THE HISTOLOGY OF THE WING PADS OF THE EARLY INSTARS OF *PTERONARCYS PROTEUS* NEWPORT (PLECOPTERA)

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Several very thorough papers have been published on the wing development of holometabolous insects. The differentiation of the wing, however, is so rapid that investigators are by no means unanimous as to the mode of formation of the blood lacunæ about which the adult wing veins subsequently form. Marshall (1913), studying *Platyphlax designatus* Walker, a trichopteran, stated that lacunæ develop in the wing Anlage of the last nymphal stage and that tracheæ do not enter until the wing has everted. Hundermark (1935) investigated *Tenebrio molitor* and reported that tracheæ grew into the newly everted wing disc and that later lacunæ formed about them. Kuntze (1935) in his exhaustive studies of *Philosamia cynthia* Drury (Lep.) claimed that lacuna formation preceded the entrance of the tracheæ and that lacunæ developed inward from the margin. Waddington (1939) speaks of the formation of vein lacunæ in Drosophila wings as being the fusion of small local lacunæ along the site of the presumptive adult vein. He does not refer to the method of formation of the small lacunæ nor are wing tracheæ mentioned in his paper.

From these studies and other investigations on holometabolous insects, it is not possible to determine the ancestral mode of lacuna formation. To elucidate the problems of lacuna formation and differentiation of wing epithelium one would logically turn first to the Heterometabola, but no histological investigation of any scope has been undertaken. Tower (1902) sectioned a first stage nymph of *Anasa tristis* De Geer (Hem.) and saw disc-like hypodermal thickenings in the dorso-pleural region on the wing bearing segments. At the time of the first moult the discs evert to form wing

pads. Formation of a disc and its subsequent eversion also occurs in holometabolous forms. Regarding *Microcentrum latifolium* (Orthoptera), Tower reported that the first instar has well developed wing pads, which everted in the embryo but do not become external until the first nymphal moult. He does not note first occurrence of lacunæ.

Sulc (1911) sectioned the early instars of *Philaneus lineatus* (Hom.) in order to reconstruct the tracheal system. He found that in the first instar tracheæ do not extend to the site of the wing pad, which is later invaded by two tracheæ in the second instar. He found four longitudinal tracheæ in the wing pad of the third instar, a pattern which is elaborated in the fourth and fifth stages. Curiously enough, he did not describe or figure lacunæ, although they have been illustrated in non-histological works on wings of Homoptera.

Beck (1920) discussed the development of the wing vein in *Phyllodromia* (*Blatta*) *germanica;* this was not histological, but was concerned with the development of the wing's tracheal system throughout the life of the roach. Neither the advent nor the differentiation of lacunæ is mentioned.

In order to investigate the differentiation and growth of wing epithelia and to ascertain the relationship between the development of lacuna and trachea, *Pteronarcys proteus* Newport was chosen. This insect is a primitive plecopteran with at least fourteen nymphal stages. The writer was fortunate in obtaining several early instars which were studied exclusively by serial sections, since whole mounts of entire individuals or of wing pads do not show lacunæ. Specimens were fixed in Kahle's fluid (two hours), sectioned in paraffin at 6μ without difficulty or loss of sections and stained with Mallory's triple connective tissue stain.

The length of the specimens is not given as it is an unreliable index of a particular instar. Body length varies among fixed specimens: some are caused to telescope by the action of the fixative, others are relaxed at full length. Head width, however, enables one more definitely to determine the stadium to which a specimen belongs.

First Instar

Head width, .408 mm. A single specimen was obtained and sectioned. The wing anlage is not a disc of cells de-

veloped in the embryo and everted upon eclosion. The only external indication of wing buds in the first instar is a small flange where the pleurum and the tergum join. This flange can well be regarded as a lateral expansion of the tergum. Hypodermal cells on the tergal portion of the thoracic expansion grade from pavement epithelium to tall columnar cells, as one proceeds laterally. Lacunæ are not yet developed, nor are tracheæ associated with the anlage.

Second Instar

Head width .54 mm. Two specimens of this stage were studied: one, in the inter-moult phase; the other, just prior to its moult to the third instar. In the inter-moult specimen both meso- and metathoracic analgen have attained the same degree of development. A well marked sinusoid runs along the outer margin of each wing expansion, and is incomplete in that it does not communicate with the hæmocœle either anteriorly or posteriorly. In the left rear wing the sinusoid is 9μ in length and is occluded in one place by hypodermal cells suspended in it. In the other metathoracic expansion the sinusoid is evidenced by 20μ and 30μ lengths, separated by an occlusion.

Histological examination shows the sinusoid to be the result of attenuated cell ends not going directly to the basement membrane of the hypodermis. Instead the processes merge with the ends of neighboring cells to leave a space. Precisely the same sort of sinusoid is found in the prothoracic expansion. It follows, therefore, that the sinusoid is not the costal lacuna. It may be termed the precostal sinusoid, for it occurs peripherad of costa in all nymphal stages. It is a false lacuna situated at the base of large bristles and may be the site of trichogen cells. In nymphs about to moult the precostal sinusoid is obliterated by large trichogen cells.

Prior to the second moult the costal lacuna is developed. It is a space between the basement membrane of the wing epithelia and is readily distinguishable from the precostal sinusoid which is an intercellular space. In the late second instar costa is a marginal lacuna which communicates anteriorly and posteriorly with the hæmocœle. Although a few blood cells are present in the posterior portion of the lacuna it is not certain that blood can circulate because the anterior

opening is very small. The costal lacunæ of the right fore, right hind, left fore and left hind wing pads measure 168μ, 162μ, 160μ and 140μ respectively.

With the exception of the right hind wing, the single trachea to the wing disappears in the dorsal epithelium of the bud, and does not approach costa or form a lacuna. In the right metathoracic flange, however, the trachea is worthy of note for its exceptional behavior. The trachea travels into the costa of this wing, the only instance where it does so in twenty-eight wing pads of early instars studied by serial section. 60μ from the site of apposition of the trachea to the dorsal wing epithelium, and 18μ past the anterior end of costa, the trachea enters the lacuna. In the next 42μ, the trachea moves across the lacuna into the ventral epithelium where it may be followed for 22μ to its end. As the trachea enters costa 18μ away from the lacunal opening it is obvious that the lacuna is not caused by a boring in of the trachea, nor does the diameter of the lacuna taper to conform to the tapering trachea.

In each segment of the pterothorax a nerve leaves the interganglionic connective at a point immediately anterior to the ganglion. The nerve associates itself with the wing trachea and enters with it into the dorsal wing epithelium. The nerve could not be traced further.

Because tracheæ are often considered to be inductors of lacunæ, it is well to digress upon the anatomy of the tracheal system. In the second instar a trachea extends to the wing for the first time. (It is the more anterior of the two branches which supply the wing of older nymphs. The posterior branch does not appear during the late third instar.) A large longitudinal trachea runs laterad of the alimentary tract, and in the wing bearing segments the longitudinal trachea receives perpendicularly an equally large branch from the anterior gill. From the junction of these large tracheæ to the corresponding junction in the succeeding segment runs a smaller trachea which arches out peripherally from the main longitudinal trachea. From the peripheral arch branches extend to the wing, leg, and posterior gill. In the late third instar a trachea extends for the first time to the posterior portion of the wing flange. This is the state of pterothoracic main tracheæ during the second, third, and fourth instars.

Third Instar

Head width, .68 mm. Killed early in period of cell division and growth preceding fourth instar. The old endocuticula is partly resorbed and the new integument is partly secreted.

The apposition of nerve and trachea is the same as that of the preceding instar. Costa communicates anteriorly and posteriorly with the hæmocœle and does not contain a trachea. Subcosta appears for the first time in the life history of *Pteronarcys proteus* during the period of hypodermal cell division in the last phase of the third stadium. Subcosta is open at the anterior end and opens posteriorly into costa at a point near to where the latter empties posteriorly.

In the right forewing 48μ posterior to the entry of the trachea onto the wing epithelium, one observes the opening of subcosta, and 30μ posterior to that opening is the point of entry of the trachea into the lacuna. The trachea is therefore associated with the wing epithelium for 78μ without having created an intercellular space, much less a lacuna. Once within the subcosta, the trachea travels for 48μ and moves from the dorsal to the ventral epithelium. The trachea ends outside of the lacuna 6μ before subcosta ends. With minor variations the behavior of the trachea in each of the three subcostas is the same. A small cross vein has already developed 60μ anterior to the distal end of subcosta.

Because the entry of the trachea into subcosta is so far removed from the anterior opening of the lacuna, subcosta could not possibly have been induced by the mechanical boring in of the trachea. Subcosta is not centered with respect to the trachea once it does enter. The lacuna does not taper, whereas the trachea tapers to nothing. Evidently the trachea does not induce the lacuna.

Head width, .79 mm. Killed late in period of cell division and growth prior to the moult to the fourth instar. Examination of the integument showed the old integument to be almost completely resorbed and the integument of the fourth instar in great part to be laid down.

The structure of the wing in this specimen differs from the early growth phase third instar. At the anterior margin of the wing a large trachea becomes applied to the dorsal wing epithelium. 24μ posterior to the point of application a lacuna appears but is not associated with the trachea. The

lacuna is divided into three smaller lacunæ by cell processes forming partions. As the wing flange broadens posteriorly, the three lacunæ become separated in space by added cell processes which form a typical middle membrane. 48μ posterior to the first appearance of the lacuna, the trachea becomes associated with the most mesal lacuna. Posterior to this point the innermost lacuna bifurcates. 120μ from the opening of costa and 120μ from the posterior margin of the wing pad, the trachea goes deeply into the ventral epithelium and tapers to an end.

If one numbers the lacunæ inward from the margin, number three appears to be the new structure, and is therefore probably radius. The subcosta in the early growth phase third instar runs midway between costa and the hæmocœle, and sends a short branch to the anterior margin of the wing near its apex. The second lacuna in the late growth phase third instar has the same position and structure as it did in the early growth phase, whereas the new (third) lacuna runs next to the hæmocœle and forks at mid-length of the wing. The left metathoracic wing has the same anatomy with minor differences. The two remaining wings were compressed locally when they were sectioned so that one cannot follow the trachea. The three lacunæ, however, are readily identified. Four additional lacunæ, each about 50μ in length, make their initial appearance at the apex of the wing pad. Further study is necessary to determine their identity.

Fourth Instar

Head width, .88 mm. Four specimens were sectioned serially and examined. The anatomy of the wing pad is the same as that of the late growth phase third instar.

General Consideration and Conclusions: Before one is able to characterize vein lacunæ, one has to study blood lacunæ in the prothorax. When the costa is formed in the second instar, a blood lacuna develops in the prothoracic flange and like the costa runs in an arc to communicate anteriorly and posteriorly with the hæmocœle. Similarly, at the time of differentiation of subcosta, the prothoracic flange adds another lacuna which is entad of and parallels the first. (Later instars show that several more lacunæ are added.) Prothoracic flange epithelia do not oppose their basement

membranes to form a middle membrane. Prothoracic lacunæ therefore differ histologically from vein lacunæ in having a diameter from 2½ to 4 times as great, and are not middle membrane structures.

From a study of the early instars of *Pteronarcys proteus* one concludes that: (1) the sole histological distinction of vein lacunæ is their middle membrane structure. One cannot say that vein lacunæ are also distinguished by tracheæ because costa rarely contains one, and costa in the early instars does not differ from subcosta or radius. (Costa, always an ambient structure, did contain a trachea in one of about 60 examined specimens of all ages. The other longitudinal lacunæ of older instars invariably contain both tracheæ and nerves. Costa never contains a nerve.)

(2) Lacunæ are not induced by tracheæ.

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Description of Plate VI.

Transverse Sections of *Pteronarcys proteus* Newport.

I. Metathoracic flange, x294, first instar, right side. The site of the wing disc is marked by the transition of pavement epithelium to tall columnar cells.

II. Mesothoracic wing pad, x294, second instar, left side. Precostal sinusoid and a middle membrane present. No lacuna or trachea developed.

III. Prothoracic flange, x294, second instar, left side. False lacuna present but a middle membrane not developed.

IV. Mesothoracic wing pad, x294, late growth phase second instar, left side. Costal lacuna is present.

V. Prothoracic flange, x294, same specimen as no. IV, left side. Blood sinusoid present.

VI. Prothoracic flange, x294, early growth phase third instar, right side. Two blood sinusoids are present. Middle membrane is not developed.

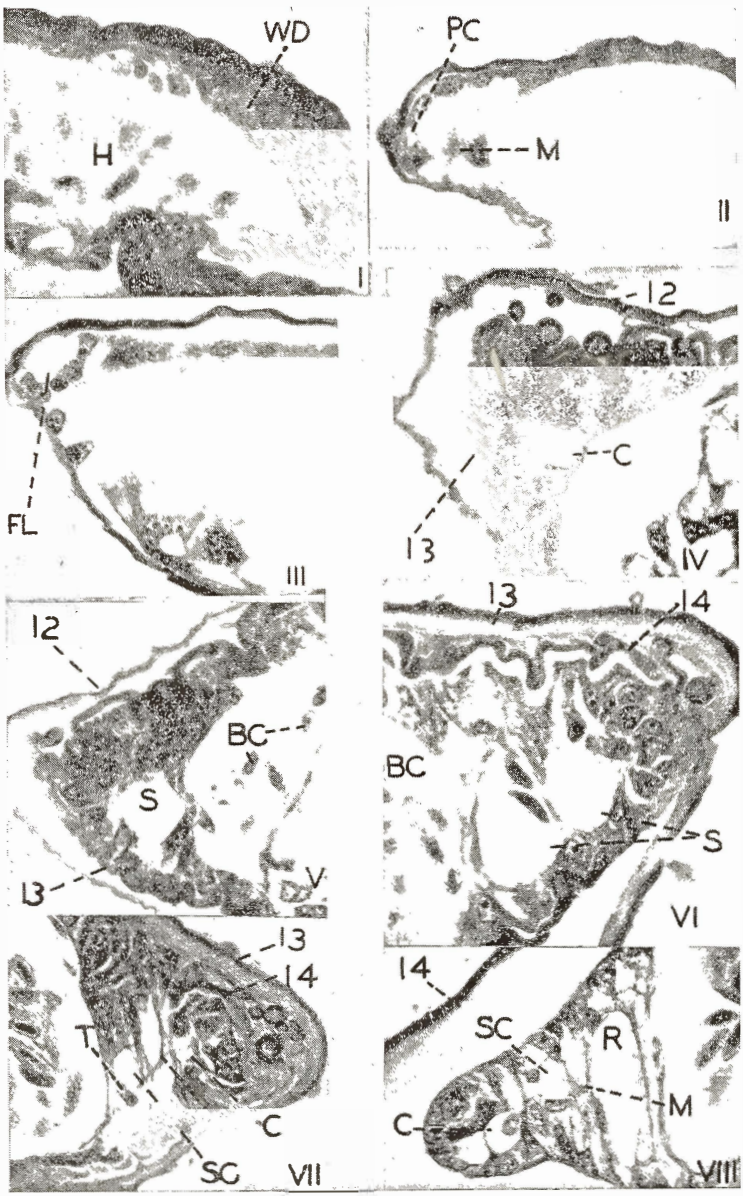
VII. Mesothoracic wing pad, x206, same specimen as no. VI, right side. Subcosta has formed.

VIII. Mesothoracic wing pad, x290, fourth instar, left side. Three lacunæ are visible.

Key to Abbreviations

BC ..... Blood cells
C ..... Costa
FL ..... False lacuna
I2, I3, I4 ..... Integument second instar, etc.
M ..... Middle membrane
P ..... Precostal sinusoid
SC ..... Subcosta
R ..... Radius
T ..... Trachea
WD ..... Wing disc

PSYCHE, 1940 VOL. 47, PLATE VI.



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