# ON THE METAMORPHOSIS OF THE ALIMENTARY CANAL IN CERTAIN EPHEMEROPTERA

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With Plates V and VI.

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## 1. Introduction.

Although the Ephemeroptera as a whole have attracted popular and scientific attention to a considerable extent from time to time, the literature dealing with the special study of the alimentary canal is relatively scanty. This is the more surprising since it is in the alimentary canal that the most profound changes have long been known to occur in these insects: changes which are perhaps unparalleled in any other Order.

The period of time occupied by a single life-history is not known accurately, but nymphal life probably extends over three or four years. The nymphal instars are succeeded by a winged subimaginal stage of brief duration—usually only a few hours—and this in turn by the imaginal stage, the existence of a subimago being characteristic of the Ephemeropteran life-history.

Both subimago and imago are capable of active flight, but the former is usually more or less quiescent unless disturbed.

Imaginal life is relatively brief, sufficient only to allow of pairing and oviposition. In some cases, the imago emerges and dies on the same day, hence the name ἐφήμερον applied to these insects by Aristotle.

In the nymph, the alimentary canal presents no very unusual features, but in the imago it is no longer capable of maintaining digestive functions. The muscular coat of the mesenteron disappears to a great extent, and this part of the digestive tract becomes excessively thin-walled and is frequently distended with gas. In consequence of this distention, the change in structure has often been regarded as an adaptation to aerial existence whereby the insect, in deflating or inflating the mesenteron, is able to alter the specific

gravity of its body at will. To the possession of such an adaptation is attributed the peculiar dancing flight of these insects. The alteration in the alimentary canal is accompanied by atrophy of the mouth-parts (12).

In his great posthumous work (13), Swammerdam suggests the possibility of such a change : " Noch een andere reeden isser waar door het Haft luchtig op het water vliegt, dewelke bestaat in dat se een subtiel blaasken met lucht binnen in haar lichaam hebben : ten sij men sou willen seggen, dat haar Maag op die tijt met Lucht vervult was. . . ." This account is accompanied by illustrations setting forth the general morphology of the nymphal gut and constitutes a valuable contribution to the subject.

Vayssière (15) gives an account of the nymphal structure in numerous Ephemeroptera and devotes a separate chapter of his paper to the digestive system in these insects. The greater part of this chapter, however, is given up to a consideration of mouth-parts. He points out that of the mesenteron, the anterior part is chiefly glandular, while the posterior part is characterised by its well-developed musculature. The whole of the alimentary canal posterior to the openings of the Malpighian tubes, Vayssière calls " rectum," in which he describes " un fort bourrelet interne . . . qui s'étend d'une extrémité a l'autre de cette région intestinale."

Palmèn (8) devotes a section of his treatise (pp. 45, 53 *et seq.*) to the development of the alimentary canal of Mayflies. He states that the mesenteron becomes widened and thin-walled in the imago, suggesting that air is taken into the alimentary canal by the subimago and that the pressure of this air is responsible for the changes in morphology. This author further describes the atrophy of the muscular coat of the mesenteron, stating that during the process of dissolution, the muscles have an appearance as if they were undergoing fatty degeneration. The function of the imaginal gut is considered to be connected primarily with the ejection of the genital products, and the probability of an aerostatic function in addition is admitted.

Fritze (5) describes the structure of the whole alimentary canal in considerable detail, dealing separately with conditions found respectively in the " larva," " nymph," subimago and imago of several species. A special point is made of the structure of a valve in the proctodaeum of the imago, which is interpreted as a modified part of the ileum. The dilation of the mesenteron and the flattening of its epithelium is regarded as due to the intake of *water* by the nymph, shortly before the emergence of the subimago, which water is later replaced by air.

Subsequent workers, Leue (6), Drenkelfort (2) and Sternfeld (12), would appear to accept this explanation of the morphological changes.

Leue considers certain thickened regions of epithelium, which he somewhat unfortunately calls " Imaginalringe," to be regions of growth, wherefrom new cells are constantly added to the epithelium of that portion of the gut immediately posterior to them, throughout nymphal life.

Drenkelfort expresses a doubt as to the possibility of such inflation of the gut that the specific gravity of the insect is diminished appreciably.

Sternfeld describes the development of the stomodaeal musculature, and shows that in the imago the stomodaeum might function as a powerful pump for forcing air into the mesenteron.

## 2. Material and Methods.

The material examined in the course of the present investigations consists of :—

*Ephemerella ignita*, Poda,
Nymphs, subimagines, imagines.
*Leptophlebia meyeri*, Eaton,
Nymphs, subimagines, imagines.
*Ephemera danica*, Müller,
Nymphs, subimagines, imagines.
*Chloeon* sp.,
Nymphs.
*Baëtis* spp.,
Nymphs.
*Baëtis binoculatus*, Linn.,
Subimagines.
*B. pumilus*, Burm.,
Imagines.
*B. rhodani*, Pictet,
Imagines.

Eaton's monograph (4) and his Concise Synopsis (3) were consulted for the identification of winged insects; nymphs were identified from Rousseau's descriptions (11).

Imagines of *Baëtis pumilus* and *B. rhodani* were taken as such in the field; all other winged insects were obtained by breeding in captivity.

The fluid of Carnoy-Lebrun was the most generally used fixative and gave good results with all stages, especially when the material was incised before fixation. Bouin's Picro-formol and Carl's formula were quite useless.

Material was embedded in paraffin or in celloidin and paraffin; sections were stained on the slide with Haidenhain's Iron Haematoxylin or Mayer's Haemalum.

Most of the work was carried out with *Ephemerella ignita* and *Ephemera danica*.

## 3. Structure and Metamorphosis of the Alimentary Canal.

### (i) *The Nymph, prior to Metamorphosis.*

*General Form.*—The alimentary canal of the nymph falls into three well-defined regions: stomodaeum, mesenteron and proctodaeum. Of these, the stomodaeum is confined to the head, extending from the ventrally placed mouth to the anterior margin of the prothorax. The mesenteron, which is much the longest division, runs from its junction with the stomodaeum to about the middle of the seventh abdominal segment, where it joins the proctodaeum. The proctodaeum opens to the exterior by the anus in segment ten. From the region of junction of the mesenteron and proctodaeum in segment seven, numerous Malpighian tubes originate and lie in the haemocoele in a somewhat irregular fashion. Apart from the Malpighian tubes, there are no diverticula of the alimentary canal, caeca and salivary glands alike being absent.

The alimentary canal attains its greatest diameter in the mesothorax; before and behind this region, the mesenteron narrows gradually until at the posterior end the diameter is about one-tenth and at the anterior end one-third of this maximum.

The stomodaeum is a much narrower tube and projects some little distance

into the anterior end of the mesenteron, forming with the anterior part of the mesenteron a simple oesophageal valve (Pl. V, figs. 1, 5).

The procotodaeum is clearly subdivided into three regions, which are respectively, from before backwards, the ileum, colon and rectum.

In the nymph, the alimentary canal is not convoluted, but nymphs of *Ephemera danica* differed from all the others examined in having the mesenteron transversely ridged internally, especially in the thoracic part of its course.

*The Stomodaeum.*—The stomodaeum is lined internally by a distinct and well-developed chitinous layer. The epithelial layer beneath this is thrown into large longitudinal folds, so that in transverse section the lumen of this region has a distinctly stellate appearance (Pl. V, fig. 3). The cells of the stomodaeal epithelium are short and columnar, with oval nuclei (Pl. V, fig. 4).

Surrounding the epithelium is a layer of circular muscles; the posterior half has also a sheath of longitudinal muscles. In addition, radial muscle bundles are present, connecting the stomodaeum with the exoskeleton of the head.

Before its entry into the mesenteron, the stomodaeum dilates slightly; this dilation is well marked in *Ephemera danica*.

The stomodaeum projects slightly into the mesenteron (about 0·05 mm. in fully-grown nymphs in *Ephemerella ignita* on the ventral side) to form an oesophageal valve, in which the epithelium of the stomodaeum joins on to that of the mesenteron without a break. The circular muscle layer continues into the fold of the oesophageal valve (Pl. V, fig. 5, c.m.) and is continuous with the circular muscle sheath of the mesenteron; the longitudinal muscles, on the contrary, end on the anterior part of the mesenteron and do not enter into the fold of the valve (Pl. V, fig. 5, l.m.). Dorsally, the oesophageal valve does not extend so far backward as it does ventrally.

Where the stomodaeal epithelium abuts on that of the mesenteron, it is raised into a ridge of *Ephemerella ignita* and *Baëtis* spp. Cell walls cannot be distinguished in this region, and the nuclei are small and crowded. Leue (6) regards this as a region of mesenteric growth, from which new cells are cut off and added to the mesenteron, thus accounting for growth in length of that division of the alimentary canal. He records having observed nuclear division in this part, which he calls an "Imaginalring." It has not been possible to verify this observation in the present investigations.

*The Mesenteron.*—The layers of the wall of the mesenteron are: on the outside, an exceedingly delicate peritoneum, which can only be discerned with difficulty, enclosing the muscular sheaths which in turn enclose the epithelium. The outer muscular layer consists of longitudinal fibres and is best developed in *Ephemera danica*; in *Ephemerella ignita*, it is absent except at the anterior and posterior ends of the mesenteron. The inner muscle layer consists of circular muscles.

The layer of epithelium consists of columnar cells having the form of fairly regular hexagonal prisms. The anterior epithelial cells, including those reflected to establish connection with the stomodaeum are crowded, especially at their inner ends, with dense, black inclusions (Pl. V, fig. 5). These inclusions are extremely dense and conspicuous, even in unstained sections. A thick and brilliant "striated hem" (Miall & Denny, 7) covers the free ends of the cells in the anterior part of the mesenteron, giving the epithelium a ciliated appearance at first sight. This striated hem disappears in the part posterior to the fifth abdominal segment. In *Ephemera danica* the striated hem is present, but is not very conspicuous (Pl. V, fig. 6). Some of the epithelial

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cells, especially the more anterior ones, have their free surfaces produced into rounded vesicles which encroach upon or even protrude through the striated hem; they probably represent a phase in the secretion of digestive ferments. Such vesicles do not appear to be produced in the region where no striated hem is visible.

A peritrophic membrane could not be distinguished in sections, but it was observed that the faeces of fully developed nymphs of *Ephemera danica*, voided prior to ecdysis, were enclosed in delicate, transparent sacs.

Food material observed in the mesenteron consisted largely of filamentous algae and diatoms; the latter were often so abundant as to injure the knife seriously during section cutting.

The muscular layer of the mesenteron is most strongly developed at the posterior end of the mesenteron, where it attains the same thickness as the epithelial layer, which is here somewhat reduced.

*The Proctodaeum.*—The proctodaeum exhibits three well-marked divisions. Of these, the ileum has the form of a short truncated cone, with the base anterior and giving off numerous Malpighian tubes as radial diverticula. The wall of the ileum, as of the rest of the proctodaeum, consists of the layers: peritoneum, circular muscles and epithelium, and has a chitinous lining. In section, the epithelium has a remarkably fibrillar appearance (Pl. V, fig. 7). At the free border of the epithelium, the striations are finer and more regular than elsewhere, giving this portion a certain resemblance to the striated hem of the mesenteron. This border is somewhat sinuous, so that the depth of the epithelium is greater in some places than in others. The nuclei are large and granular; towards the anterior and posterior limits of the ileum they decrease a little in size. Radial cell-walls are not evident and are probably entirely absent, at least in *Ephemerella ignita*.

In the colon, two kinds of epithelium may be distinguished, one containing large nuclei (Fritze, *loc. cit.* "*grosskerniges Epithel*") and one much more delicate, with small nuclei (*ibid.* "*kleinkerniges Epithel*"). The arrangement of these epithelia varies with the species. In *Ephemerella*, *Baëtis* and *Leptophlebia*, a transverse section (Pl. V, fig. 8) shows that there are three bands of "*kleinkerniges Epithel*" alternating with three of "*grosskerniges Epithel*," with the latter dorsal and ventro-lateral in position. The ventro-median edges of these large bands frequently project into the lumen of the colon; it seems possible that it may be such a projection and not a definite structure which Vayssière describes as ". . . . un fort bourrelet interne" (cf. Vayssière 15, fig. 38). In *Ephemera danica* there are only two bands of each type; those of "*kleinkerniges Epithel*" are dorsal and ventral in position. According to Fritze and to Drenkelfort, *Ecdyurus fluminum* (Lin.) and *Siphlurus lacustris*, Eaton, have only one band of each kind. In general appearance the "*grosskerniges Epithel*" closely resembles the epithelium of the ileum; it is fibrillar, with large, granular nuclei, and, except in *Ephemera danica*, with no suggestion of radial walls (Pl. V, figs. 8, 9 and 10). Each of its bands is pressed closely against the muscular sheath, except at the edges, where it tends to become free and to project into the cavity of the gut as described above. The bands of "*kleinkerniges Epithel*" are continuous with these; each is very narrow and rather irregular, containing small nuclei which enclose two or three chromatic bodies (Pl. V, fig. 9).

Towards the posterior end of the colon the "*kleinkerniges Epithel*" enters more and more into the composition of the gut-wall by encroaching on the "*grosskerniges Epithel*" and finally replacing it altogether. In this way, the

colon merges into the rectum, in which the epithelium is all of the small nucleate type (Pl. VI, fig. 11).

The depth of the rectal epithelium varies to some extent, but is, in the main, greater than that of the "*kleinkerniges Epithel*" in the colon. Surrounding it is a layer of circular muscles, which, in transverse section, show very fine histological details. Radial muscles also are present, and, just anterior to the anus, the circular muscles are greatly developed to form a sphincter. In the first half of its course in *Ephemerella ignita* the wall of the rectum is depressed all around in such a way that the lumen is almost obliterated for a little distance and a rectal valve (Pl. VI, fig. 11, rect. v.) is thus formed. The muscular coat as well as the epithelium is involuted to form this structure.

Outside the anus, the rectal epithelium is continuous with the hypodermis.

(ii) *The late Nymph and the commencement of Metamorphosis.*

The approach of the ecdysis which is to liberate the subimago is evidenced by a very marked darkening of the colour of the nymph in all the species examined. This darkening begins on the dorsal side of the thorax and extends slowly in all directions until, at the end of two or three days, the nymph has assumed a very dark grey coloration over its whole surface. Such nymphs become sluggish in habit, even the respiratory movements of the gills being a little slowed down. In most cases, the gut is emptied of food during this time, though exceptionally, the whole process may take place with food remaining in the alimentary canal.

When darkening of the cuticle is completed, bubbles of gas may be noted beneath the nymphal cuticle, especially in the thoracic region; the wing rudiments in particular are filled and often distended with gas. In consequence of the increased buoyancy imparted thereby, the nymph rises from the substratum and floats passively at the surface of the water. Should the nymph be disturbed in this condition it makes violent and aimless swimming movements, recalling those of the pupae of many of the higher aquatic insects. After a time the nymphal cuticle splits longitudinally in the thoracic region and the subimago rapidly makes its way out.

The subimago of *Ephemera danica* emerges about three hours after the nymph has commenced to float; males on an average emerge more quickly than females, and the male subimago and nymph tend to be rather smaller than those of the female.

Considerable internal changes take place at this time. The diameter of the mesenteron is considerably reduced, apparently as a direct consequence of the absence of gut contents: if the alimentary canal of a living nymph be dissected out, small portions of it can be emptied of food by gentle washing, when it is found that such portions contract to a very small size.

A striking appearance observed in nymphs of *Baëtis* spp. and of *Leptophlebia meyeri* at this stage is that blood corpuscles are aggregated in dense swarms along the course of the mesenteron and proctodaeum.

As a result of the contraction, the epithelial cells of the mesenteron become somewhat distorted; the cells also lose their well-defined boundaries, the radial walls ceasing to be visible and the free surfaces becoming very irregular. At the same time the protoplasm near the free surface becomes very coarsely granular (Pl. VI, fig. 13).

This condition is followed by a very striking one in which cell material is extruded in the form of vesicles into the lumen of the alimentary canal. Each of these vesicles is a spherical body containing a clear, refractive sub-

stance, and a few bodies which take up nuclear stains very readily (Pl. VI, fig. 14). Indeed, many of the vesicles are very similar to nuclei in appearance and size at this time. The true nuclei of the epithelium have meanwhile become reduced in size and contain a few chromatic granules. It has not been possible to correlate these changes with any nuclear division or reduction process. In consequence of this loss of cell material the epithelium becomes reduced to a much thinner, somewhat irregular layer, containing degenerate nuclei.

The process of epithelium reduction described above appears to be the typical one and was observed in every case examined. In *Ephemerella ignita* and *Leptophlebia meyeri*, however, the process occurs in a modified form in most parts of the mesenteron, though the typical process does occur in some parts, particularly at the posterior end. In these cases the cell boundaries are not lost in the early stages, but the contents of the cells appear to separate into two fractions—one optically denser and more granular, which collects at the base of the cells, and a clear fraction which fills that half of the cell nearer the gut lumen (Pl. VI, fig. 15). The epithelium thus comes to present the appearance of a squamous epithelium with granular contents, supporting a series of delicate arches which are the cell-walls enclosing the clear material.

This is apparently a specialisation of the more typical process, in which the whole of the inner part of the epithelium cell is converted into one vesicle of rejected cell material.

In *Ephemera danica* the muscular coats of the mesenteron also undergo a change while these alterations are proceeding in the epithelium. The bundles of muscle fibres, which are distinct in younger nymphs, now become ill-defined, the peritoneum breaks down in places and blood cells enter through the openings so formed (Pl. VI, fig. 13). The other species examined also show the beginnings of a similar change in the muscular coat, but pronounced muscular degeneration is more characteristic of the next instar.

The stomodaeum does not undergo so marked a change; only in the neighbourhood of the oesophageal valve is any alteration observed. Here, the stomodaeal epithelium entering into the formation of the valve becomes apparently reduced in amount, and in all the material, except *E. danica*, the valve itself tends to become directed transversely across the lumen instead of backwards as in earlier stages. It has not been possible to obtain preparations showing histological change in this tissue. Congregation of blood corpuscles does not take place to any great extent around the stomodaeum except in the neighbourhood of the oesophageal valve.

In metamorphosing nymphs of *Baëtis* sp., blood corpuscles were observed to cluster quite as abundantly around the proctodaeum as they did around the mesenteron; there was, however, no reason to believe that they penetrated into the muscles and no histological change was observed in this region.

It is of interest to note that the blood corpuscles of the late nymph consist almost exclusively of small cells, oval or circular in optical section, 5–8 $\mu$ in diameter; younger nymphs have a considerable proportion of large fusiform corpuscles exceeding 12 $\mu$ in length (measurements obtained from *E. danica*). This alteration in the constitution of the blood is worthy of further study.

### (iii) *The Subimago.*

The processes of metamorphosis of the alimentary canal in the subimago are a direct and probably unbroken continuation of those recorded above in the full-grown nymph. In some individuals the alimentary canal was contracted even to a greater extent than in the late nymph, so that the dorsal

and ventral walls of the mesenteron were actually contiguous in parts (Pl. VI, fig. 16). This extreme contraction characterises newly emerged subimagines.

The state of degeneration of the mesenteron at the time of the emergence of the subimago varies considerably, but appears to be more or less constant for a given species. In general, the newly emerged subimago of *Ephemera danica* seems to be most advanced in this respect and that of *Ephemerella ignita* least so.

Apart from local changes in general form, associated with the reduction of mouth-parts, the stomodaeum remains practically unchanged. Histological change does occur, however, in the stomodaeal portion of the oesophageal valve, slightly anterior to its apex. Here for a little distance in *Ephemerella ignita* the epithelium shows distinct signs of degeneration: the radial cell walls are lost, the protoplasm becomes coarsely granular, and the tissue generally becomes indefinite in structure.

In the mesenteron a wide range of conditions may be found in this instar, even in different parts of one individual. The protoplasm of the epithelium condenses still more towards the bases of the cells, and there is a certain amount of extrusion of clear material in the form of vesicles into the lumen of the gut. In *Ephemerella ignita* large terminal vacuoles are still present in most of the epithelium cells when the subimago is newly emerged (Pl. VI, fig. 16). As these processes continue, the cytoplasm still remaining becomes very coarsely granular with the granules aggregated into clusters (Pl. VI, fig. 17); the nuclei become very indistinct by loss of their contents and ultimately disappear for the greater part. The vacuoles also become detached or ruptured, leaving behind an extremely thin and almost structureless layer to represent the epithelium. As this condition is attained in the several parts of the mesenteron, inflation of that part seems to occur, so that the completely metamorphosed mesenteron is distended along its whole length. The portion of the mesenteron lying in the prothorax retains its nuclei and its cellular nature generally, but a striated hem is absent and the cytoplasm is free from granules.

While these changes take place in the epithelium, dissolution of most of the muscular coat of the mesenteron proceeds apace. The process is essentially similar to that described as occurring in the late nymph of *Ephemera danica*: the individual muscles lose their definition, the layer becomes coarsely granular in parts and invasion by blood corpuscles occurs. Certain blood corpuscles become swollen and contain dark, granular inclusions. The muscular coat becomes thinner and thinner, ceases to be sharply marked off from the degenerating epithelium and ultimately disappears, except at the anterior and posterior ends of the mesenteron.

In *Ephemera danica* the epithelium of the ileum and colon was observed to be reduced in thickness at this stage, though normal nuclei were retained. The development of this condition, which is not found in the other species, was not traced.

Distention of the reduced mesenteron causes displacement of certain parts of the alimentary canal, but apparently without any fundamental morphological change in those parts. The ileum is particularly involved: in *Ephemerella ignita* it becomes completely telescoped into the colon, or displaced to one side of it, so that ileum and colon both lie in the same segment. In *Ephemera danica* the anterior part of the ileum is diverted to lie across the lumen of the alimentary canal, and forms, with the hinder part of the mesenteron, a valve between the mesenteron and the proctodaeum.

Eclosion of the imago occurs about the time when all the foregoing changes are completed, but the exact state of completion is subject to slight variation.

### (iv) *The Imago.*

In some few cases the last stages of mesenteron reduction may occur after the final moult. With this exception the alimentary canal persists for the remainder of the insect's life in the condition described above for the subimago immediately before moulting finally (Pl. VI, fig. 20).

## 4. Conclusions.

The observations of Palmèn and of Fritze led those investigators, as indicated in the introduction, to regard the change in structure of the mesenteron as the simple and direct result of mere mechanical pressure. The former considers that *air* pressure is responsible for initiating and completing the change, while Fritze contends that *water* pressure is first exerted, just before the subimago emerges: "Nach Ausstossung der festen Nahrung durch die Nymphe unmittelbar vor dem Ausschlüpfen des Subimago tritt das Wasser mit ziemlicher Kraft in den Darm, welcher hierdurch ausgedehnt wird. . . . Diese Ausdehnung halte ich für die einzige Ursache der Verwandlung" (5, p. 17).

It would appear, since no account of actual observations of the process is given by either author, that these views are simply hypotheses to be accepted or rejected on their merits. In effect, Fritze accepts his predecessor's explanation, but suggests another dilating agent—water instead of air—because, on bisecting a subimago under water, no gas escaped from the alimentary canal, while gas did escape when an imago was so treated. On the other hand, Eaton (4, p. 9) states distinctly that gas is present in the alimentary canal when the subimago is about to emerge: "The change from nymph to subimago is effected while the insect is floating at the surface of the water, buoyed up by gas which has accumulated within the alimentary canal and between the new and the old integuments of the body" (*loc. cit.*).

Direct observation of the alimentary canal in living specimens is almost impossible; in the late nymph the accumulation of air between the old and new cuticles renders the body-wall quite opaque, while, in the subimago, the chitin is characteristically dull and impenetrable. Repetition of Fritze's experiment of bisecting the living insect under water confirms his view in the case of the subimago of *Leptophlebia meyeri*, that this time the alimentary canal does not contain gas under pressure, since two minute bubbles alone appear at the cut end of the body, and these only when the body is pinched gently. This experiment has been repeated many times with young subimagines of *Leptophlebia meyeri*, always with precisely the same results.

It has been shown above that in all cases examined, where the gut contents had been expelled before the transformation of the alimentary canal commenced, the lumen was narrower in the late nymph and early subimago than in younger nymphs, and dilation was not observed in the sections until this transformation was nearly completed. The regularity with which this occurred would seem to preclude the possibility of its being due to an artefact, and such a condition is obviously incompatible with a state of high internal pressure. The explanation given by Palmèn and Fritze cannot therefore be accepted.

In following the fate of the muscles in the wall of the mesenteron, Palmèn observed a gradual resolution of the fibres into a homogeneous granular mass "etwa als waren sie fettig degeniert," and he does not suggest that this change is due to the same cause as is the alteration in the epithelium.

Histological research on the higher insects since Palmèn's time has shown

that in many cases the blood corpuscles play an important part in the process of metamorphosis, probably by ingesting and removing from their original situations various tissue elements which are no longer required there. It has been shown earlier in the present paper that prior to the emergence of the subimago there is a characteristic crowding of corpuscles around the mesenteron, and there seems to be no doubt that a process of true phagocytosis of the muscular coat of the mesenteron occurs in the Ephemeroptera. This view is supported by the fact that free cells with large inclusions may be seen in the haemocoele, which are regarded as blood corpuscles containing debris from the break-up of muscle. In many places, corpuscles may be distinguished lying among the muscles at this stage, beneath the very delicate peritoneum which they have penetrated. It is interesting to recall in this regard Palmèn's statement that the condition of the muscles resembles fatty degeneration—an appearance which, according to Pérez (9), constantly accompanies phagocytosis.

In a Chrysomelid beetle, Poyarkoff (10) describes a metamorphic process in the hypodermis which resembles in many respects the change here described in the mesenteric epithelium of *Ephemera danica*. According to Poyarkoff, certain cells in the affected tissue elongate and divide mitotically while the larva is still active. A single large granule appears in the nuclei of such cells and is passed into the cytoplasm, thence into the haemocoele or an intercellular space, together with a mass of cytoplasm which surrounds the granule. Poyarkoff regards this as a process of "rejuvenation" whereby a cell "peut passer a un état moins différencié en rejetant une partie de sa substance, et se différencier ensuite dans un autre sens."

The process described for the Ephemeroptera, however, differs from "rejuvenation" as defined by Poyarkoff in the following particulars:—

(1) It has not been possible to correlate the process in Ephemeroptera with a preceding nuclear division.
(2) The vesicles of rejected cell matter contain granular inclusions which are not definitely known to be of nuclear origin.
(3) Eliminated matter is passed into the lumen of the gut and not into the haemocoele or an intercellular space.
(4) Cells which have undergone the process remain reduced.

In spite of these differences, it may be possible to reconcile the phenomena in the Ephemeroptera with true rejuvenation up to a point.

(1) It is doubtful to what extent cell division is an essential part of the process of rejuvenation. Tiegs (14) considers that metamorphic processes such as rejuvenation occur when the volume-surface ratio of cells becomes too great for efficiency in nutrition and respiration; the natural reaction to such a condition in cells forming a tissue would be volume reduction either by throwing out cell material or by cell division, but probably not both in the simplest cases.
(2) Although the granules present in the eliminated vesicles have not been traced to a nuclear origin, it seems probable that nuclear material is voided by the cells since there may be reduction of chromatin material to the point of disappearance of the nuclei.
(3) The mode of disposal of eliminated matter may be explained by a consideration of the situations of the tissues involved in the two cases. It must be admitted, however, that material passed into the haemocoele might become available for a subsequent histogenesis,

while material passed into the lumen of the gut is not likely to become available.

(4) Although in the Ephemeroptera, cells which have parted with the bulk of their substance remain in a reduced condition throughout the rest of the life of the insect, it is probably incorrect to say that re-differentiation does not occur in the mesenteric epithelium. At the beginning of the reduction process there is one type of tissue, and at the end there is another—both adapted to special purposes.

There seems to be at least a prima-facie case for considering tissue reduction in the Ephemeroptera homologous with rejuvenation.

Carpenter (1, pp. 192 and 333) regards the Ephemeropteran subimago as the homologue of the pupa of Holometabola. He suggests that one ecdysis after the development of functional wings was originally the rule, and that the first winged instar is represented in Ephemeroptera by the subimago, in Holometabola by the pupa. In support of this, he adduces the large size of the external wing rudiments in the less specialised pupae, such as those of many Coleoptera and Neuroptera and the relatively great activity of those pupae.

This suggestion is in accordance with the facts observed in the present study, and, together with those facts, prompts the following view of the development of the insectan metamorphosis. In the Ametabola, formation of the imago occurs gradually during nymphal life and the extent of the development becomes apparent externally after each ecdysis. During the course of the evolution of higher insects, this development becomes postponed to the later instars in the case of a greater or lesser number of organs and tissues, either by a progressive holding up of development or, on Tiegs's view, by a loss of the power of division by the nymphal cells. In the latter case, formation of imaginal tissue only begins when the volume-surface ratio is too great for efficiency and cellular death is threatened. Development in either event would tend to be concentrated into the later nymphal instars and even to extend into the subimaginal stage which we are postulating as common to all insect life-histories. Such a condition persists, as we have seen, in the life-history of some Ephemeroptera such as *Ephemera danica*. Still later in phylogeny, more of the tissue transformation became postponed until the subimaginal stage was attained, so that although the processes continued to be initiated in the nymph, they became more and more definitely associated with the subimago. Greater specialisation in the form of both nymph and imago resulted in a more profound metamorphosis occurring in the subimago, which consequently became incapable of active life, and so the quiescent pupal stage was derived. Although the recent Ephemeroptera cannot be regarded as on the direct line of evolution of the Holometabola, the Order exhibits within itself a parallel to part of such a course of development, for, as has been shown, in *Ephemerella ignita* and *Baëtis* spp., more of the transformation is held up until the subimago emerges than in the case of *Ephemera danica*. A study of wing-venation in these types tends to the view that *Ephemera danica* is the most primitive of the three and *Baëtis* the most specialised.

## 5. Summary.

1. An extensive change in the structure of the mesenteron occurs in the types studied, during which the epithelial and muscular layers become reduced and form in the imago an extremely thin membrane.

2. This change commences in the last nymphal instar shortly before the emergence of the subimago, and is completed in the latter stage or immediately after the imago emerges.

3. The attenuation of these layers is not brought about by simple mechanical pressure, as has been suggested, but is accompanied by phagocytosis and a phenomenon considered to be comparable to "rejuvenation."

4. The mesenteron is the principal region of the alimentary canal affected, but a specialised region of the stomodaeum is involved, and there may be some attenuation of the ileum and colon in addition.

5. Arguments are adduced in support of the view that the subimago is the homologue of the pupa of Holometabola.

## 6. Acknowledgements.

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## Explanation of Plates V and VI.

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### *Abbreviations.*

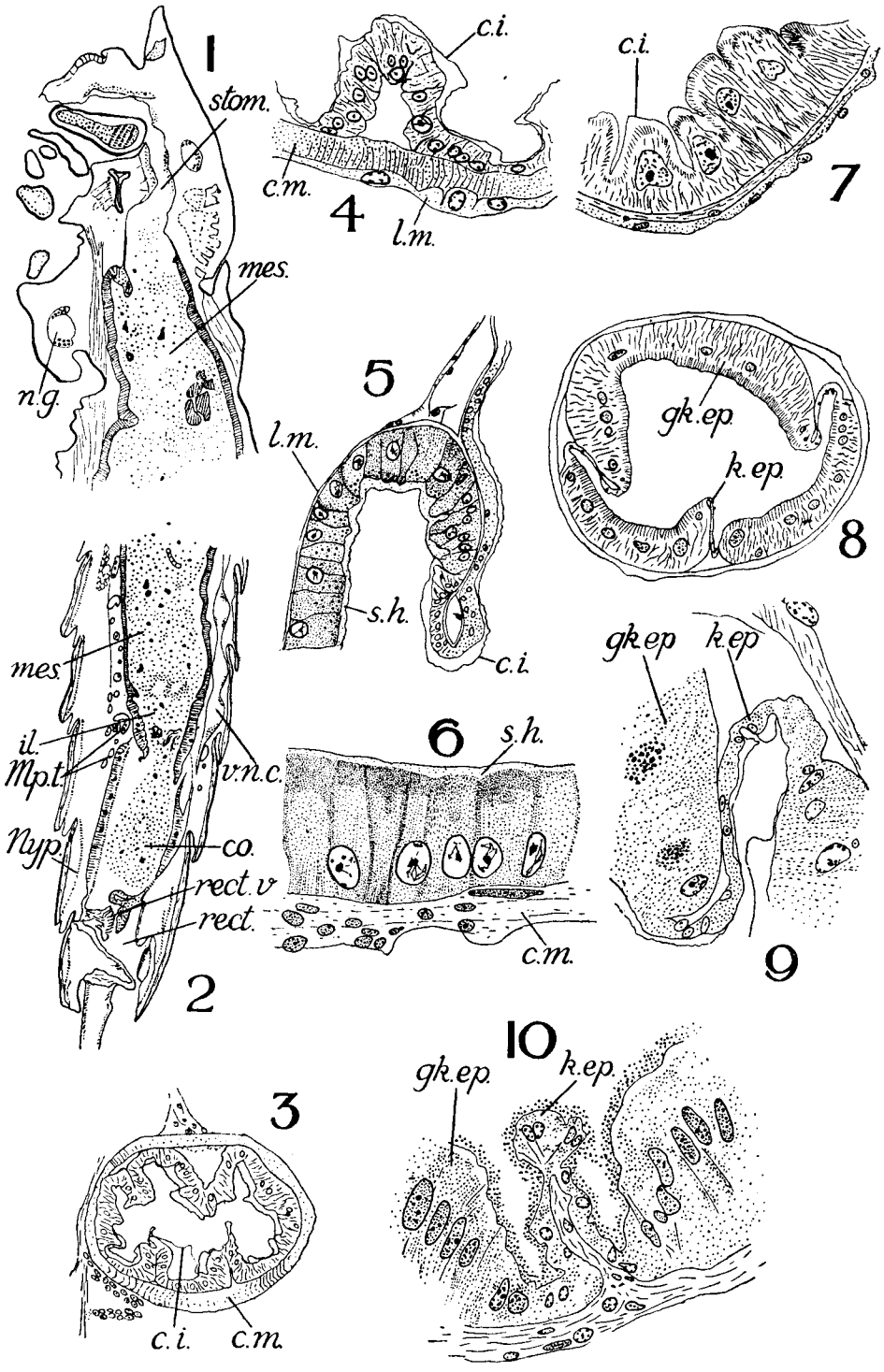
| | | | |
|---|---|---|---|
| an. | anus. | mes. | mesenteron. |
| b.c. | blood corpuscles. | n.g. | nerve ganglion. |
| c.i. | chitinous intima. | od. | oviduct. |
| c.m. | circular muscle layer. | ov. | ova. |
| co. | colon. | oes.v. | oesophageal valve. |
| ep. | epithelium. | p. | peritoneum. |
| gk. ep. | "grosskerniges epithel." | rect. | rectum. |
| hyp. | hypodermis. | rect. v. | rectal valve. |
| il. | ileum. | s.h. | striated hem. |
| k. ep. | "kleinkerniges epithel." | stom. | stomodaeum. |
| Mp. t. | Malphigian tubes. | v.n.c. | ventral nerve cord. |

### Plate V.

Fig. 1. *Ephemera danica ;* nymph; head and thorax, L.S.
2. *Ephemerella ignita ;* nymph; abdomen, L.S.
3. *Ephemera danica ;* stomodaeum, T.S.
4. *Ephemera danica ;* part of stomodaeum, T.S., more highly magnified.
5. *Ephemera danica ;* nymph; L.S., through the oesophageal valve.
6. *Ephemera danica ;* nymph; T.S., through part of wall of mesenteron.
7. *Ephemerella ignita ;* nymph; T.S., through part of wall of ileum.
8. *Ephemerella ignita ;* nymph; colon, T.S.
9. *Ephemerella ignita ;* nymph; part of colon, T.S., more highly magnified.
10. *Ephemera danica ;* nymph; part of colon, T.S.

### Plate VI.

Fig. 11. *Ephemerella ignita ;* nymph; rectum, T.S.
12. *Leptophlebia meyeri ;* nymph; part of mesenteron, T.S., showing an early stage in metamorphosis.
13. *Ephemera danica ;* nymph; part of mesenteron, L.S., showing an early stage of metamorphosis.
14. *Ephemera danica ;* nymph; part of mesenteron, L.S., a later stage, showing a phase of the supposed "rejuvenation."
15. *Ephemerella ignita ;* nymph; part of mesenteron, L.S., showing a stage in metamorphosis.
16. *Ephemerella ignita ;* subimago; mesenteron, T.S.
17. *Ephemerella ignita ;* subimago; part of mesenteron, L.S.
18. *Ephemerella ignita ;* subimago; part of mesenteron. A later stage.
19. *Ephemerella ignita ;* subimago; part of mesenteron. A later stage.
20. *Ephemerella ignita ;* ♀ imago; abdomen, T.S.

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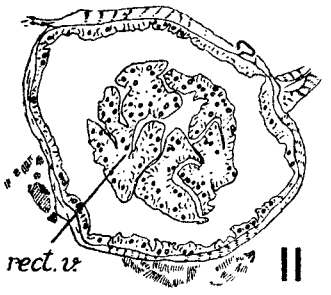


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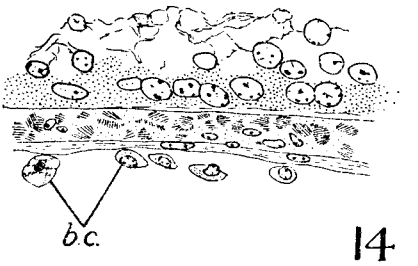


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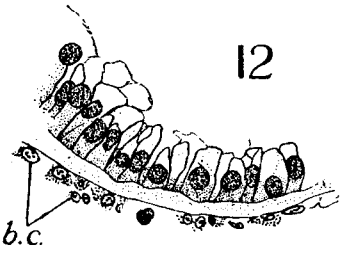


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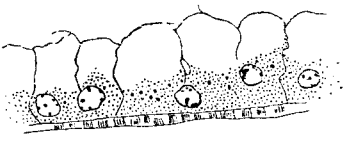

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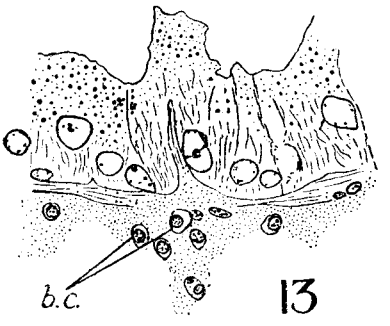


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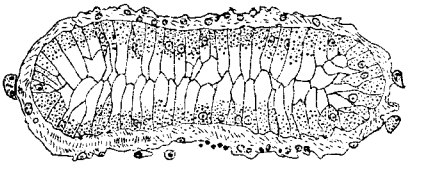

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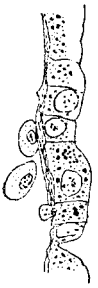

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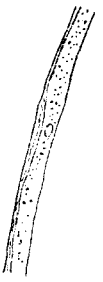

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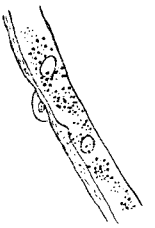

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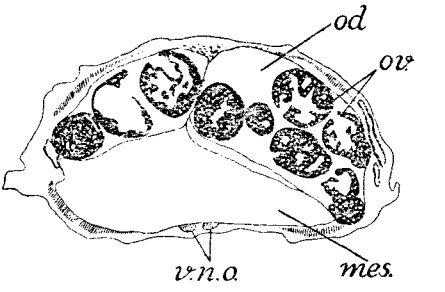


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