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THE DEVELOPMENT OF THE WINGS IN THE LEPIDOPTERA.

PLATES I–V.

By William Fairfield Mercer.

The development of the wings inside of the body of the larvæ of insects with a complete metamorphosis is one of the most striking features in the transformations of insects; it is, however, one that is not generally understood. Although the fact that caterpillars have wings was discovered by Malpighi (1687) and by Swammerdam (1738), it is only in the last half of the present century that any exact information as to their method of development has been obtained; and this information is only just finding its way into the general text-books.

The beginning of the solution of the problem was made by Weissmann('64 and '66) in his remarkable papers on the development of flies. These papers were followed by important contributions by Landois ('71), Ganin ('76), Dewitz ('81), Pancritius ('84), Schäffer ('89), Mayer ('96), Gonin ('92), and others. The most complete account that has yet appeared is that of Gonin.

Nearly two years ago, in the course of some studies of the development of the wings of *Pieris*, Professor Comstock came to the conclusion that in some respects the excellent account by Gonin was inaccurate. And at his suggestion I undertook the repetition of the tracing of the development of the wings in this insect. The following paper is the result of this investigation.

As Gonin's contribution appeared in a journal which is to be found in but few libraries in this country, it seems worth while to publish for American readers my complete results even though in many particulars they duplicate the results of Gonin.

It is with pleasure that I acknowledge the aid of Professor Comstock and his able corps of assistants; without this and the resources of the entomological department of Cornell University, which were put at my disposal, this investigation would have been impossible.

THE LIFE-HISTORY OF *Pieris*.

The observations on the number and duration of the stages of this insect were made on the July brood, which is one of the four broods of the year. The butterfly lays its eggs upon the food-plant of the caterpillar, sticking one at a time upon the leaves at varying distances. The egg hatches in from four to five days. As the caterpillar emerges, the head measures .36 mm. Excepting the first, the ratio of increase in head measurement at each moult is 1.55; in the case of the first moult it is slightly greater.

The caterpillar moults for the first time in from 30 to 48 hours after hatching. The head measures after this moult .6 mm. The caterpillar moults for the second time in from two to three days after the preceding moult, and the head measures .93 mm. In one day it moults again, and the head measures 1.44 mm. In one day more it moults again, and the head measures 2.22 mm. After four days it moults for the pupa, and seven days later the adult emerges. The butterflies pair when about 24 hours old, and the female at once begins to lay the eggs for a new brood. The whole life-cycle covers a period of about 22 days. The time of these changes varies somewhat with the nourishment and the temperature. These points are summed up in the following table:

		HEAD MEASUREMENT.
Egg	4 to 5 days.	
1st larval stage	30 to 48 hours	.36 mm.
2d " "	2 to 3 days	.6 mm.
3d " "	1 day	.93 mm.
4th " "	1 day	1.44 mm.
5th " "	4 days { 3 days active. 1 day hung up. }	2.22 mm.
Pupa	7 days.	
Emergence to egg laying (Pair in 24 hours, begin to lay almost immediately).		

The caterpillar eats much and grows rapidly, and seemingly has nothing to do but to store up energy and fat to bring out the adult insect.

In moulting, the cuticle splits down the dorsal line of the first and second thoracic segments. During the latter part of the last larval stage the caterpillar ceases to eat and prepares to hang itself up for the pupal life. The approach of this event may be detected from the red color of the excrement, which is supposed to come from the cast-off cells of the epithelial wall of the alimentary canal. At the same time the caterpillar is searching about for a suitable place to hang itself up. It soon forms a button of silk to which it holds with its caudal prolegs and at once begins its girdle of silk over the thorax, fastening the ends to its support on either side of its body. The caterpillar is now shorter and changed in form somewhat as a preparation for pupation, which will take place in about 24 hours.

Throughout this discussion the term prepupal period is applied to the time the caterpillar is hung up before pupation. This period is characterized by the fact that the wings are outside of the body wall although beneath the larval cuticle, the change in position taking place while the caterpillar is spinning the silken support for the pupa stage.

TECHNIQUE.

The caterpillars were killed in hot water, small caterpillars being left in one-half minute, and large ones one minute.

Perenyi's fluid gave the best results as a fixing reagent. In fixing for wings the caterpillars were cut caudad of the third thoracic segment, and also the head was removed before placing them in the fluid. The chitinous coat that covers insects is very hard to penetrate, therefore to get a good fixation the greatest care is necessary. A large amount of the fluid in proportion to the caterpillars was used, the thoracic segments of from six to ten caterpillars being placed in from 30 cc. to 50 cc. of the fluid. The fluid was heated to 50° C., and the caterpillars transferred directly from the hot water and left from 8 to 12 hours in the fixing fluid. They were then hardened in successive grades of alcohol as follows:

70% Alcohol	24 hrs.
80% "	24 "
95% "	24 "
Absolute alcohol	12 "

Absolute alcohol and cedar oil, equal parts, 12 hrs.

Cedar oil, 24 hrs., or until the specimen was thoroughly cleared.

In the process of imbedding, the infiltration must be carefully done. To insure the best results, it is well to get the specimens into the hard paraffin and set them back into the oven for a few hours. This insures a uniform medium for cutting.

Sections cut from ten to fifteen microns thick gave the best results for the general outline, while for the finer cell structure sections seven microns thick were better. Sections were stained on the slide with hematoxylin and counter-stained with safranin to bring out the chitin. When staining with safranin it is necessary to leave the slides in for at least 24 hours, then differentiate very rapidly, dehydrate, clear, and mount all within a very few minutes. Orange G. was also found to give good results as a counter-stain for chitin.

For the study of the wings as a whole it is necessary to dissect them from the body and mount in glycerine jelly; the jelly must be cooled quickly in order to insure the tracheæ not being filled with it. To accomplish this, lay open the larva along the dorsal line and pin the cut edges of the tegument to the wax bottom of a dish, all being done under water. The wings are recognized as small flat bodies a little longer than wide. Figure 12 shows a drawing made from a photograph taken and mounted as above. The dissection of the pupal wings to bring out the tracheæ, for the study of the wings as a whole, is nicely brought about by placing the pupa in 4% formal for from six to ten hours; this hardens the tissues and at the same time leaves the air in the tracheæ, which appear as black lines under the microscope.

THE FIRST LARVAL STAGE. (Plate I, Figs. 1, 2, and 3.)

The wing-buds are found in the very earliest stages of the larval life; embryos were not examined. Figure 1 represents a section through the body-wall of one side of a thoracic segment at a point where a wing-bud is developing; *c* represents the cuticle and *h* the subjacent layer of cells, the hypodermis. The section was taken from a larva which was not more than one-half hour old. At a point opposite a trachea (Fig. 1, *t*) the cells of the hypodermis are elongated; these elongated cells constitute the rudiment of a wing or a wing-bud. Rudiments like this of organs that are not to be functional till the adult stage are often termed imaginal discs or imaginal buds. Near the center of the outer surface of this group of cells forming the

imaginal bud there is a slight depression beneath the cuticle (Fig. 1, *b*).

As the larva advances in age during this stage (Figs. 2 and 3) the elongation of the cells is increased, the depression enlarged, until finally the imaginal bud (Fig. 3, *ib*) is quite distinct and a thickening of the cuticle appears opposite a depression in the imaginal bud. This thickening began to show even in the early part of the stage. Near the trachea and the wing-bud there is in each case a group of cells which bear some resemblance to leucocytes and which were termed by Verson embryonic cells (*ec*); these are believed by Schäffer ('89) to be a sort of lymphatic gland, a center in which leucocytes are developed. From the study of these figures it is easily seen that the imaginal bud (*ib*) in this stage is simply a thickening of the hypodermis.

THE SECOND LARVAL STAGE. (Plate I, Fig. 4.)

In passing from the first stage to this, the growth of the wing-bud has been gradual and not very extensive. The cells have elongated somewhat and the small pit, referred to in the first stage, has increased to a definite invagination into which extends a chitinous plug (Fig. 4, *b*) from the cuticle.

THE THIRD LARVAL STAGE. (Plate I, Figs. 5, 6, and 6*a*.)

After the second moult the cells of the wing-bud have increased in length, the invaginating process has been going on until the wing-bud is deeply sunken in the body cavity; it remains, however, connected with the hypodermis by a thin membrane, the peripodal membrane (Fig. 5, *e*). The term peripodal membrane was proposed by Van Rees ('88) and was suggested by the fact that in the internal development of the legs of flies a similar membrane is formed; as the leg-buds and the wing-buds closely agree in structure the terms applied to parts of the leg-buds are also applied to the wing-buds. Figure 6 represents a section at a point which shows the peripodal membrane (*e*) on both sides. The trachea has increased in size. The plug of chitin at the opening of the invagination (Fig. 5, *b*) is readily noticed. To get a clear idea of the invagination it is necessary to study a series of sections through it. A section through the center of the invagination will show the chitinous plug (Fig. 5, *b*) and the opening into the

invagination. At a point two or three sections distant from the center, the invagination is closed in by the hypodermis of the body-wall (Fig. 6, *b*). The opening of the invagination is, therefore, a narrow pore.

The single layer of the hypodermis appears to pass into two or more layers in the wing-bud, but upon a careful examination it will be seen that there is but one layer, the nuclei of the cells being at different levels, while the cell walls may be traced from one side of the wing-bud to the other. In this stage, at the point of nearest approach of the trachea to the wing, there is a slight depression and the cells of the trachea are elongated slightly on the side toward the depression (Fig. 6).

Different specimens of larvæ in the third stage exhibit a great difference in the appearance of the basement membrane of the hypodermis of the body-wall. In some it is distinguished with difficulty, being merely a line limiting the inner ends of the hypodermal cells ; which, in this case, are the same diameter throughout their length. In others the inner ends of the hypodermal cells are prolonged into delicate fibers between which are spaces ; in this case the basement membrane connecting the tips of their fibers is remote from the main body of the cell (Fig. 6*a*, *bm*). In both cases the basement membrane of the wing-bud, which is directly continuous with that of the hypodermis of the body-wall, appears merely as a line bounding the ental surface of the bud. The tissue of the bud is solid, without the spaces represented in Figure 6*a*. It is, therefore, difficult in some specimens to distinguish the basement membrane of the wing-bud in this stage.

THE FOURTH LARVAL STAGE. (Plate I, Figs. 7–11 : Plate II, Fig. 15.)

Sections of a larva taken in this stage (Figs. 7 and 8) show that the process of invagination has been going on at a rapid rate. The slight depression in the region of the trachea in the third stage (Fig. 6) has increased to a prominent evagination. It will be observed that in the course of this internal development of the wing there is first an invagination of the hypodermis, and later an evagination of a portion of this invagination. The evaginated portion is what is destined to become the wing ; while the remaining, thinner portion of the invagination forms the peripodal membrane.

It is in this stage that a temporary set of tracheoles is developed. The cells of the epithelium of the large trachea near the wing-bud

(Fig. 7, *T*) are elongated on the side toward the evagination of the wing (Fig. 7, *mct*). A little later in this stage these cells appear very much larger with large nuclei (Fig. 8, *mct*). These cells are destined to form the tracheoles; and may be termed the mother cells of the tracheoles. In some of these cells, very fine tubes are seen, through which the light passes in the section. This is the first appearance of the temporary respiratory organs of the wing, or the tracheoles. Later in this stage the cells have wholly disappeared except the nuclei, and in their place are great bundles of capillary air tubes, the tracheoles, which differ from tracheæ in lacking the spiral thickening of the intima (Fig. 15, *tl*). The nuclei of these mother cells of the tracheoles do not disappear until after the development of the tracheoles (Figs. 10 and 11). It is evident from this that the tracheoles are not formed, as Landois supposed, from the nuclei of the cells, but from the body of the cells as described by Gonin. Figure 11 shows a group of cells in cross section, very much enlarged. In two of them the nucleus is cut through and appears, while in the other two the razor has not passed through the nucleus. In cutting across a group of cells containing a bundle of tubes, the section would show round holes, which is the case with these cells. Two other cells (Fig. 10) are shown in longi-section. These show the nuclei with a bundle of tubes surrounding them.

During this stage the tracheoles have no communication with the lumen of the large trachea about which they are developed. But as the new intima (Fig. 15, *nin*) is formed, which is to line this trachea in the next stage, it is not extended over the mouths of the tracheoles. It will be seen, therefore, that on the removal of the old intima (Fig. 15, *oin*) at the following moult, the air in the lumen of the trachea will have free access to the mouths of the tracheoles (Fig. 15, *mt*) which now become functional.

Proliferation of the Tracheæ.—At the very beginning of the fifth stage at the latest, and there is considerable evidence that in the latter part of the fourth stage, there begins the development of the permanent wing-tracheæ. These arise as a pocket-like evagination of the epithelial walls of the trachea that gives birth to the tracheoles. This is shown diagrammatically in Figure 9, *et*. This evagination is increased in size, and others are formed, some of which branch and extend into the lumen of the wing, following in a general way the course of the bundles of tracheoles. Figure 9 is diagrammatic to show more clearly

what is suggested in Figure 14, *T*. These evaginations are extended into the lumen of the wing, which process takes place while the tracheoles are becoming completed and straightened out. The lumen of these small tracheæ in the wing has no connection with the main lateral tracheal trunk (Fig. 9, *lt*), since they are closed by the pre-existing intima, therefore these tracheæ are not functional. As the tracheæ lengthen the tracheoles straighten out and extend to the margin of the wing. The tracheoles occupy the space, the vein cavity, along the course of the tracheæ as they push out into the wing. Verson thought that the tracheoles never reached the margin of the wing, but my observations bear out those of Gonin who says they do extend to the margin of the wing unless hindered by the more dense portion of the edge of the organ. It is probable that the pressure of the air is the main agent in straightening out the tracheoles.

THE FIFTH LARVAL STAGE. (Plate II, Figs. 12–14, 16, 17. Plate III, Fig. 23.)

In the study of this period two methods were used, that of direct observation of the entire wing-bud and that of the study of sections.

The external lateral trachea sends out branches to the wing and these enter it at about one third of the distance from the hypodermal attachment to the margin and give the appearance, as Gonin states, of the stem of a nasturtion leaf (Fig. 12). The tracheæ seem to enter as into an arm pit and the tracheoles extend from this point to all parts of the organ, even to near the margin where the cells are denser and do not allow them to penetrate. The tracheoles extend in masses of bundles from the trachea as previously described. Tracheæ, being filled with lymph at this stage, are not easily seen in the entire wing-bud, but the photograph for Figure 12 was taken under very favorable conditions and they are faintly outlined.

Upon the study of sections (Fig. 14) it will be observed that the process of evagination has gone on from that described in the stage preceding this until the wing is nearly surrounded by the peripodal membrane (*e*). The evaginated portion is better shown in Figure 16 which is a frontal section of the larva and gives a longi-section of a part of the wing. In cutting the section shown in Figure 14 five layers of hypodermal origin were passed through, viz., the outside body wall or hypodermis (*h*), the external part of the peripodal membrane (*e'*), the external boundary of the wing-bud (*u*), the internal

boundary of the wing-bud (*w*), and the internal wall of the peripodal membrane (*e*).

The Parts of the Wing.—These can be best studied from the sections of the wings in various planes. Figure 13 shows a cross section of a wing through a vein cavity (*lv*). This vein cavity contains a trachea (*t*) which is not functional, is filled with lymph, and contains no chitinous intima. This trachea consists of a single layer of epithelial cells which will finally change somewhat upon their internal margin and form the chitinous intima in a manner to be described later. In this vein cavity sections of tracheoles, both cross and longitudinal, are found; those in cross section appear as holes through a mother-cell (*tl*). Leucocytes (*l*) are also found.

In a frontal section (Fig. 16) the lateral trachea (*T*) is cut in longi-section, and the razor has passed through a wing trachea (*t*) in longi-section. This trachea has passed into the lumen of the wing (*lw*). The tracheoles (*tl*) are connected with the lumen of the main trachea at a point (*mt*) while at the same time the chitinous intima (*in*) extends across the mouth of the trachea (*t*) which, as yet, has no chitinous intima and is not functional.

Figure 17 is a section of a wing in a slightly different plane than Figure 14; enough so that a mass of tracheoles (*tl*) appears instead of the bases of the developing tracheæ (Fig. 14, *T*).

During this stage the wing has been growing very rapidly and the ventral margin has extended toward the feet, and at the latter part of the period the wing changes from the inside of the body to the outside. This is all brought about while the caterpillar is searching for a suitable place to hang itself up.

The basement membrane (*bm*) is not easily distinguished in some sections in this period though quite distinct in others; it is shown in Figures 16 and 23. In many places between the vein cavities in the wing the two layers of the basement membrane appear as coming together and are somewhat fused. In some sections (Fig. 13) the line of union is seen with difficulty, but even in this section the line of contact is faintly made out by careful focusing. The union of these two layers forms the so-called middle membrane (Fig. 23, *bm*) which will be discussed more fully later.

THE CUTICLE OF THE WING-BUD.

There has been a long dispute as to whether or not a cuticle is formed around the wing-bud in the younger stages.

Pancritius thinks it is and figures it as a thick layer of chitin with the same structure as the outside cuticle of the body. Gonin thinks it is not formed at all until the latter part of the last larval stage. In *Sanninoidea exitiosa* (Fig. 20, *ic*) there is no doubt of a definite layer of cuticle surrounding the wing. In *Automeris io* there is a much more definite layer of cuticle ; this is true in an early stage, even before the proliferation of the tracheæ (Fig. 21, *c*). The tracheoles alone are functioning at this period. The thick hard cuticle in this specimen may be traced quite a distance into the invagination ; and it will be noticed that it gradually thins and finally becomes very thin and delicate. In *Pieris* the layer of cuticle extends in the form of a plug as described in the discussion of the first period, and the wing-bud is not surrounded by a chitinous layer until the fifth stage when a very thin, delicate, structureless membrane of cuticular nature appears which is destined to become the cuticle of the wing of the pupa.

THE ORIGIN OF THE CUTICLE.

My observations indicate that the cuticle is a product of a transformation of the outer ends of the hypodermal cells instead of a secretion thrown out from these cells. By the use of chitin stains, the chitinous nature of the ends of the cells in the pupa wings (Fig. 35, *c*) is brought out. As more evidence that it is not a secretion, the cuticle in the development of the prepupal wing (Fig. 28, *pc*) is seen to gradually decrease in density as it approaches the hypodermal cells and gradually fades into the cells themselves. These facts have been observed many times and in many places upon the insect other than upon the wings, during the course of this investigation.

Several observers have noted that the cuticle is often divided into polygonal areas corresponding to the subadjacent hypodermal cells. It is not probable that this would be the case if the cuticle were a secretion thrown out from the cells ; for in such a case the products of the different cells would naturally merge into a continuous layer. But the formation of a cuticle by a transformation of the outer portions of the body of the cells would result in the formation of such areas.

Ordinarily this division of the cuticle into areas is not very evident in sections. But in the case of one larva of *Sanninoidea exitiosa* the areas were remarkably distinct ; so much so that the sections of the cuticle broke up into little blocks corresponding to the hypodermal cells (Fig. 37).

THE PREPUPAL PERIOD. (Plate III, Figs. 22–25; 27–28. Plate IV, Fig. 29.)

In all the previous stages of the caterpillar the wings are inside of the body cavity. In this stage they are found upon the outside. If one dissects the wings out in this stage, he finds it necessary to dissect away the body wall before he can get to the wings if he works from the inside as he did in the other stages. But if he takes the experience of Swammerdam and plunges the caterpillar into hot water a few times, then carefully takes off the chitinous covering, he will find the wings as external appendages the same as the legs.

For some time before the last moult, that is before the larva hangs itself up for the pupal period, the wings are transparent in the body, and their position is recognized only by their accompanying tracheæ. As the wing becomes gradually opaque it also becomes very much smaller and the contour is irregular and folded. The wings have now disappeared from the interior of the body, but are easily found in their new position by the method given above.

This change in position takes place in a very short time, while the caterpillar is searching for a place to hang itself up and spinning the button of silk to which it attaches itself. This is a very curious phenomenon, and one that needs explanation. Herold explains it easily. "The germs of the wing" he says "although hidden under the skin have a great tendency to come to the outside." Landois, who considered the wings as appendages of the tracheal trunk instead of an invagination of the hypodermis, thought that these germs opened a way through the muscular bed with their points and that then the hypodermis withdrew, thereby letting it appear under the form of an evagination of the integument. Weissmann and others have advanced the idea of the breaking down of the hypodermis to allow the wings to reach the outside. Dewitz does not refer to the breaking down of the hypodermis. According to him the opening of the cavity of the invagination is enlarged just enough to give a free removal of the wing and it remains for some time like a picture in a frame. Soon the wing becomes free and the hypodermal frame becomes a part of the body wall. Goffin says, "This theory raises two principal objections. First, it is difficult for the orifice of the peripodal cavity to be able in a few hours to enlarge itself without a tear or rent; in the second place, in order that all of the envelope could find a place upon the thorax it would be necessary that the

circumference of it should be much increased. Now this is not the case. The second and third segments are swollen only while they contain the germs of the wings. After these organs issue the diameter does not exceed that of the neighboring segments." Gonin explains the transition of the wings according to the theory of the destruction of the envelope. As evidence of this he cites the fact of there being a large amount of debris around the wing and between the two layers of the integument. He also refers to the fact of having a series of slides in which the front wing is entirely exterior while the hind wing has not completed the transformation.

I have observed all this, but I believe that this structureless substance referred to by Gonin is none other than the moulting fluid. The reagents act upon this substance, coagulating it, which gives it the appearance of degenerating tissue. Stains act upon this substance very vigorously, even more so than upon the hypodermis. But since I find this structure around the entire larva (Fig. 29, *mf*) as well as around the wing, in about equal quantities, I believe it has no connection with broken down tissue.

A careful study of Figures 18, 19, and 20 which were taken from the peach-tree borer, Figure 18, from a caterpillar just before spinning its cocoon, Figure 19, from one which had begun to spin its cocoon, and Figure 20, from one which had just completed its cocoon, will show the method of the transition of the wing from the inside of the body cavity to the outside. It is found that the dorsal part of the wing (*d*) emerges first. Figure 18, *x* shows the two layers of the hypodermis, the one the external hypodermis and the other the peripodal membrane, drawn back a little but not fused as shown in Figure 19, *x*. The fusion of the two layers and the drawing dorsad in the direction of the arrow in the latter case is very evident. In the stage shown in Figure 20 the dorsal fold at *x* has entirely straightened out. During this time the wing has grown considerably as shown by the relative size of the figures, Figures 19 and 20 both being drawn to the same scale. The same result is shown by the ventral fold *y*. In the stage represented by the first two figures the walls have not fused, while it is easily seen that they are coming closer together in the course of the development. In the third case, represented in Figure 20, the walls have fused at *y* and drawn ventrally in the direction of the outside arrow. During the course of the development the wings now grow very rapidly in the direction of the

feet. From the very nature of this growth and the extent of it the peripodal membrane on the internal ventral side is swung dorsad in the direction of the inside arrow. Finally the ventral fold has straightened out as the dorsal fold did and the hypodermis has assumed the position shown in Figure 29, *y*. The wing is now on the outside of the body. The fused portion of the hypodermal wall is broken down and disappears with the development of the adult hypodermis. The ventral hypodermis approaches the dorsal hypodermis as the larva increases in age until there is only a narrow opening into the lumen of the wing (Fig. 29, *lw*) which corresponds more nearly to the lumen of the adult wing.

Gonin's objection to the idea of Landois, who thought the folds entirely straightened out thereby placing the wing on the outside of the body-wall, is not sustained. Gonin observes correctly that the thorax is not larger after the transition than it was before. According to my observations given above there would be no necessity for the enlargement referred to, for what slack there is is needed to form the ventral wall (Fig. 29, *y*) after the transformation has taken place. It will be seen also from the above description that the part of the fold that disappears is a small portion of the peripodal membrane and a piece of the hypodermal wall.

During the time of the last larval stage and especially during the latter part of it (prepupal period) the wings have been growing very rapidly. Now after the wings are on the exterior of the body this growth is even more rapid. The wings have reached the region of the feet and yet the growth is increasing. In order to allow this growth and still have the wings confined in the limits of the segments of their origin they become folded and wrinkled, so that the surface appears convoluted (Fig. 28). During this stage the wings become free and conspicuous in the section. A thin layer of cuticle is laid down upon the surface and follows the contour of the wings (Fig. 28, *pc*). This cuticle is thicker on the portions of the wing that will come to the air upon pupation, at the same time it must be very different in texture from the outside cuticle of the body for upon pupation it must needs straighten out as the wing expands.

On studying the wing-buds as a whole the tracheæ can be seen, not because they are filled with air but on account of their chitinous intima (Fig. 22, *T*). Tracheoles (*tl*) are shown in the wing with their point of attachment in the axis of the wing. At the time the

caterpillar spins up, the tracheæ show very faintly but as the age increases they appear more prominent and the intima is very evident. During the early part and the middle of the last larval stage the tracheoles (Fig. 12, *tl*) are in great abundance, but in the prepupal stage they have begun to decrease in number and extent (Fig. 22, *tl*). As the caterpillar moults for the pupa the intima of the main trachea (Fig. 16, *in*) is pulled out which opens the mouths of the wing tracheæ and allows air to enter; and the tracheæ thus become functional. It is a question as to what now becomes of the tracheoles. Gonin says that they are drawn out at the time of this moult. He also says that this can be easily demonstrated. But the results of my observations are very different, as will appear later.

THE PUPAL WINGS. (Plate III, Figs. 24 and 26. Plate IV, except Fig. 29 and Plate V.)

During the pupal stage several important changes take place in the structure of the wing: the temporary or larval tracheoles disappear; a secondary or pupal system of tracheoles arises; important changes take place in the tracheæ; and a complicated series of changes takes place in the form of the hypodermal cells and of the basement membrane.

The Disappearance of the Temporary Tracheoles.—The temporary tracheoles are found in pupal wings (Fig. 26, *tl*) but not in such great numbers as in larval wings. They run in straight lines much the same as in the larval wings, but they are found only in the axial region of the wing, and that only in very young pupæ. They are attached to the epithelial wall of the trachea (Fig. 33, *tl*). This evidence will add to the proof of the theory of Landois and Pancritius who thonght that the tracheoles of the larva disappeared by absorption in the pupa, and disapproves the statement of Gonin referred to above.

The Development of the Secondary or Pupal Tracheoles.—A new system of tracheoles now arises from the tracheæ. This may be called the secondary system of tracheoles. The two systems are entirely different and have no relation the one to the other. The temporary or larval system has its origin from the main tracheal trunk or from the bases of the wing tracheæ, while the pupal system has its origin from the tracheæ in the wing itself and functions during the pupal life. The secondary system extends at right angles to the tracheæ and penetrates the wing substance (Fig. 24, *tl*) while the

temporary system extends in straight lines from the axial region and follows the course of the tracheæ (Fig. 26, *tl*) practically parallel with them and functions only during the larval life.

The Changes in the Structure of the Tracheæ.—In the very young pupa the tracheæ (Fig. 30, *t*) retain the characteristics of the tracheæ of the preceding stage except that the cells of the epithelial layer are not so distinct. This indistinctness increases with the age of the pupa until in the old pupa (Fig. 35, *T*) the walls between the cells have disappeared. At this time the epithelium appears under low powers to be thrown into folds; under high powers its degeneration is revealed. The nuclei are still quite distinct; but the cell body is becoming semi-transparent except in a branched central area where the protoplasm is of greater relative density. In this stage of the degeneration of the trachea the intima appears as a thin membranous structure in the lumen. Sometimes it appears merely as a line, at other times as a loop in a great variety of forms (Pl. V, Fig. 38, *in*).

In a later stage the inner boundary of the cells is broken down and the cell body is evidently disappearing. The central branched part of the cell body is the last to go (Pl. V, Fig. 39) and projects for a time into the lumen of the trachea.

A degenerating trachea in this stage is figured by Schäffer (his figure 37) and described as Semper's Flügelrippen (p.646). He regards the branched remains of the cell body described above as tree-like processes of an intima, and the remains of the intima he believes to be a secretion from the walls of the tube.

Semper's original description of the so-called wing-ribs ('57, Taf. XV, Fig. 6) was evidently based on an examination of a trachea which had just begun to degenerate. The intima had separated from the epithelium and was mistaken for a central nerve, but the epithelial cells were still distinct and of the usual form.

This mistake of Semper has found its way into a large part of the literature on this subject and has been the cause of much confusion.

It is evident that this degeneration of the wing tracheæ finally becomes complete; for in the wing-veins of the adult the tracheæ are lacking.

The Changes in the Hypodermis and the Basement Membrane.—Our knowledge of the changes that take place in the course of the development of a wing has been greatly confused by what are evidently misconceptions regarding the nature of the membrane limiting the inner

ends of the hypodermal cells. This membrane is easily seen in the early larval stages to be continuous with the basement membrane of the hypodermis of the body wall and is obviously a homologous structure. In the fourth larval stage, the upper and lower hypodermal layers of the bud come together and the two opposed basement membranes appear as a single membrane; this has been termed the *middle membrane* of the larval wings, and its homology with the two basement membranes of the upper and the lower hypodermal layers of the wing overlooked. At the end of the fifth larval stage just before the evagination of the wings, which is characteristic of the prepupal period, the wing-buds become transparent so that they are seen in dissection with difficulty; their position, as already indicated, is recognizable only by the accompanying tracheæ. In sections of the wing-buds made at this time, the so-called middle membrane is seen only with difficulty. This has given rise to the belief that it disappears at this time. Later when the wings become more opaque, *i. e.*, in the pupa stage, the two basement membranes are again easily seen. Semper ('57) believed that the reappearing basement membrane was a new structure, which he named the *grundmembran*; and he describes its formation from mesenchymatous cells that had wandered into the wing. The result has been that students of the subject have been confused by descriptions of three different structures, the basement membrane of the hypodermis, the middle membrane of the larval wing-buds, and the grundmembran of the pupal wing; when in reality there is only a single structure, the basement membrane.

The Development of the Venation of the Wings.—As stated above, during the fourth larval stage, the upper and lower hypodermal layers of the wing-bud come together, and the two opposed basement membranes appear as a single membrane, the so-called middle membrane. This union of the two layers of the wing does not take place, however, throughout the entire extent of the bud. Along certain lines, spaces occupied by tracheoles and lymph remain. These are the forerunners of vein cavities, and into these the tracheæ extend during the fifth stage (Fig. 12). In the fifth stage, therefore, the venation of the wing is outlined, each vein consisting of a cavity filled with lymph and containing a trachea. It is not, however, till the pupa stage that the wing-veins in the same sense in which the term is used by Entomologists (*i. e.*, the cuticular framework of the wing) are developed. These so-called wing-veins are merely thickened lines of the cuticle bounding the pre-existing vein-cavities.

In some cases the formation of a distinct vein-cavity follows the development of the trachea within. This is shown in the section represented in Figure 14. Here there is one large vein-cavity containing a trachea; but the other tracheæ are closely surrounded by the hypodermal tissue of the wing. It is probable that this surrounding tissue is forced away later by blood pressure thus forming the large vein cavities characteristic of later stages.

The Formation of the Hypodermal Pillars.—During the prepupal period, that is between the time at which the wings pass out from the body cavity and the time of moulting the last larval skin, there is a great increase in size of the wings. This expansion is doubtless brought about by blood pressure. If there were no provision to prevent it, the forcing of the blood into the lumen of the wing would distend it like a filled bag. But the upper and lower sides of the wing are tied together by fibers of hypodermal origin, which have been termed the hypodermal pillars. The result is that the expanding of the wing is in a single plane.

According to the observations of Mayer ('96) the formation of these hypodermal pillars takes place in a complicated manner; but I have not been able to confirm his observations. In *Pieris* it takes place as follows:

During the period in which the two basement membranes are closely applied to each other, thus forming the so-called middle membrane, these membranes fuse in places, thus uniting hypodermal cells of the opposite sides of the wing. Later, at these points of fusion the fused basement membranes disintegrate, or at least are not to be distinguished from the bodies of the united cells, which form a fiber passing from one cuticle to the other.

When the wing is expanded, the hypodermal cells, each of which must cover a much larger area of the surface of the wing, are greatly shortened; and thus the two basement membranes are pulled apart except in those places where they have fused. Thus is presented the appearance shown in Figure 32, *ls*, which represents a section of a vein cavity and several spaces between hypodermal pillars.

Later in the pupal stage, the hypodermal pillars appear to contract, for the two surfaces of the wing are brought even closer together (Figs. 31 and 35). In fact, in sections of wings of the adult very little hypodermal tissue can be found; and in many places the two cuticular layers are closely applied. The details of the disappearance of the hypodermal pillars have not been worked out.

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LIST OF ABREVIATIONS USED IN THE FIGURES.

b . . beginning of the invagination of the wing.
bm . . basement membrane.
bmt . . basement membrane tubes.
c . . cuticle.
d . . dorsal.
ec . . embryonic cells.
et . . epithelium of trachea.
h . . hypodermis.
hp . . hypodermal pillars.
ib . . imaginal bud.
ic . . inner cuticle.
in . . intima of trachea.
l . . leucocytes.
ls . . lymph spaces.
lt . . lumen of trachea.
lv . . lumen of wing vein.
lw . . lumen of wing-bud.
mct . . mother cells of tracheoles.
mf . . moulting fluid.
mt . . mouth of tracheoles.
nlt . . new lumen of trachea.
oc . . outer cuticle.
pc . . pupal cuticle.
T . . principal trachea.
t . . small trachea.
tl . . tracheoles.
v . . ventral.
vl . . vaculoated leucocytes.

EXPLANATION OF PLATE I.

Pieris rapæ.

Fig. 1. Wing-bud from cross section of larva one-half hour old.

Fig. 2. Wing-bud from cross section of larva a little older than the one represented in Figure 1.

Fig. 3. Wing-bud from cross section of larva a little older than represented in the two preceding figures, but in the same stage.

Fig. 4. Wing-bud from cross section of larva after the first moult (second stage).

Fig. 5. Wing-bud from cross section of larva after the second moult (third stage).

Fig. 6. Section of wing-bud three sections ahead of the one represented in Figure 5.

Fig. 6a. A few cells from a wing-bud of the same stage as represented by Figure 6.

Fig. 7. Wing-bud in cross section of larva after the third moult (early part of the fourth stage).

Fig. 8. Wing-bud in cross section of larva a little older than the one represented in Figure 7 (fourth stage).

Fig. 9. Diagrammatic figure showing the proliferation of the trachea.

Fig. 10. Longi-section of two mother cells of tracheoles.

Fig. 11. Cross section of four mother cells of tracheoles.

EXPLANATION OF PLATE II.

Pieris rapæ.

Fig. 12. Wing-bud of the fifth larval stage dissected out for direct observation.

Fig. 13. Cross section of wing-bud from the fifth stage.

Fig. 14. Section of the wing-bud in cross section of larva after the fourth moult (fifth stage).

Fig. 15. Section of trachea showing the connection of tracheoles (fifth stage).

Fig. 16. Section of the wing-bud in frontal section of larva (fifth stage).

Fig. 17. Section of wing-bud in cross section of larva cut in a different plane than the one represented in Fig. 14 (fifth stage).

EXPLANATION OF PLATE III.

Sanninoidea exitiosa.

Fig. 18. Wing-bud in cross section of larva just before spinning cocoon.

Fig. 19. Wing-bud in cross section of larva just spinning cocoon.

Fig. 20. Wing-bud in cross section of larva just in cocoon.

Automeris io.

Fig. 21. Section of wing-bud in cross section of larva before the proliferation of the trachea.

Pieris rapæ.

Fig. 22. Prepual wing dissected out for direct observation.

Fig. 23. Section of the wing-bud in the fifth larval stage just before hanging up for the pupa stage.

Fig. 24. Trachea and secondary tracheoles in the young pupal wing.

Fig. 25. Cross section of prepupal wing under very high power.

Fig. 26. Trachea and larval tracheoles in pupal wing.

Fig. 27. Section of prepupal wing in cross section of larva.

Fig. 28. Section through one side of prepupal wing under high power.

EXPLANATION OF PLATE IV.

Figures all from *Pieris rapæ* except Figures 34 and 37 which are from *Sanninoidea exitiosa.*

Fig. 29. Cross section of the larva of the prepupal stage through the third thoracic segment.

Fig. 30. Cross section of very young pupal wing.

Fig. 31. Cross section of pupal wing about four days old.

Fig. 32. Cross section of pupal wing two days old.

Fig. 33. Trachea and larval tracheoles in pupal wing.

Fig. 34. Cross section of young pupal wing of the peach-tree borer.

Fig. 35. Cross section of pupal wing four days old. Section is through a vein cavity.

Fig. 36. Longi section of pupal wing two days old.

Fig. 37. Cross section of hypodermal layer of larva with cuticle. It shows the structure of the cuticle. (Peach-tree borer.)

EXPLANATION OF PLATE V.

Pieris rapæ.

Fig. 38. Cross section of trachea in old pupal wing.

Fig. 39. Portion of trachea in cross section a little older than the one represented in Figure 38.