the exuviae are found epithelial cells with large nuclei **(fig. 9 EC).** This fact misled Hagen to take these epithelial cells for the whole of hypodermal lining of the gill. In my opinion, although they doubtless are those detached from the gills, but they are not the whole of the gill structure; for the gills, though partly lost, are so clearly to be conceivable in a surface observation, that it needs not to speak of an observation of a series of sections.

[1]) The material was fixed when the nymph made a slit on the thorax from which it was to emerge, and through that slit the soft whitish body of imago was slightly visible.

The epithelial tissues arranged in a unicellular layer are variable in their size but usually not more than several times the length of a cell, and these cells or epithelial tissues are from the epithelial cushions of the gills. Such cells could be found nowhere but in the cushion, and a close study shows that these two are identical. On one margin of this detached epithelium, or the tissue which was once the epithelial cushion, is recognizable a thin cuticular covering passing into other parts of cuticula of gill surface, while its opposite margin shows occasionally irregular appearance whithout any coverings. Among the foldings of gill exuviae, furthermore, there are found many like tissues in degeneration or decay. This fact suggests that these tissues would, sooner or later, decay leaving behind only the cuticular substance in the nymphal skin.

The Remaining Rectal Gills.

The rectum modified into the rectal gills, is eliminated behind the ileum by the considerable growth of the postbranchial rectum. It shows as a whole, a semilunar shape on sagittal sections of the hind intestine; the gill lamellae remaining within are arranged somewhat radially **(fig. 9 Gl)**, directing their tips to the centre of this part of the rectum, and the fat cells in the basal cushion are still visible in the same part. Of course the gill lamellae, in this period, has no longer cuticular covering, and the hypodermal cells of it are fused together including many tracheal branches. At the part of the basal cushion, where the epithelial cushion is situated, epithelial cells entirely disappear, leaving the basement membrane only, while at the opposite wall many small cells are yet to be seen lying upon the same membrane **(fig. 10 ep)**. From these data we are able to conclude that the cushion cells are cast off from the gills, together with the accompaning cuticular coat and leaving the basement membrane in the adult body. The irregularity of one margin of detached cushion above noted is due to this process of gill transformatton during the last ecdysis of nymphal life **(fig. 10 C)**. The basement membrane remaining usually shows many convolutions near the gill lamella **(fig. 10 bm)**, denoting the contraction which took place at the same time whith the elimination of the epithelial cushion.

Periodical falling and regeneration of the epithelium has been studied at first by Bizzozero (1893), later by Rengel (1898) and more recently by Deegener (1902), in the mid-intestine of several water beetles [1]), and the mode by which the epithelium falls off, leaving the basement membrane behind it, shows very close resembrance to the case of epithelial cushion in Odonata (compare with Rengel's figures [2]) **2** und **3**).

[1]) Such as Hydrophilus, Hydrobius and Hydrous.
[2]) Figure 1 and 3 were cited by Deegener (Handb. Ent. p. 281, 282).

It is interesting that the similar occurence is found also in the hind intestine. Whether any secretion [1]), however, gives rise to the falling operation or not, is not yet clearly determined. The renewal of the epithelium is completed in the case of such beetles by the multiplication of crypts, which are found scattered around the mid-intestine and by the spreading of those newly formed cells upon the naked basement membrane, while in dragonfies the regeneration is effected in an other way as will be stated later on.

The intermediate space of the basal cushion is still occupied by a large mass of cells and several leucocytes as well as the muscle fibres of circular layer which have gradual grown. The development of the longitudinal muscle layers between every two double gill rows is very remarkable.

V. The Rectal Tracheal Gills of Immature Imago. [2])

Surface Observation.

Though the abdomen is not yet completely stretched to full length, it attains a length much greater than that of the nymph, also causing the alimentary canal to grow much longer. The stomach still holds the longest portion of the whole canal, being scarcely modified from the preceding stage, and remarkable changes have taken place in the parts posterior to the ileum. The ileum, which was slender in the preceeding stage having fine longitudinal foldings on its surface, has become as thick as the stomach in its diameter, develops on the surface much deeper folds, both longitudinal and transverse. This phenomenon is caused by a vigorous multiplication of epithelial cells which has effect to increase the intestinal surface. The anterior part of the rectum where the gills rest, is considerably shortened in length, showing many tranverse parallel folds which indicate the positions of the lamellae **(fig. 11 RG)**, and six double rows of gills are quite distinctly observed, owing to constriction of intermediate portions of the rows. The posterior or postbranchial rectum gives rise to several evaginations just behind the branchial part **(fig. 10 und 11 ev)**, these being due to an intensive incerment of the epithelial cells.

Of the changes of the tracheal system the most notable is that all of the tracheal branches entering the gills have now become scarcely visible.

Observation in Sections.

Owing to the approach, of both the anterior and posterior ends of the branchial port of rectum, which occured in the previous stage,

[1]) In the mid-intestine, by a certain fluid secreted out of the crypt-neck-cells the contraction of crypt muscles, is loaded with some pressure and subsequently floes between the chitin lamella of epithelium and the basement membrane, so as to result complete separation of both the structures (Rengel, p. 446).

[2]) An imago that has completely emerged from the nymphal skin, has soft wings in which the pigment is not yet developed, and the abdomen is incompletely stretched.

and owing to the new formation of the epithelial tissue after the last ecdysis **(fig. 12 ne)**, all of the rectal gills are completely eliminated from the inner surface of the alimentary canal. Subsequently the gill lamellae fuse together into compact cell masses and the boundaries of each lamella are entisely lost. There are still observable the sets of fat cells, thongh they are decreased both in size and number. The muscles, a considerable development of which was already observable in the preceding stage, in the space of the basal cushion, become more and more conspicouus in this stage, and those situated in the intermediate parts of every double gill rows also grow prominent. Pigment granules are gathered at the tips of these degenerating gills.

VI. Rectal Tracheal Gills in Mature Imago.

The hind indestine reaches in the mature imago, its extreme prolongation in accordance with the complete stretch of the abdomen, and the ileum extends straight instead of being more or less covuluted as was seen in the precedings stages. The whole lengfh of it surpasses three times the length of the rectum. The part where the ileum joins with the rectum is strongly constricted, and the latter becomes again dilated. Slightly posterior to this constricted portion and on the external surface of the rectum are six small brownish patches **(fig. 13 RG)**, of which three, together with three raised streaks between them, are to be seen only on the dorsal side. These patches are the last remnants of the rectal gills, and the streaks are muscles in their highest development. Examining sections throuhgt this region **(fig. 14)** we find that the inner surface of the part with the brown patches is lined with thin epithelial cells, under which many pigment granules are collected and is surrounded by thick muscle layer. No trace of the branchial epithelium is left, all the epithelial cells disappear after the extreme degeneration, and it is due to the fate of the respiratory organ out of use in the last stage of life.

Development of six muscles between two double gill rows is striking but two of them in the ventral position are scarcely distinct from each other, as they are closely connected with the muscles in the periphery of the degenerated gills.

VII. Histological Study on the Elimination of Rectal Gills and the Regeneration of the Epithelium and Fat Cells.

Prolongation of the Epithelial Tissues of Both the Ends of the Branchial Part.

In the foregoing pages I have described: how useless gills are eliminated and how they degenerate at last. Now I will briefly deal with the histological changes of them. After the last ecdysis, each of the double gill rows assumes, by approach of both ends of branchial part

of the rectum on one hand and by development of muscle layers at the intermediate parts of the rows on the other hand, the form of a pocket with the opening facing to the central space of the rectum. This opening decreases gradually in diameter by a new formation of epithelium which has budded out from the impaired edge of the rectum, and finally has in results total closure. Turning to sections (Textfigure), we find an epithelial tissue (ne) which originates at the impaired margin of the rectum. This new tissue increased, is extended gradually to be joined at last with the corresponding structure of the opposite edge. The cells in this new epithelium are generally smaller in size, enabling one to distinguish them easily from those of the original epithelium of the rectum, from which they arose. The epithelial cells of the hind intestine are not arranged strictly in single layer, but another series of cells, which is not

A section of the impaired margine of the rectum (Zeiss. obj. D. Oc. 2). bm. basement membrane; cm. circular, muscles: F. fat cells; g. germinal cells of the epithelium; gc. germinal connective tissue cells transformed from tp; l. leucocyte; ne. newly formed epithelium; R. rectum.

regularly arranged in layer, is found at the bottom of the epithelium: these cells of this series contain protoplasm of fine granular appearance and stain rather intensively with the anilin color. They are supposed to be the germinal cells which compensated in the course of development, the decayed cells and probably the cells of newly formed epithelium also originate in this germinal cells.

In connexion with the new formation of epithelium, takes place new formation of the connective tissue which support the epithelium from beneath. The basement membrane passes over into the fibrous substance, the tunica proparia under the rectal epithelium (tp). There is no reason to separate the basement membrane of the branchial epithelium from the tunica propria in the other part of the rectum, since both are the same in nature and origin but the tunica propria in this case not only contains cells arranged in a few indistinct layers but also shows an irregular fibrous appearance, and serves to connect the epithelium with the underlying muscles. Besides these structures the free cells are scattered about the

spot where th eterminal gill lamellae pass over into the rectal epithelium. These cells have the nuclei more or less spindle shaped of elongate form usually much larger than those of branchial cells. These cells are probably the indifferentiated cells destined to be converted afterwards into the connective tissue, and they are exactly similar to the cells found beneath the rectal epithelium (tp). Or, in other words, they have come out of their original positions through the wounds of the epithelium resulted from the ecdysis of gills, to form the connective tissue under the newly formed epithelium (ne). Leucocytes and pigment granules imbedded in a protoplasmic fragment or some of the branchial cells are also to be seen together with these cells. I have often observed that some cells of the branchial epithelium are crowded just beneath the new epithelium, mingled with the connective tissue cells described, but they make by no means a permanent tissue, but are absorbed sooner or later probably by leucocytes, leaving pigment granules behind.

Significance of Falling off of the Epithelial Cushion.

That the rectal gills correspond to rectal glands or at least to a part of them in other insects has been considered by previous authors to be very probable. I also incline to this view after observing the glandular nature of the cushion cells as above mentioned. Still remains obscure. the question, why the cushion cells must fall off in the course of metamorphosis, from other parts of branchial epithelium. However from the accounts given obove it is clear that the detachment of the epithelial cushion causes the destruction of epithelial arrangement of cells, and subsequently makes path through which the germinal cells of connective tissue would come forth, unless these cells beneath the epithelium are not able to wander out. On the basement membrane of the gills are for some time to be found such germinal cells, but I cannot say with certainty, whether it happens so invariably or not. I believe that some of these cells would be detected by a closer observation on the basement membrane; they form probably a part of supporting tissue under the new epithelium. If this be the case, the significance of the falling off of epithelial cushion Iwould become far clearer than before.

Fat cells in the Basal Cushion.

Here I shall touch briefly upon the nature of cells or tissue filling up the space of the basal parte of a gill or the basal cushion. These cells or tissue, generally known as the fat cells or the fat tissue, have already been described by Chun (1876) and Sadones (1896) in *Libellula depressa*, and also by Faussek (1887) and by Sadones (1896) in some of Aeschna. It has been considered, at the same time, that these cells are very distinct in their nature as compared with the ordinary fat cells or adipose tissue in other parts of the insect body; each cell has a large roundish nucleus which is always found near the centre of the cell **(fig: 15)**; the cell contents show a fine granular appearance, but with no fat

grobule. But it is evident that they are quite homologous with other connective tissue (and true fat cells) both in being formed from mesodermal cells in early stages of development, so that they may be called merely connective tissue cells, as Faussek[1]) describes as follows.

„Was das Bindegewebe, das den inneren Raum der Darmkiemen füllt, anbelangt, so besteht es aus mit Kernen und Hüllen versehenen Zellen, welche aber nicht regelmäßig im Gebiet der Darmkiemen verteilt, sondern in einzelne Komplexe von runder oder länglicher Form zusammengefaßt und von einer dünnen, deutlich sichtbaren Hülle (Membrana propria?) umgrenzt sind". He observed, therefore, with certainty the membranous covering in the periphery of the cell-complex and supposed them to be the Tunica propria. I have, however, not detected such a membrane except the basement membrane underneath the epithelium, to which I have repeatedly referred. Though the functions of these peculiar cells are still obscure, it seems very probable that they serve in means similar to other connective tissues and that they have other functions, which are noy yet explained.

VIII. Summary.

1. The rectal tracheal gills undergo striking changes during a remarkable short time, and these changes are necessary to the supplement of the wounded portion of the rectal wall by ecdysis for the insect maintaining the same condition of life without pupal resting stage.

2. At the last ecysis, the epithelial cushion is detached from the basement membrane and subsequently is cast out of the adult body, together with the cuticular coating of the gills, but they never include the whole of the branchial epithelium. The view of Hagen who denied Palmen's opinion is, therefore partly correct.

3. The falling off of the epithelial cushion is a destruction of epithelial wall of the rectum; this destruction gives rise to the new formation of epithelium and opens the path for the germinal connective tissue cells wandering out, and from these cells is formed finally a new supporting element under the newly formed epithelium.

4. The gill lamellae are transferred, with little modification, to the adult body. So the view of Palmen is proved partly to be the fact, as a part of the epithelial cushion is certainly thrown off at the last ecdysis.

5. The gills are eliminated from the inner surface of the rectum in consequence of prolongation of the postbranchial rectum; at the same time, the longitudinal muscles of intermediate portions of every double gill row develop enormously, and at last, six double rows are converted into six pockets which open by a small path to the interior of the alimentary canal.

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[1]) Faussek: Zeitschr. f. Wiss. Zool. XLV. 1887. p. 710.

6. These openings are gradually fused together through the new formation of epithelium brought about by multiplication of cells of rectal epithelium at both the anterior and posterior ends of the branchial part, and the results of this fusion are the new formation of supporting tissue or the tunica propria.

7. The remaining gills aggregated into rather compact masses and degenerate gradually, until all of the elements composing them, except the pigment granules, are entirely absorbed probably by action of leucocytes At last all of the six double gill rows are reduced in mature imago, into six brown spots surrounded by muscles at the anterior part of the rectum.

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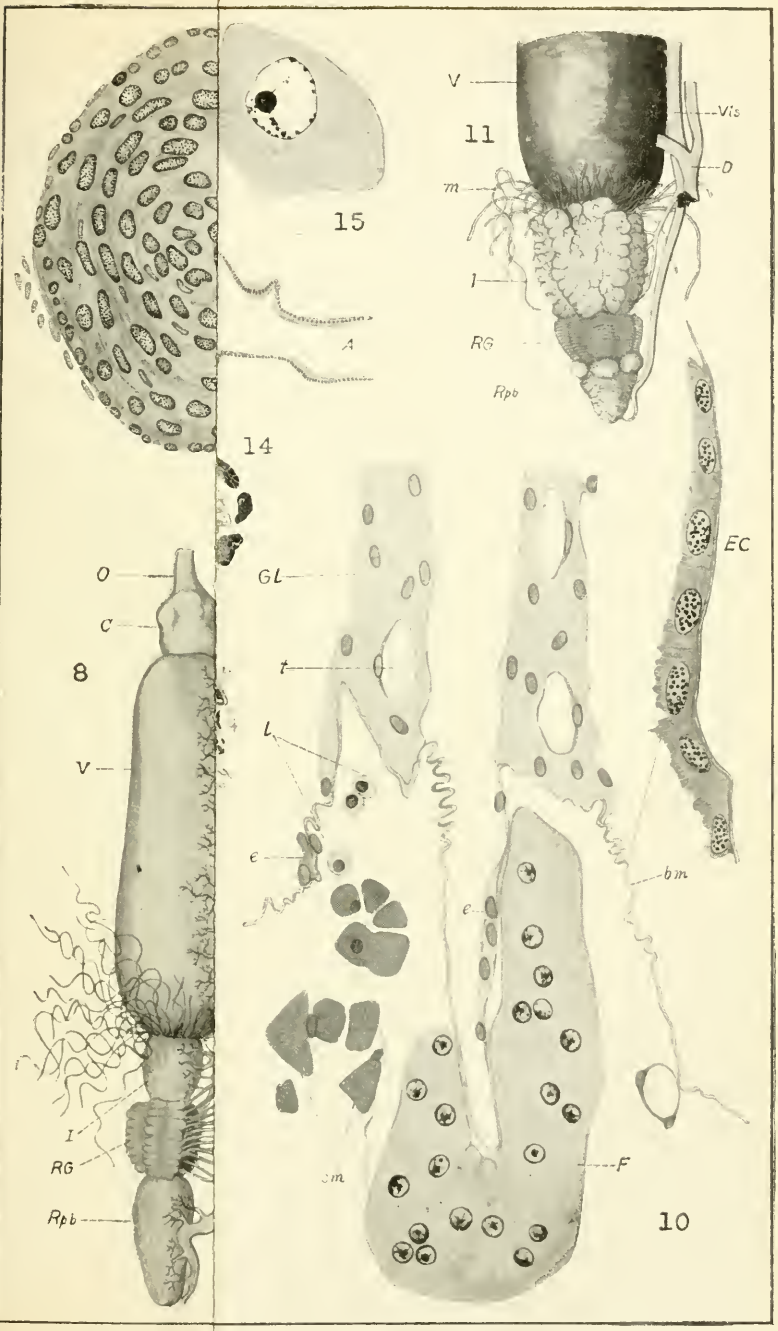
Explanation of Plate.

Abbreviations.

A = anus; BC = basal cushion; bm = basement membrane C = crop; c = crticula; cm = circular muscle; D = dorsal tracheal trunk: EC = epithelial cushion; F = fat cell or fat tissue; Gl = gill lamella; I = ileum; l = leucocyte; lm = longitudinal muscle; M =



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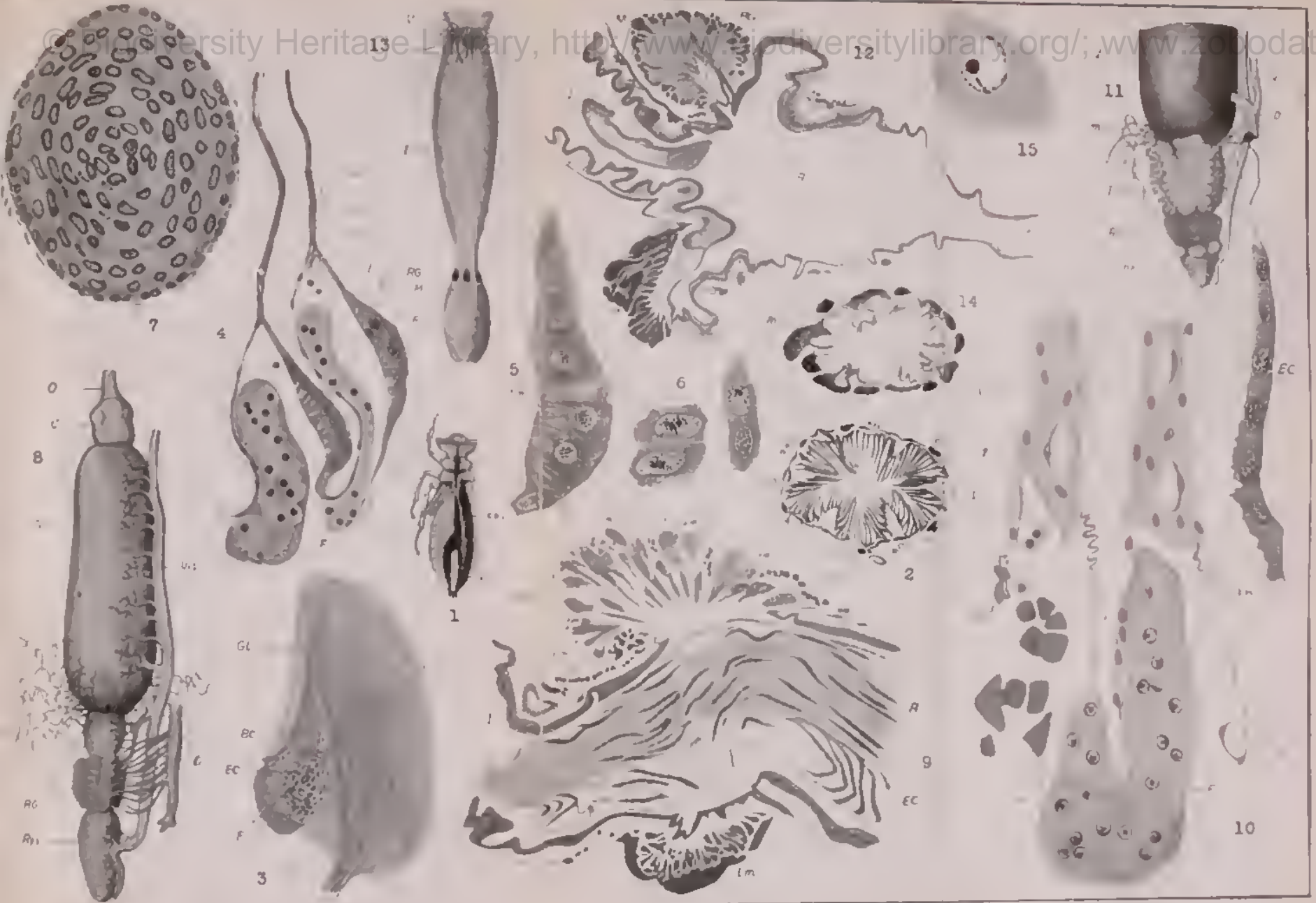

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muscle (all kinds of); m = Malpighian tubules; O = oesophagus; R = rectum; RG = rectal tracheal gill; S = lateral tracheal trunk; Sp = dorsal thoracical spiracle; t = trachea; tb = tracheal branch; V = ventricle or stomach: Vis = visceral tracheal trunk.

Fig. 1. Exuviae of the last nymph. ET = exuviae of trachii; EG = exuviae of gills.

Fig. 2. Cross section trough the rectum of a full grown nymph, showing the arrangement of gills. Leitz. Obj. 1: Oc. 2.

Fig. 3. Two gills; the arrow denoting the direction in which the section of **Fig. 4** is made. Leitz. Obj. 3: Oc. 1.

Fig. 4. Transverse section of the same. Obj. D: Oc. 2.

Fig. 5. Epithelial cushion of the same, more enlarged. Zeiss. Apochr 4 mm: Comp. Oc. 8.

Fig. 6. Cells of the same in tangential section (drown from detached cushion). Zeiss. Apochr. 4 mm: Comp. Oc. 8.

Fig. 7. Epithelial cushion. Zeiss. Obj. D: Oc. 2.

Fig. 8. Alimentary canal and trachii of an imago ready to emerge (trachii of the left side removed).

Fig. 9. Median longitudinal section of the same; note the cushion cells among the exuviae of gills. Leitz. Obj. 3: Oc. 2.

Fig. 10. Remaining gills with detached epithelial cushion. (EC). Zeiss. Apochr. 4 mm: Comp. Oc. 8.

Fig. 11. Hind instione with trachii (trachii of the left side omitted), of an immature imago.

Fig. 12. Median longitudinal section of the same. Leitz. Obj. 3: Oc. 2.

Fig. 13. Hind intestine of a mature imago.

Fig. 14. A cross section of the same through position where the brown spots lie. Leitz. Obj. 3: Oc. 2.

Fig. 15. A single fat cell. Zeiss. Apochr. Homol. Im. 2 mm: Comp. Oc. 12.

All the figures are reduced about $^1/_5$.