

The DEVELOPMENT of the
LEGS, WINGS and HALTERES in
WILD TYPE and CERTAIN MUTANT STRAINS
of Drosophila melanogaster.

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Introduction.

One of the most important aims of present day genetics is to study the relationship between genes and their end products, in order to gain a better insight into the intricate methods of gene action. The fundamental problem "How does a mutant gene influence development?" is still very far from being solved, and at the present state of our knowledge offers extreme difficulties to the research worker. Only the general outline of a hypothesis on gene action has been given by R. Goldschmidt ('27), and has been accepted by most modern geneticists. According to this conception, normal development consists in a great number of processes interacting with each other in a highly complex, but completely balanced way. A mutant gene disturbs this balance, presumably by initiating a chain of reactions which, at some definite stage, interfere with the normal developmental processes. A determination of the time when the gene thus first enters actively into the process of development would bring us a step nearer to the solution of the fundamental problem of gene action. Numerous experiments with various physiological and genetical methods have been tried for this purpose. However, the first preliminary

for this investigation is a thorough knowledge of the normal development; until this is available, all such experiments lack a reliable basis. Therefore, the first aim of the present investigation was to study in detail the morphogenesis and histogenesis of the thoracic appendages of *Drosophila melanogaster*, organs which are specially valuable for genetical work. So far, the legs have received less consideration from geneticists than the wings. Very few leg mutants⁺ are known, possibly not only on account of a lower mutation rate of the leg determining genes, but also because such mutants as appeared, were often discarded owing to their poor viability or variable manifestation. It would have been very interesting to investigate, for example, a mutation like Hoge's ('15) reduplicated legs, both owing to the manner in which its development deviates from normal, and also because its manifestation is influenced by temperature, a fact which might throw some light on the physiological nature of gene action. Incidentally, many mutations discarded by geneticists on account of variable manifestation would, for that very reason, have been particularly interesting from a developmental

⁺ e.g. dachsous (Stern and Bridges '26), stubble and stubbloid (Dobzhansky '30), bent (Muller '14), crippled (Komai '26), reduplicated legs (Hoge '15).

point of view. Furthermore, the fact that leg modifications have been induced by temperature (Goldschmidt '35, Gottschewski '33) and by embryonic trauma (Geigy '31, Howland and Child '35), also suggests that the legs are a favourable object for a study dealing with the interrelation of genetics and development.

The second task of the present investigation, to which the study of normal development was only an introduction, consists in the comparative study of the development of some wing mutants. This should provide the answer (at least in the case of the particular genes involved) to the question as to when a mutant gene produces the first visible deviation of normal development. A preliminary report on the results obtained so far forms the second part of the present paper. In the case of one mutant, the temperature-effective period was determined, in order to correlate the morphological findings with the results emerging from physiological experimentation.

Acknowledgment.

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A survey of literature.

There exists a rich literature on insect development. The development of the wings in particular has been investigated by Tower('03) for Coleoptera, by Tannreuther('10) for Lepidoptera. Kähler('32) gives a very thorough description of the development of the wings of *Ephesia kühniella*, with a view to provide a basis for studies on the relation of genetics and development in this species. Schlüter('33) has done the same for *Habrobracon* which has been introduced as an object for genetical research by the studies of Whiting and Kühn.

For a study of the imaginal discs of the cycloraphous Diptera, with their highly specialised mode of development, Weismann's (1864) investigations still remain a standard work, indispensable to every student in this field of research. They were followed by others (Kowalewsky 1887, van Rees 1889, Pratt 1901, Vaney 1902, Pérez 1903, 1910, Stange 1907, Keilin 1915, Snodgrass 1924), but most of these papers are little concerned with the initial stages of the thoracic discs in the young larva. A histological description of these stages has been given only for the very modified development of *Melophagus* (Pratt 1901, Stange 1907).

Until recently, very little was known of the

anatomy and development of *Drosophila*. Only a few years ago, a very thorough description of the tracheal system of the larva, of the intestine and of the nervous system has been given by Rühle ('32), Strasburger ('32) and Hertweck ('31), but these authors do not deal with organs of special importance for pure genetics, except the eyes. Chen('29) was the only one who tried to trace the development of the imaginal discs,⁺ but he did not deal with several important questions, such as the first anlagen of the discs in the young larva, the relationship between the larval instars and the stages of the discs, or the details of wing development in the early pupa, and moreover he did not study the development of the legs. Therefore it seemed important to reinvestigate the development of the thoracic appendages, including the legs, from the very beginning of larval life, on the basis of histological studies.

⁺ When the thesis was practically finished, there appeared a paper by Medvedev ('35, Zt.ind. Abst. Bd. LXX, Heft 1) which deals with the development of the imaginal discs of the eyes.

Alpatov('29) has described the three larval instars in *Drosophila melanogaster*. This is very helpful for comparing developmental stages. Bliss ('26) gives data on the prepupal development of *Drosophila melanogaster* at different temperatures.

The morphogenesis of *Drosophila* mutants was studied by Chen in the paper referred to above. He dealt with some eye mutants, the haltere mutant *bithorax* and the wing mutants *no-wing* and *vestigial*. As *vestigial* has been frequently used for physiological experiments, and as many points in its development have been left unexplained by Chen, it was desirable to re-examine the morphogenesis of this interesting mutation in greater detail.

In recent years, several physiological and genetical methods have been tried to determine the time of gene action in *Drosophila*. Of special importance among these are the attempts to influence the manifestation of a gene by temperature. For *Bar* and its allelomorphs, including the wild type eye, this was done in a series of papers by Zeleny and his school (Driver '26, '31, A.H. Hersh '24, '27, '28, '30, '34, R.K. Hersh '24, Kraffka '20, '24, Luce '26, Seyster '19, Zeleny '23). Plunkett ('26) formulated a biochemical hypothesis of gene action based upon

the results of his temperature experiments on the manifestation of *Dichaete*, a bristle mutation. Timoféeff-Ressovsky('29, '34) determined the temperature-effective period for the manifestation of a vein mutation in *Drosophila funebris*. He used his results for speculating on the nature of the underlying developmental processes. Stern('27) and Samohvalova('33) found temperature-effective periods for two wing mutations, jaunty(Stern) and drooping wing (Samohvalova).

The mutant vestigial which formed one of my objects of investigation, has been used frequently for studies for studies of this nature. Roberts('18) noticed that the vestigial wing of *Drosophila melanogaster* is lengthened when the flies are bred at higher temperature. Nadler('26) confirmed this observation for *Drosophila virilis* and determined the temperature coefficient of this process for the range between 12° and 30° . In *Drosophila melanogaster*, Harnly ('30) found a critical temperature for this effect to be 30° for the males and 31° for the females. Stanley('31) , in an extensive series of experiments, confirmed these results and moreover

determined the temperature effective period for vg males and females. Hersh and Ward('32) discovered that temperature also influences normal wings, but the effect is opposite to that in vg, the normal wing decreasing in length as temperature rises. Heterozygotes were shown to react like wild type. Stanley('35) in continuing and extending his former studies, determined the temperature effective period not only for vg at different temperatures, but also for wild type strains and the heterozygous offspring of vg and wild type. In comparing his data with those of Chen, he came to the conclusion : " that the effects of the vg gene are manifested a considerable time before the primordia of the wings can be identified as such"; this conclusion, as will be shown in this paper, cannot be upheld following a fuller study of the normal development. One of his most striking results was that "the time of initiation and termination of the effective period at the different temperatures does not maintain a constant relative position in the developmental periods." Further he states that "without a knowledge of the embryology of Drosophila at the different temperatures, it is impossible to say whether

or not these limits correspond to a shifting of the time of imaginal bud formation. Riedel ('34) examined in greater detail the influence of temperature on vg and normal wings, paying special attention to sex dimorphism and the changes in the different parts of the wing. Her analysis of the results, based on Goldschmidt's theory of timed reactions, starts from the assumption that the basal parts of the wing are formed first during development, the more distal ones being added later in the course of ontogeny. She writes: "Die Eigenart der Mutation vg besteht darin, dass die Entwicklung des Flügels so stark verlangsamt ist, dass bei Auftreten des definitiven Determinierungspunktes im Sinne Goldschmidt's im Vergleich zu dem Flügel der normalen Wildform erst der Basalteil gebildet ist". This idea, derived from a comparison of adult normal wings with those of the different vg allelomorphs and with the wing forms produced by breeding vg flies at higher temperatures, seems surprising from a purely developmental point of view. It will be discussed later in relation to my observations on the development of the normal and the vg wing.

Another attack on the problem of the time of gene action consists in the production of

modifications by exposing certain developmental stages to sublethal temperatures. This method was used by Goldschmidt ('29, '35), Jollos('30-'34), Gottschewski('33), Plough and Ives('34) and J. Schultz('34). In all these experiments modifications of the thoracic appendages were produced which often were phenotypically identical with known mutations or with mutations produced by the same treatment, a fact which makes them useful for gaining an insight into developmental processes, as will be discussed later.

A purely genetical method of analysing the time of action of different genes consists in studying the effects of interaction of genes affecting the same character. Poole('27) found that the effect of *vg* is epistatic to those of several other wing mutants. Csik('34) applied this method to a number of wing mutants with the view to:

"gewisse Schlüsse auf die Art zu ziehen, in der sie (the mutant genes) in den Entwicklungsgang kontrollierend eingreifen".

The problem of the determination of the thoracic appendages in the embryo was attacked by Geigy('31) and Howland and Child('35). They

used the method to destroy circumscribed parts of the embryo, Geigy by the action of ultraviolet rays, Howland and Child by mechanical deletion with a micro needle.

While this thesis is being written, Goldschmidt('35) publishes a paper in Biolog. Zentralblatt, Bd. 55, Heft 9/10, in which he reports investigations on the development of several wing mutants in the pupa. Some of his mutations are the same as mine, but as his observations begin only with the formation of the pupa sheath, whereas mine, beginning in the mature larva, do not proceed much beyond this stage, there is but little overlapping between our results. Where it occurs, both series confirm one another.

Material.

The normal development was studied on two wild type strains of *Drosophila melanogaster* for which I am indebted to the Kaiser-Wilhelm-Institut für Biologie in Berlin-Dahlem. These strains had been inbred for a long time, and inbreeding was continued here for more than ten generations before starting the investigations. One of these two strains, Lausanne, was used only in the beginning, as it proved to be not free from wing abnormalities (crumpled wings). The other one, an Oregon stock, is excellent in every respect.

For the study of wing mutations I used vestigial and dumpy stocks from Berlin-Dahlem. They had been inbred before, and the inbreeding was continued here for at least twenty generations.

The dumpy(dp) wing is truncated obliquely, the distal parts having^{been} cut off, especially from the submarginal and the first and second posterior cell, and furthermore the area of the entire wing is reduced by 34.2% (Csik '34). (Phot. 2)

The vestigial (vg) wing is characterised by a striking reduction owing to the terminal and marginal parts having been trimmed away. (Phot. 4)

Through the kindness of Prof. Jollos, I obtained two of his heat mutations, 13d and 17b.

l3d is a mutant with narrow wings, cut off along both lateral margins (Phot.3). In a homozygous condition the gene causes the wings to crumple and blister very shortly after hatching. It is sex-linked and dominant, but the heterozygotes show the character in a slighter degree. In order to locate it, l3d flies were crossed to sc ec ct v g f⁺. The F₁ females were backcrossed to sc ec ct v g f, and the male offspring of this cross were classified in relation to l3d, f and sc. The recombination value with sc was found to be 47, with f 3.4, if all the flies used were considered. But if corrections were made for the apparently lower viability of some classes, the recombination value with f was 4.3. Before computing the recombination value with the genes v and g which affect the eye colour, it was necessary to remove from the l3d stock the gene for white eye, which was present in Jollos' stock; for white, being epistatic to all other eye colours, would have concealed the presence of v and g in those cases where crossing-over would have brought them together on the same chromosome. Therefore, l3d flies were outcrossed to wild type, and, as the recombination value l3d-w is 50%, a sufficient number of l3d offspring with red eyes was obtained,

⁺ sc scute I,0	ec echinus I,5.5
ct cut I,20.0	v vermilion I,33.0
g garnet I,44.4	f forked I,56.7

and a stock was established. Individuals from this stock were mated to sc ec ct v g f. The heterozygous females were backcrossed to sc ec ct v g f, and their male offspring were classified as to recombination of l3d with v, g, and f. Table one gives the results.

Table 1

l3d	300
vgf	250
l3d v	26
gf	35
l3d vg	30
f	51
l3d vgf	11
+	17
l3d g	2
vf	0
l3d gf	0
v	0
l3d f	0
vg	1
l3d vf	0
g	0

n = 723

From these data l3d is found to be located to the right of f. The recombination values are:

l3d with v	23.5 ± 1.6
" " g	15.4 ± 1.3
" " f	4.2 ± 0.7

As the locus of f is 56.7, l3d was thus located at 60.9. The recombination value with v is too

small, even taking into account the double crossing-over to be expected from the curves for the differences between map distance and percentage of recombination (Genetics of *Drosophila*, 1925, p. 103). Since, however, all classes containing *v* are smaller than the corresponding non-*v* classes, they appear to be less viable. If this ^{is} taken into account and only non-*v* classes are used for computation, the recombination value of *l3d* with *v* becomes 26.0, which agrees very well with expectation. Unfortunately, in this case, the standard error becomes very high (6.9), which somewhat impairs the validity of the computation.

The mutant *l7b* was called "unequal wings" by Jollos (1934), asymmetry being its most characteristic effect. It reduces wing length in a varying degree; often the shortened wing is blistered. Manifestation is very variable and greatly affected by temperature. Males have a lower percentage of manifestation than females. The degree of expression varies from a slightly shortened wing to a tiny wing rudiment. The gene is autosomal and recessive. In order to locate it, the Star-Dichaete method was used. Star is a second

chromosome dominant, Dichaete a third chromosome dominant. Through the kindness of Dr. Timoféeff-Ressovsky, of the Kaiser-Wilhelm-Institut für Hirnforschung in Berlin-Buch, I obtained a Star Dichaete stock which, by means of a system of balanced lethals and crossing-over inhibitors, breeds true. 17b flies were mated to flies of this stock. In the F_1 , males exhibiting both Star and Dichaete were selected and backcrossed to 17b females. These males carry a 17b gene on one of their autosomes. As crossing over in the male does not occur normally, this gene cannot become transferred to the same chromosome as Star or Dichaete. In the present experiments, all the individuals of the F_2 which had unequal wings, failed to show D, whereas a number of flies were found exhibiting both unequal wings and Star. This indicates that 17b is located in the same autosome as Dichaete, i.e. the third chromosome. As a matter of fact, there should have been an equal number of 17b flies exhibiting Star and not-Star, but the low manifestation of 17b obscured this proportion. For the same reason, an exact location of 17b

proved difficult and was not followed up, as it is irrelevant for the present study.

As an object of comparison for the earliest larval stages *Calliphora erythrocephala* was used.

Technique

For studies on development, exact determination of the age of the larvae and pupae is of special importance. Great care was therefore taken to render this determination as reliable as possible. Among different methods tried out, the following was finally adopted. About twenty females (sisters) were isolated after hatching and put into vials with food along with a number of brothers. In the following days they were often changed to fresh food so as to prevent eggs from being withheld in the uterus. When the best egg-laying age was reached (3-6 days), the flies were once more changed to a fresh vial, and about 12 hours later transferred to Petri dishes by means of a glass plate into which a hole of the size of the mouth of the vial had been drilled. The bottom of the Petri dish was covered with filter paper thoroughly soaked with a well fermenting banana yeast mixture. The flies were allowed to deposit eggs on the paper for one hour, in some few cases for two hours. After that time they were etherised through the hole in the glass lid and removed. The eggs were then removed carefully with a bent dissecting needle and placed into another Petri dish containing filter paper spread with a layer of the usual

food (maize meal, treacle, agar). In order to avoid overcrowding, not more than 30 to 50 eggs were placed into one dish. The food was always prepared in exactly the same way. In order to provide as nearly as possible homogeneous conditions for the developing larvae, the food was thoroughly mixed with yeast one day before it was put into the dishes; immediately before transferring the eggs to it, it was flooded with a very thin suspension of yeast in water, which helped to keep it moist and fresh. The dishes were kept in an incubator at a temperature varying between 24° and 27° , but mostly constant at 25° . At different hours larvae were brought to the surface of the food by gently heating the bottom of the dish by steam. Before fixing the larvae were at first examined under the microscope as to their instar (see following section), but later this examination was often omitted, since the sections themselves present sufficient evidence of the instar (see following section).

In spite of all the precautions taken, no absolute uniformity of development could be reached. Apart from some obvious stragglers in hatching and further development, which occurred in almost every dish and were discarded, the time differences in hatching, moulting, growth and beginning of pupation always exceeded one hour. They naturally increased with the process of development,

so that at pupation the range of variation was several hours, up to ten. But as both in the living larvae and on sections, it is possible to estimate the developmental stage relative to the previous and the following moult, no serious difficulties in seriating the specimens were met with. The age of the larvae will be given in hours, starting from the hour of hatching, usually 24 hours after the egg-laying period.

Since, according to Chen('29), the first hours of prepupal life represent the critical period in the development of the wing, special care was taken to secure an exact time determination for this stage. Mature larvae were removed from the culture vials and placed in Petri dishes with moist filter paper, in the centre of which a lump of food had been placed. When the paper is well soaked, the larvae will pupate on the ring of paper outside the food. The dishes were kept in the incubator and the pupae collected every hour from the paper only, as those pupating on the food are liable to be overlooked on account of their yellow colour. The beginning of pupation⁺ was taken

⁺Pupation proper takes place only about twelve hours after the eversion of the anterior spiracles. In this paper, however, ~~the~~ the terms "pupa" and "pupation" will be used for prepupal and pupal stages likewise, so that e.g. "a pupa of 3 hours" means a prepupa 3 hours after eversion of the anterior spiracles

to be the moment when the anterior spiracles are everted and the larva has ceased to move. The white colour of the skin during the first hour provided a good check on the correctness of the age determination. The pupae were kept moist in the incubator until dissection. As it often took an hour to dissect all the pupae collected simultaneously, the age of each pupa is given correctly only within ± 1 hour. In some batches of pupae this range was reduced to $\pm 1/2$ hour by fixing all the pupae to be dissected simultaneously at the beginning of the dissection hour.

Dissections were made in Ringer solution under a Zeiss binocular. The larvae were dissected without fixation, but most of the pupae were fixed by a momentary immersion in boiling water. This has two advantages: 1. the coagulation of the mass of fat cells, degenerating tissue and phagocytes, which in the early pupa stage fill the body cavity and render the search for the imaginal discs difficult, 2. the consolidation of the cellular body liquid which enters between the lamina of the newly formed wing and is very liable to be squeezed out when dissecting. The discs were fixed in 95% alcohol and stained with alcoholic borax-carmines or with Ehrlich's acid haematoxylin. A comparison of this

primitive method of fixation with other fixatives (Carnoy, sublimate, Bouin) showed no difference in the results.

Different fixatives were tried for the larvae and pupae used for histological preparations. Of these, Bouin proved to be by far the best and in later series was used exclusively. For the larvae it was taken hot, almost boiling, as cold Bouin does not kill them instantaneously. In order to secure good penetration of the fixing fluid, the abdomen was torn open with a pair of sharp forceps after fixation. For embedding the methylbenzoate-celloidin method of Peterfy (Methodik der wissenschaftlichen Biologie, Berlin 1928, Bd. 1, p 616) was used. It gave excellent results, even with pupae inside their brittle cases. Serial sections were cut at a thickness varying from 4μ to 10μ , 5μ being usual. The staining was done with Heidenhain or with Ehrlich's or Delafield's haematoxylin counterstained with eosin. All the drawings of sections and of ~~wings~~ wings dissected out in toto were made with an Abbe camera lucida.

The method of the temperature experiment carried out with the 17b stock will be described in the section on the development of the wing mutations.

An outline of the general development of the
larva and early pupa.

Under the conditions of my work, the larva hatches about 24 hours after the egg has been deposited. The first moult takes place about 24 hours after hatching, the second moult about 24 hours later. Pupation occurs during the fifth day of larval life. According to Alpatov, the three instars are easily identified by the state of the tracheal system. In the first instar, the anterior spiracles are still lacking. In the second, the dorsal tracheal trunks end with club-like enlargements which, however, are still closed exteriorly. In the third instar, they communicate with the outer air through a bunch of seven to eight tubules (cf. fig. 31 C). The anterior spiracle (in the first instar a characteristic thickening of the hypoderm preceding it) is situated at the posterior margin of the first thoracic segment⁺. As the positions of the inner organs shifts according to the degree of retraction of the larval head, the position of the anterior spiracle is a useful mark for identifying the segments

⁺The larval head is followed by the three thoracic segments (I, II, III in the figures), and these are followed by the abdominal segments (1A, 2A, etc)

on sections.

A simple means of determining the instar on sections without previous microscopic examination of the larva is to observe the changes in the rib-like folds on the ventral wall of the pharynx. In the first instar, these are very slender and bend towards the middle line. Fig 1a shows diagrammatically their view on cross-sections of a first instar. In the second instar they are more rigid and, on cross-sections, appear T-shaped (fig 1b). A cross-section of the third instar would present a similar picture, except that there are nine ribs instead of seven. During each instar the cuticle, the pharyngeal ribs, the spiracles and other cuticular organs of the following instar, can be seen under those already present, and the degree of their development can be used as an indication of the interval elapsed since the last moult. This seems a more satisfactory method of comparing the stages of different specimens than the mere counting of the hours elapsed since egg-laying.

In a mature larva, all the thoracic discs are present in the same shape and position as described by Weismann for *Musca*. They are arranged in two groups. The ventral group consists of the ventral prothoracic and the ventral mesothoracic buds. Fig. 301C shows diagrammatically this group and its position relative to the larval brain. The lateral group consists of the dorsal prothoracic, mesothoracic and metathoracic

buds, and the ventral metathoracic buds. Apart from the dorsal prothoracic buds, which are not dealt with in this paper, a diagram of the shape of these buds, of their relative position and connection with the dorsal tracheal trunk is given in fig 31 .

It is well known that the thoracic buds, in the course of their development, give rise not only to the thoracic appendages, (wings and halteres from the dorsal buds, legs from the ventral ones), but also to the integument of the imaginal thorax. For the sake of simplicity, however, in this paper they will often be referred to as leg-buds, wing-buds, haltere-buds.

Pupation begins with a contraction of the larval body. The anterior spiracles are everted, movement stops. The larval skin hardens into the pupa case which assumes first a yellow, later a light brown colour. The larval head and the greater part of the prothoracic segment are retracted, thus loosening the connection with the puparium at the anterior end of the body. The mouth armature, however, retains this connection, and if the puparium is removed, remains attached to it. About 6 hours after pupation, the wings, halteres and legs are everted, the head still remaining invaginated.

The anterior end of the pupa inside the case is now covered by the thoracic discs and their appendages. The discs adjoin each other and combine to form the imaginal thorax. At this stage, the appendages are hollow sacs into which the cellular body liquid enters freely. Contrary to the observations of Bliss ('20), I have always noted a marked time interval (5-6 hours) between this stage and the eversion of the head. The pupa sheath forms during this interval. The puparium now contains the pupa proper.

The development of the legs.

The legs develop from the three ventral pairs of thoracic buds. The mode of development of all three pairs is very similar.

In a newly hatched larva the anlagen of all the ventral buds are seen as thickenings of the hypoderm. Figs 2,3,4 show them in a *Drosophila* larva 2 hours after hatching. Fig 5 represents cross-sections through one pair, the ventral prothoracic buds, in a *Calliphora* larva immediately after hatching. The magnification is the same in both cases. A small sensory organ consisting of two or three hairs is present in the hypoderm over each ventral bud. It was described by Keilin ('15) and in this paper will be referred to as Keilin's sensory organ. Immediately underneath it there is a shallow depression in the hypoderm, the first indication of the subsequent invagination of the buds.

The anlagen of the prothoracic buds are fused, to form a common midventral thickening of the hypoderm of the first thoracic segment(fig.2). Its double origin is proved by the presence of two sensory organs and two hypodermal depressions. Posteriorly,

the central mass of tissue is drawn out into two little knobs; each of these is connected with the ventral ganglion by a posterior process into which a nerve enters, and with the dorsal tracheal trunk by a lateral process into which a trachea enters. In the following pages, these processes will be referred to as the neural and the tracheal process respectively. On longitudinal sections the neural process is very distinct (fig.6).

The ventral mesothoracic(fig.3) and metathoracic(Fig. 4) buds arise from two pairs of anlagen in the ventral hypoderm of the second and third thoracic segment. Each pair is arranged symmetrically to the median line of the larval body, the metathoracic pair a little more laterally than the mesothoracic. In shape and histology they closely resemble the prothoracic buds, and they are also connected with the tracheal system. The mesothoracic bud likewise has the ~~same~~ kind of neural connection as the prothoracic, but no connection of the metathoracic bud with the nervous system has ever been described in Diptera. In my own material there was only one case (fig 16b) where a nerve originating directly in the ventral ganglion seemed to enter the metathoracic bud, but this question was

not further investigated as having no importance for the present study. In the diagrams fig. 30 a possible neural connection of the metathoracic buds with the ventral ganglion has been indicated by dotted lines.

During the first instar the ventral buds undergo very little change. Figs 7,8,9 show them in a late first instar shortly before the first moult. The difference between the imaginal and the larval cells of the surrounding hypoderm has become obvious, both the imaginal cells and their nuclei being smaller than the larval. Invagination is beginning, but the buds are still embedded in the hypoderm, from which only their knob-like processes are protruding.

During the first moult and the greater part of the second instar, the differentiation of the leg buds proceeds no further. Fig 10 represents a longitudinal view of all three buds in a larva in the second half of the second instar. The buds still form part of the hypoderm with posterior solid outgrowths. Fig. 11 gives a clear view of the branching of this outgrowth into the neural and tracheal processes.

Towards the end of the second instar three important changes take place in the ventral buds, namely 1. the formation of a lumen inside the bud, 2. the formation of a stalk connecting the bud with the hypoderm, 3. the first indication of a differentiation of the bud into the imaginal disc proper and the peripodial sac.

Of these changes, the first two are both due to the invagination of the imaginal bud into the interior of the larval body. This proceeds by two steps which follow each other immediately. Firstly there is formed a little sac protruding into the body and connected with the hypoderm by a short broad tube with a distinct lumen. Figs 12,13 represent the beginning of this process in the prothoracic and mesothoracic buds in a 46 hours' larva. The prothoracic buds seem a little farther advanced in development than the mesothoracic, while the metathoracic buds have not yet invaginated. In another larva of the same batch and age, but obviously slightly more advanced in development (figs 14,15,16), all three buds have developed a distinct lumen; the stalks of the two anterior pairs have become longer and thinner, and their lumina very narrow, so as to be

partly invisible. Fig. 17 represents longitudinal sections of a larva of the same age.

The second step of the invagination process occurs during the second ecdysis. Figs. 18, 19, 20 are cross-sections through a larva in the process of moulting. The stalks of the buds are being drawn out into thin threads. As the buds are pushed backward by the outgrowth of the stalks into the neighbourhood of the brain, the neural connections become correspondingly shorter, presumably through retarded growth-rate. (figs 30A and B).

The third change at the end of the second instar is the differentiation of the bud into the disc proper and the peripodial sac. After invagination, the wall of the bud is composed of a single layer of cubical cells arranged regularly round the lumen. The first indication of a differentiation of the bud is a slight dorsal thickening of its wall brought about by elongation of the cells. The wall remains one-layered throughout. The thickened part will later form the disc proper, while the remaining portion will become the peripodial cavity into which the anlage of the leg will protrude.

After the second moult, the stalks of all the discs are long and thin (fig. 30 C). The prothoracic one (fig. 21) is the longest, extending

from the posterior part of the first to the end of the second thoracic segment, where it branches into the two buds. Those of the mesothoracic buds (fig.21) are a little shorter, extending from the end of the second to the middle of the third thoracic segment. The stalks of the metathoracic buds are the shortest, originating and ending in the middle of the third thoracic segment. In some cases they extend straight in ventro-dorsal direction and may be completely visible in one section (fig.23). The connections of the buds with the ventral ganglion remain short. The buds are therefore lying much nearer to one another, and in an advanced third instar all of them are crowded together in the third thoracic segment. Sometimes all the six buds may be visible on the same cross-section (phot. 16, where the dorsal mesothoracic and metathoracic buds and the cephalic complex are present as well).

In the course of third instar the following changes take place:

1. The disc invaginates into the peripodial cavity. This proceeds in the same way as during the gastrulation of Vertebrata, when the entoderm invaginates into the blastula giving rise to an embryo with two walls, the ectoderm and the entoderm, and two cavities, the reduced blastocoel and the gastro-coel. In the mature leg bud the two walls are the

peripodial wall and the wall of the young leg, while the two cavities are the reduced cavity of the peripodial sac and the newly-formed cavity of the leg.

2. Into the legcavity mesenchyme immigrates. Its origin has been much discussed, but the present investigation was not concerned with this question.

3. By means of oval concentric folds, the disc becomes differentiated into the anlage of the sternal part of the thorax and the various segments of the legs. The formation of these segments progresses in a distal-proximal direction, the anlage of the last tarsal joint being the first to be formed. This process in *Drosophila* closely resembles the same process in *Musca* described by Weismann, and it will therefore suffice to illustrate its various stages by a series of figures. (figs 22-29).

The entire development of the leg disc in the larva is summarised in figs 30 A,B,C,, which are diagrammatic reconstructions based on numerous cross- and longitudinal sections. To make a comparison between them easier, they have all been drawn to the same scale. In order to get the true proportions of size in the different instars, B should be reduced by one half and A by two third.

The diagrams show the ventral hypoderm with the attached buds, as seen from the interior of the body. Keilin's sensory organs with the underlying hypodermal depressions, which of course would not be visible in this view, have been represented in their proper place. The brain and the dorsal tracheal trunks have been projected into the plane of vision. The connections of the buds with the dorsal tracheal trunks are given in a purely diagrammatic form and are not claimed to be true to nature in detail, for their real course is much more complicated.

During the prepupal stages the buds, by a shortening of their stalks, are drawn to the surface and finally everted, in the same way as described by van Rees in *Musca*. At the same time, their thoracic portions spread, replace the old larval hypoderm and eventually meet to form the imaginal thorax. Photos. 18-23 represent some stages of this process. 2 hours after pupation (photos. 18-19) the larval hypoderm is still intact, and the buds have not started evagination. The thoracic portions have spread out; on the photographs this is best seen in the ~~right~~ ventral mesothoracic bud. The peripodial sacs have widened, and inside their cavity the legs unfold, as is clearly visible on phot. 19. As described by Weismann, the coxa is marked off by

a constriction in the anterior end of the disc. The remaining portion of the disc becomes drawn out telescopically to form the other segments of the leg. 2 hours later, 4 hours after pupation (phot. 20) the discs are ready for eversion, but still covered by the degenerating larval hypoderm. 7 hours after pupation (phot. 21-22) all the thoracic appendages are everted. The fusion of the thoracic portions of the buds is not yet completed. The larval hypoderm is still present between the newly formed portions of imaginal hypoderm. 10 hours after pupation (phot. 23), when the head is ready to evert, the larval hypoderm has disappeared, and the body is completely covered by imaginal hypoderm. The pupa sheath begins to be secreted in form of a very thin cuticula, which, however, cannot be seen on the photograph. The histological differentiation of the appendages has also made progress, but this is a question which was not considered in the present study.

The development of the wing buds and haltere buds in the larva.

According to Chen, the first trace of the dorsal mesothoracic bud is found 16 hours after hatching, in the shape of a small group of cells attached to the hypoderm. The dorsal metathoracic bud is indicated 8 hours later by a similar formation. This seems a priori improbable, since in other Diptera all the imaginal buds are formed in the embryo (Weismann, Pratt), and also in view of the experiments by Geigy and Howland and Child which indicate at least a predetermined pattern in the late embryo. The cell groups described by Chen may be the knob-like processes of the ventral mesothoracic and metathoracic buds; this is the more probable as Chen does not recognise the presence of these buds before 32 hours.

A very careful examination of newly hatched larvae, however, never showed any pronounced hypodermal thickenings apart from the leg buds. This led me to the conclusion that at the time of hatching, the wing and halter buds are already invaginated into the interior of the body. As the histological examination of very young *Drosophila* larvae offers great difficulties owing to their extremely small size and complex organisation,

the earliest stages were first studied in *Calliphora*. As figs. 32,33 show, in a newly hatched *Calliphora* larva both pairs of discs are not only present, but already invaginated and constricted off from the hypoderm in the form of small sacs with a distinct lumen. The position and shape of the discs in *Calliphora* having been determined, it was easier to find them in newly emerged *Drosophila* larvae. Figs. 34,35 represent cross-sections through the second and third thoracic segment of a newly hatched larva. The wing and haltere buds are present as small groups of oval cells with a clearly demarcated margin, situated between the muscular sheath of the body wall and the fat body. They are already invaginated to the same degree as in *Calliphora*. Figs. 36,37 show them 6 hours after hatching. No lumen is visible; taking into account the probable origin of these buds by invagination of the embryonic ectoderm, it may be assumed that a very small lumen is present, but is obscured by a slight shrinkage. The connection with the hypoderm, which is visible on longitudinal sections of older larvae (figs. 54b, 55b), could never be clearly established in these youngest specimens, probably merely owing to technical difficulties. The strand of cells in figs. 32,33,

34, 36, 37 cannot represent this connection, for apart from the fact that it is composed of a single row of cells without a lumen, it consistently originates at the posterior (instead of the anterior) end of the bud and runs to the hypoderm of the following segment which it touches closely, but has never been observed actually to penetrate. A connection with the tracheal system is apparent from the very beginning (figs. 32, 34, 37, 40), a fact which led Weismann to the assumption that the dorsal buds originate from the peritoneal sheath of the tracheae. A connection with the ventral ganglion was never found, which is consistent with the observations of all other workers.

In a more advanced first instar, the wing and haltere buds are elongated sacs, each closely attached to a branch of the dorsal tracheal trunk. They show a distinct lumen, round which the imaginal cells are regularly arranged (figs. 38, 39, 40).

The chief progress in the course of the second instar is the differentiation of the bud into the disc proper and the peripodial membrane. This process begins early in the second instar and is finally completed when the second ecdysis occurs. Figs. 41-45 represent the consecutive stages.

At about 30 hours after hatching the inner wall of the bud exhibits a thickening⁺ brought about by an elongation of the cells. The wall remains one-layered throughout. Incidentally, some longitudinal sections through larvae of the same age (figs. 46,47) furnish clear pictures of the close connection between the bud and the tracheal branch attached to it. The relation of the tracheal branch to the main dorsal trunk can be recognised on fig. 48.

In the second half of the second instar (figs. 43,44) the differentiation of the bud has become very distinct. The cells of the disc proper have assumed a very long and sometimes fusiform shape. Since their nuclei are not all at the same level, superficial inspection may give the wrong impression of several layers of cells. By an outgrowing of the stalks, the buds have been pushed backwards, the mesothoracic buds into the third thoracic segment, the metathoracic to the border line between the third thoracic and the first abdominal segment (fig. 45)

⁺In fig. 42 this thickening seems to occur in the dorsal wall, owing to a slight pivoting of the whole bud

At the second ecdysis the buds stretch considerably (figs. 49,50). During the third instar there occurs the process of folding by which the wing disc - and to a lesser degree also the halteres disc - gradually assumes the form characteristic for the bud in the mature larva and indicative of the future destination of its parts. This process has been described by Chen from total preparations. Obviously all the stages represented in his paper (except the alleged 16 hours stage) belong to the third instar, both because a successful dissection of a second instar is all but impossible, and also as judging by the shape and size of all the represented buds, characteristic of the third instar. It is true that his youngest stage - 40 hours - usually belongs to the second instar, but the variability in the rate of development, even under controlled genetic and environmental conditions, is so great that a second ecdysis at 40 hours is still inside the range of normal variation. It was observed once in my own investigations.

Figs. 59-62 represent a series of total preparations showing the gradual formation of the cross ridges, the marginal fold and the concentric folds. On some of the preparations the stalk and

the attached tracheal branch are visible. For further details I refer to the paper by Chen, whose drawings and descriptions of these stages are fully corroborated by my findings. However, the "lateral notch" to which Chen points repeatedly, seems to be of no special importance, for in my preparations it appeared only occasionally.

A slight bend is always present between the anterior and the posterior portion of the bud, but the sharp notch is probably only due to the pulling of the trachea or the peripodial membrane while dissecting.

The pattern of folding of the haltere buds during the third instar follows that of the wing bud, but without attaining the same degree of complexity. Details will be found in Chen's paper.

The histology of the wing and haltere buds in the third instar is shown in figs. 51-55. The peripodial membrane has been drawn out into an extremely thin wall. The disc is protruding into the peripodial cavity the lumen of which is considerably reduced. In a very early third instar (figs. 51, 52) the metathoracic bud is not yet folded, while in the mesothoracic bud the first cross-ridge has appeared, separating the anterior, thorax-forming portion from the posterior wing-forming portion. In a medium third instar (fig. 53) the second cross-ridge has appeared. On longitudinal

sections through a mature larva of 96 hours the folding is completed (figs. 54,55). On fig. 54a the two cross-ridges as well as the circular wing portion have been cut in the median line of the bud. The tracheal branch is visible running along the ventral side of the disc and merging into a loose tissue which connects the tracheal sheath with the imaginal tissue of the bud⁺. Another section (fig. 54b) through the same disc shows the stalk of the bud connecting it with the posterior edge of the third thoracic segment.

In comparing sections of the mature leg discs and wing discs (figs. 27, 54), a characteristic difference is noticed. In the leg disc, differential growth leads to the formation of a folded hollow pouch, into which mesenchyme migrates. The future thoracic portion remains unaffected by the process of folding and surrounds the outgrowing leg pouch in the shape of a ring of imaginal tissue forming part of the peripodial sac. In the wing disc, on the other hand, the folding of the anterior portion seems to

⁺for a diagram of the course of the tracheal branch along the disc see fig. 31

be merely the result of the continued growth of a sheet of cells inside a limited space. The folds thus formed possibly are of no greater importance for the morphology of the adult thorax than are the folds of the old pupa wing inside its sheath for the adult wing. In the posterior portion of the bud, true differential growth takes place and starts the outgrowth of the wing pouch, but contrary to the leg pouch no mesenchyme migrates into the cavity of the invagination.

Figs. 52, 55 show that the development of the metathoracic bud during the third instar follows similar lines to those of the mesothoracic one.

The development of the wings in the pupa.

During the first 6⁺ hours after pupation the wing develops from the dorsal mesothoracic bud. This process has already been described in brief by Chen.

The wing is formed by the invagination and subsequent growing-out of the posterior circular portion of the disc. The first signs of this invagination are noticed in a mature larva, shortly before pupation, as a dorsal pouch with a ventral opening. (fig. 62 and phot. 19). During the hours immediately after pupation the invaginated area develops into an oblong pouch stretching posteriorly. Figs. 56 - 58 show this process semi-diagrammatically; they are drawn from discs dissected out without previous fixation. The discs are seen from the ventral aspect, and the attached tracheal branch and the opening of the invagination are visible. It is noticeable that the posterior margin of the original disc

⁺The number of hours given for each developmental stage are averages for the conditions of these experiments. Variation, however, is considerable.

persists during the invagination and forms a thick fold beside the opening.

A more exact inspection shows, that the area which invaginates is already demarcated in the mature larva by folds (fig. 62 and 63 and phot. 17) which play a rôle in the subsequent development. This is shown by a series of fixed and stained total preparations (figs. 64 -70). During the first two hours after pupation the thick margin of the invagination is the most conspicuous feature of the disc (fig. 64). It was termed by Chen the "horse-shoe" on account of its form. Chen claims to have found it in mature larvae; a lateral opening was present which during the first hours after pupation was brought, by a pivoting of the ridge through 90° , into its final, anterior position. Such a process was never observed in the present studies. The wing pouch always grows out posteriorly, thus giving rise to a "horse-shoe" with an anterior opening. In the mature larva, however, the first indication of the invagination appears in the form of a shallow depression which may happen to be a little further advanced and therefore deeper on one side than on the other. After fixation this

might perhaps sometimes give the picture seen by Chen.

Meanwhile the folds in the invaginating portion become more pronounced and subdivide the pouch. They are seen as lateral incisions of the outgrowing pouch. The main fold (g) separates the distal part from the proximal by a very deep groove. The distal part forms the wing proper, the proximal its basal articulation with the thorax. The proximal part becomes subdivided very early by folds which appear as a scalloping of its margin (figs. 65 -67,). During the third to fifth hours the distal portion of the wing pouch grows out until it has become approximately square in outline. In the fifth or sixth hour the young pupal wing develops from it. As the changes in the length and relative position of the cross-folds indicate (fig. 68a and b), this process is brought about by an unequal expansion in the different parts of the pouch. In this way the pouch expands on the side of the groove so that this becomes obliterated and the pouch rotated through about 90° . In the examination of the development of mutant wings this point is important because changes in length and width of the wing correspond to changes in width and length of the pouch before

the expansion.

About 6 hours after the beginning of pupation the developing wing is ready to evert from the peripodial sac. Its antero-dorsal position on the back of the prepupa has to be changed now to that of the pupa wing lying flat against the side of the pupa. Fig. 69 shows a wing in the process of bending-over required for this change of position. In the recently everted pupa wing the future venation is already indicated by a pattern of pale lines, but during the following hours this pattern becomes obscured by the formation of the pupa sheath (photos. 5,6)

The photographs 18-23 show the histological appearance of some of the described stages. In the present study, the histology of the pupa wing was not examined in detail.

A preliminary report on the development of some wing mutations.

13 d(pointedoid)

A great number of total preparations of wing discs of early pupae and mature larvae were made. A series of them is shown in figs. 71-78. Up to the formation of the wing pouch no visible difference from wild type-exists or at least was detectable by comparing fixed and stained total preparations (comp. figs. 64 and 71). Slight differences sometimes observed in the size of the whole bud were considered insignificant, in view of individual variation, of shrinkage during fixation and of the change of size of the bud (mentioned by Chen) just at the time of pupation. The first difference becomes visible when the outgrowing pouch is subdivided by folds. As these folds are marked off already in the mature larva, it is ^{not} unlikely that a detailed histological comparison would be able to detect the difference between wild-type and 13d a little farther back. As soon as the fold g is seen to separate the distal wing-anlage proper from the proximal anlage of the basal articulation, the distal portion is visibly smaller than in wild-type.

(fig. 72). In the following hours it grows out (figs. 73,74) without ever reaching the stage of the square outline, as does wild-type at about 5 hours after pupation (comp. figs. 74 and 67). Stretching and eversion occur in the same way as in wild-type. As a consequence of the rotation of the wing pouch during stretching, described on p.47, the short pouch develops into a narrow pupal wing (fig. 78). About 24 hours after pupation the characteristic shape of the narrow 13d wing has been attained (comp. photos. 8 and 10). Venation is indicated by light lines.

vg(vestigial)

In accordance with Chen I found that the difference between vestigial and normal is already very marked in the wing discs of the mature larva (comp. figs. 79 and 62). The circular portion, which begins to invaginate, is markedly smaller in vestigial than in the wild type. In sections it will probably be possible to follow the difference back to earlier stages. On the other hand, my results do not indicate, as do those of Chen, that a consistent difference in the size of the whole buds of mature vestigial and wild-type larvae exists. In those larvae where such a

difference seemed to exist, I prefer to attribute it to environmental or developmental variation, for the reasons given in discussing the size of the 13d bud (p. 49). Figs. 79-87 show how the basal wing-rudiment, characteristic for vestigial, develops from the circular area. The outgrowing wing-pouch has a narrower basis than has the wild-type (figs. 80, 81), and during the growth stage it remains very small. Apart from this, it shows from its very beginning a constriction marking off a small lateral portion. (fig. 80). After eversion (figs. 84, 85) when the young pupal wing has been formed, this constricted-off portion, having changed its position from lateral to distal by the rotation referred to previously, forms the tip of the young pupa wing. It is still present in older stages after the formation of the pupa sheath (fig. 87, representing a 10 hours' stage), but apparently is obliterated in the course of subsequent development. The proximal portion of the wing alone persists and develops into the vestigial wing.

dp(dumpy)

A few dissections of the dumpy prepupae made it clear that the first visible difference in shape between a wild-type and a dumpy wing must occur after the eversion of the pupa wing.



The recently everted wings of both types are still of the same shape, no indication of the typically cut posterior margin being as yet visible in the dumpy wing (comp. photos. 5, 11). Even 15 hours after pupation the shape of the dumpy wing does not differ from that of the normal one at the same stage (comp. photos. 6, 12). During the following 20 hours, however, the characteristic shape of the dumpy wing is completed, presumably by an obliteration of marginal parts. The first sign of this process was seen in a dumpy wing 17 hours after pupation. At about 20 hours the difference is distinct, the dumpy wing being cut off obliquely at its tip in contrast to the symmetrically shaped tip of the normal wing. At 30 hours the difference is still more pronounced (comp. photos. 9, 14), and a wing 35 hours after pupation is a reduced image of the adult wing (comp. photos. 1, 15).

The difference in size between the normal and the dumpy wing is also established during this period, but in my studies no measurements were made to follow up this process more exactly.

17b(unequal wings)

In order to detect possible relations between the period of physiological activity of a gene and the time of its first influence on morphogenesis, several wing mutants were tested for changes of manifestation with temperature. Among these, the manifestation of 17b proved to be highly susceptible to temperature. Under the conditions used in this laboratory, the penetration⁺ of 17b never reached 100%. With falling temperature it decreases. At 16° all the flies, with very rare exceptions, are normal. Their offspring, bred at 25°, shows again the percentage of 17b wings normal for this temperature.

For a rough determination of the temperature-effective period, the method described by Stanley('31) was adopted. A number of well fed females in their best egg-laying activity were made to deposit eggs in a vial during two hours. All the vials used belonged to the same batch and contained approximately the same amount of the same food. The eggs in the different vials were either laid by identical females or by their sisters or other nearly related females. All vials were kept in

⁺penetration=percentage of individuals exhibiting the character(Vogt 1926, Timoféeff-Ressovski)

an incubator at 26° . At different times during the larval period, batches of vials were removed to the low-temperature incubator at $15-16^{\circ}$, and kept there until hatching. The times for changing the vials were chosen so as to give a series of flies which have spent from 0 to 120 hours⁺ at the high temperature, the intervals being 8 hours.

These experiments cannot be compared to the numerous and exact experiments by Stanley. Their only purpose was to serve as a guide and object of comparison in my studies on morphogenesis. Rough as they are, they fulfill this purpose, as is shown by the diagram fig.88. The temperature-effective period occurs between 96 and 104 hours after hatching, that is to say at the end of the larval stage, shortly before or at the time of pupation.

Discussion.

I. General.

The problem of the time of gene action can be approached from two different angles, namely the genetical and the developmental. From the first with the question : during what stage does a given mutant gene alter the course of ~~normal~~ development?, from the second with the question: what are the successive steps of determination⁺ by which the developing organ becomes restricted in its capacity to respond to the influence of the mutant gene? The present observations are concerned with both questions, but conclusions can be drawn only when they are correlated with the results obtained by other methods. Before doing this, it appears desirable to review those methods briefly in relation to the fundamental problem and to state the reservations necessary in drawing conclusions from them. Only the work on *Drosophila* will be considered.

A The genetical problem.

The determination of the period during which a particular gene influences development can

⁺ Here and elsewhere the expressions "determination" and "determined" are used in the sense given to them by Spemann and Mangold.

often only be made by fixing separately the initial and terminal points of its action. The morphogenetic method used in the present studies indicates the stage at which the divergence of the mutant from normal development is first visible. If from this stage, the mutant organ develops in a normal way, it may be assumed that the terminal point of the action of the gene has been found. If not, the terminal point can be determined by examining successively later stages of development. The morphogenetic method cannot be applied for the determination of the initial point of gene action, for the mutant gene may exercise an influence on development long before a visible difference appears.

Less direct methods must be used for this purpose. Among these, temperature experiments have been prominent. They may be of two types.

1. The production of modifications by the exposure of eggs, larvae or pupae of definite age to sublethal temperature.
2. The determination of a temperature - effective period for the manifestation of the mutant character.

The first type was used by Goldschmidt ('29, '35), Jollos ('33, '34), Plough and Ives ('34), Gottschewski ('33) and J. Schultz ('35).

Since the development of the artificially produced modifications must have been normal at least into the exposure, the time of exposure represents a lower limit for the beginning of the altered development. Such artificial modifications are often phenotypically identical with natural mutations or with mutations induced by the same treatment. The lower limit for the initiation of a developmental change would be identical for such a modification and the corresponding mutation only, if the same process is affected in both. In using the results of such experiments for drawing conclusions on the starting point of gene action, it must be borne in mind that this will not always be true. As regards the endpoint of gene influence, this type of temperature experiment offers no clue.

The second type of temperature experiment, viz. the determination of a temperature-effective period (TEP) was used by a number of *Drosophila* workers (Stanley '31, '35, Timoféeff-Ressovsky '32, '34, Plunkett '26, Riedel '34 a.o.). Since any stage in the chain of reactions connecting the gene with its end result may respond to temperature, no exact conclusions concerning the time of gene action may be based on the results obtained by this method.

But as it is generally assumed that the TEP coincides with a period of increased genic activity, and as obviously the period of gene influence on development is one of great genic activity, the existence of a TEP often gives an indication of the time of action of the gene in question, which must be verified by other methods. If, during the TEP, future manifestation can be wholly suppressed, this second type of temperature experiment will provide a further means of determining a lower limit for the initial point of gene action.

A quite different way of approaching the problem from its genetical aspect consists in studying the interaction of genes influencing the same character. Csik('34) by this method could draw conclusions on the relative position of the effective periods of different mutant genes. If this period were known for some genes, this method would help to determine it for the others.

B: The developmental problem.

One of the favourite methods for establishing the successive steps in the determination of an organ consists in destroying definite parts of this organ during development. In *Drosophila* this method offers great technical difficulties and

has until now been used successfully only in the embryo (Geigy'31b, Howland and Child'35)- During later stages, the methods used for the solution of the genetical problem and discussed in the precious paragraphs may also prove helpful for approaching the developmental problem, because an organ may respond to many mutant genes in the same way as it would to external agents. If, e.g., a certain mutant gene influencing shape has a temperature-effective period, it may be concluded that at the time of the TEP shape was not yet determined finally. If, on the other hand, a mutant organ characterised by missing parts shows the deletions from the first formation of its outward shape, it may be inferred that the parts which are missing in the mutant organ, must have been determined already in the unshaped anlage of the mutant, and the normal organ.

II.))Special.

With the help of the principles discussed above, my observations may be used for drawing conclusions on the process of determination in the legs and wings of *Drosophila* and on the action of the mutant genes examined. The developmental aspect will be discussed first, as it forms the basis for dealing with the genetical.

A. The developmental problem.

My studies established contrary to Chen that the imaginal buds of all the thoracic appendages are present in the newly hatched larva. It is therefore reasonable to assume that they were formed during embryonic life. This is in accordance with the results of Geigy who produced imaginal defects by treating eggs with ultraviolet rays. Similar results were obtained by Howland and Child. In Geigy's experiments there was a marked difference in the response of different organs at different times of exposure. All the wing defects were obtained by earlier exposures than the leg defects. Further the predominant effect of early irradiation was to induce defects of the fore legs, but as the time of exposure was shifted to later embryonic stages, more defects of the middle and finally of the hind legs were obtained. To explain these effects, Geigy suggests different sensitive periods for the different organs. Now, it is generally assumed that periods of increased sensitivity in a developing organ coincide with periods of increased developmental activity. From this point of view it is interesting to correlate Geigy's results on the sensitive periods with my observations on the different

degree of development which the appendages have reached at the time of hatching of the egg. The wing buds, having already invaginated into the body cavity, are more advanced in development than the leg buds. In the latter, no morphological difference is apparent at hatching, on account of their primitive structure, but the fact that the third pair is definitely lagging behind during the first and second instar, suggests that its development is already retarded at the time of hatching. On similar, but very weak evidence, the second pair is possibly slightly retarded relative to the first pair. If we make the justified assumption that the more advanced in development an organ is at the time of hatching, the earlier it has started development, we get the following sequence for the starting points of development: wings, first pair of legs, second pair of legs (possibly simultaneous with the second pair), third pair of legs. The analogy of this series to the series of Geigy's sensitive periods is noteworthy and suggests that the periods of increased developmental activity, as indicated by increased sensitivity, are the periods of the first histological differentiation.

Geigy's experiments also throw some light on the process of progressive determination inside the anlage of the leg. Irradiation at the earliest susceptible stage leads to complete suppression of the leg, a little later it produces total reduplication, crippling and partial reduplication, the latest and weakest effect being a modification of the distal segments only. This means that, whereas in the early stages the anlage is still undetermined and has the capacity to produce two legs out of the material intended for one, a little later the pattern of the future parts has been formed so that the effects are more localised. Therefore it must be inferred that the concentric folds which divide the anlage during the third instar, are not the cause, but the consequence of the determination of the different parts of the leg.

Certain genes influence leg development in the same way as does injury to the embryo. For instance the gene described by Hoge('15) caused a reduplication of the legs. It may be assumed therefore to have exercised its influence on the young anlage. This assumption agrees well with Hoge's experiments on the influence of temperature on the manifestation of this gene, since a TEP seems to

exist at a very early stage. Another mutant gene for abnormal legs, crippled(Komai '26) is interesting on account of its variable effects. It affects either the middle or the hind legs (very rarely the fore legs), causing suppression, crippling or reduplication in accordance with various physiological and environmental influences. All these phenomena would find a common explanation if it was assumed that the type of manifestation depends on the time of gene action in relation to the stage of determination of the leg anlage. A very slight shift of this time due to an environmental or physiological agency would account for a great change in the type of manifestation.

Some conclusions on the successive steps in the determination of the wing can be drawn from a comparison of my results with those obtained by other methods discussed above (p.56 ff)

Chen calls the first 5 hours of prepupal life (under the conditions of my work they correspond to the first 6 hours) the critical period of wing formation, because during this period an organ resembling in shape the adult wing is formed from a circular area in the wing disc. However, from a more complete study of the development of the wing it would appear that there are at least four periods of morphogenetic importance in wing development, namely:

1. The formation in the embryo of the anlage of the dorsal mesothoracic bud as a whole.
2. The subdivision of the bud in the early third instar into the thorax-forming and the wing-forming portion.
3. The growing out of the wing pouch in the mature larva and prepupa.
4. The final development of the details of the wing in the pupa.

During the first period complete suppression of the wing or the production of abnormal wings was obtained in the experiments of Geigy and Howland and Child.

The second period seems to be important in the development of the vg wing. At this time the differentiation of the circular area, which is much reduced in size in vg, takes place.

The third stage of morphogenetic importance is Chen's critical period. The following data suggest that the shape of the wing as a whole is determined during this period.

a Exposure to heat at the beginning of this period (Goldschmidt, Jollos, Plough and Ives) leads to modifications of the wing shape, e.g. the mutants 13d and 17b were produced in this way.

b The manifestation of l7b is suppressed completely by exposure to low temperature during this period.

c Also for the normal wing this stage has the importance of a TEP (Hersh and Ward '32). In contrast to vestigial, the length of the wild type wing decreases with increasing temperature during this time. According to Riedel('34) the change of wing area is not, as in vestigial, brought about by loss or addition of distal parts, but by an approximately proportionate reduction or enlargement of the whole area.

d At the end of this period, the l3d wing differs markedly from the normal wing in a way which clearly foreshadows its future narrow shape.

The first three observations show that at the beginning of the third period of morphogenetic importance the determination of the shape of the wing as a whole has not yet occurred, while the last suggests that at the end of this period it is completed. The actual determination must occur between these limits, i.e. during the third period of morphogenetic importance.

Furthermore the development of the vestigial wing proves that in this period also the determination of the parts of the wing has begun, for

otherwise it would not be possible for a fold in the outgrowing wing pouch to constrict off the future distal part of the wing.

Even when the shape of the wing as a whole has been determined during the third period, its details are still subject to the influences of mutant genes or external agencies. Goldschmidt ('35) in his heat experiments obtained modifications of the wing shape by a treatment as late as the seventh day after the eggs had been laid. My observations, on the other hand, show that the recently everted pupa wing of dumpy still resembles in shape the normal and assumes its final shape only 20-30 hours after pupation. Therefore an alteration of the shape determined roughly in the third period is still possible in the fourth period, probably by obliteration of parts (Goldschmidt).

The size of the wing may be affected by genetic or environmental influences during any of the four periods: in the first through an effect on the anlage of the dorsal mesothoracic bud as a whole (small wing obtained in the experiments of Howland and Child), in the second through change in size of the wing forming area (vestigial), in the third and fourth through indirect results of the alteration of shape.

Gottschewski('33), from his experiments on the production of mutations by exposure of larvae to sublethal temperatures, postulates the existence of a critical period for alterations of the wings, which would coincide with none of the four morphogenetically important stages. He locates it at the end of the first instar, a period which (following Chen's statement) he believes to coincide with the first formation of the dorsal mesothoracic bud. However, as he is chiefly concerned with mutations, and not with modifications, and as his age determination is very inexact, no valid conclusions on the existence of an additional critical period can be drawn from his data. In any case, it cannot be maintained that the production of wing alterations is restricted to his critical period.

B. The genetical problem.

In the following paragraphs will be discussed when and how the mutant genes used in my investigations influence the development of the wing.

13d(pointedoid).

The fact that a modification exactly resembling 13d was produced by the same treatment as the mutant 13d at the end of the larval or the beginning of the pupal period (Jollos'34) indicates the point

before which the action of the gene has not begun (under the reservations discussed in the general part). In the present study, the first visible influence of the gene was observed during the stage of the outgrowing wing pouch. The groove separating the distal wing anlage proper from the proximal anlage of the basal articulation marks off a smaller distal portion than in wild type. A detailed histological examination might establish a deviation from normal even in the arrangement of the ridges in the circular, wing-forming portion of the disc in the mature larva. In any case, the results of the physiological and morphogenetic methods agree well enough to suggest that the gene begins to exercise its influence on development at the beginning of the third period of morphogenetic importance. At the end of this period the narrow shape of the wing is apparent; its final shape is obtained during the rest of the first day of pupal life. It seems therefore that *l3d* influences development in two successive steps: first by a modification of the subdivision of the wing pouch in the third period, thereby outlining roughly the narrow shape of the wing, and secondly by finally modelling

the peculiar outline of the 13d wing during the fourth period, probably by subsequent obliteration of the already formed marginal parts. (Csik'34).

17b (unequal wings).

17b was produced as a mutation and as a modification by the same treatment as 13d. Its TEP was found to be at the same stage at which 17b was produced by heat treatment. Low temperature during the TEP suppresses manifestation completely. Both these facts prove that the decisive action of the gene does not take place before the end of the larval or the beginning of pupal life, i.e. our third period of morphogenetic importance. This makes it appear very probable that the mechanism of action of the 17b gene consists chiefly or solely in an alteration of the process of growing-out of the wing pouch. Morphogenetic studies to test this hypothesis are in progress.

dumpy The dumpy gene acts on the pupal wing after the formation of the pupa sheath, presumably by obliteration of marginal parts. This agrees well with the results of Goldschmidt ('35) who produced modifications like dumpy by treating normal young pupae 6 days after the eggs had been laid.

vestigial

It seems that the gene vestigial exercises its influence over a long period or else at different periods, separated by intervals. At an early developmental stage it is highly susceptible to temperature (Stanley '31, '35). Contrary to Stanley's assumption, based on Chen's studies, the TEP at the end of the first instar does not coincide with the time of formation of the dorsal mesothoracic bud. The only morphogenetic process with which it might coincide, is the differentiation of the bud into the disc proper and the peripodial sac. However, it is not necessary to assume that the TEP coincides with any important morphogenetic process at all. It may conceivably affect some link in the chain of reactions started by the gene before there are visible effects. The shifting of the TEP to the third instar for larvae bred at 30° (Stanley '35) cannot be explained without histological examination of the development at different temperatures.

On purely morphological grounds it may be assumed that the vestigial gene first exercises its influence on development in the second period of morphogenetic importance, by reducing the wing-forming area. This results later in a reduction of the size of the outgrowing wing pouch as a whole. At the beginning of the third period, another effect

of the gene is visible in the constricting-off of the future distal part of the wing by a fold. During the fourth period this portion is obliterated; whether this is only a consequence of previous constriction, or whether the gene continues to exercise a direct influence during the process of obliteration, cannot yet be decided.

Goldschmidt's ('35) experiments show that the effect on development produced by the weak allelomorphs of vestigial can also be induced by heat treatment of normal larvae. In showing further that the heat treatment and the vestigial gene can have a joint effect in producing weak vestigial manifestation, Goldschmidt proves that both affect the same process. The fact that the treatment was effective only at the end of the larval period, suggests that this process is the formation of the fold in the outgrowing wing pouch, and that it (and not the preceding determination of the size of the wing forming area) is influenced by the action of the weak vestigial allelomorphs.

Summary

- 1.) The development of the thoracic appendages in *Drosophila melanogaster* has been studied morphologically and histologically. The successive steps of development have been arranged in a series according to their relation to the three larval instars. Criteria are given for determining the developmental stage of a larva on sections.
- 2.) The imaginal discs of the wings, halteres and legs are all present in the newly hatched larva.
- 3.) The leg buds at the time of hatching are represented by ventral thickenings of the hypoderm.
- 4.) They invaginate into the body cavity towards the end of the second instar. At the same time they differentiate into the disc proper and the peripodial sac.
- 5.) The anlage of the leg is formed from the disc during the third instar.
- 6.) All the leg discs are connected with the tracheal system.
- 7.) The first and second pair have a direct connection with the ventral ganglion, but for the third pair, such a connection could not be clearly established,

- 8.) During the first 6 hours after pupation the legs become everted. The remaining portions of the discs participate in the formation of the thorax.
- 9.) The wing buds and halter buds, at the time of hatching, are already sunk into the body cavity of the larva. About 15 hours after hatching each forms a small sac with a distinct lumen.
- 10.) Both pairs of buds are attached to branches of the dorsal trunks.
- 11.) No connection of the dorsal buds with the nervous system was observed.
- 12.) During the second instar differentiation of the bud into the disc proper and the peripodial sac takes place.
- 13.) During the third instar, the disc proper, by a complicated process of folding, becomes divided into several portions, each of which has a different destiny.
- 14.) In the mature larva, the dorsal mesothoracic bud consists of an anterior portion, which will take part in the formation of the thorax, and a posterior portion, which will form the wing.
- 15.) In the dorsal metathoracic bud, the process of folding follows the same lines, but not to the same degree of complexity as in the mesothoracic.

- 16.) The wing develops from a circular portion in the posterior part of the dorsal mesothoracic bud. Invagination of this portion begins in the mature larva and is finished about 6 hours after the beginning of pupation.
- 17.) The outgrowing wing pouch is subdivided into a proximal portion, the future basal articulation of the wing, and a distal portion, the future wing.
- 18.) During the 6th hour of pupal life, the wing pouch stretches and changes its shape by different expansion of its parts.
- 19.) About 6 hours after the beginning of pupation the young pupa wing is everted from the peripodial sac.
- 20.) A preliminary report on the development of the wings in several mutants is given.
- 21.) In l3d (pointedoid) the first marked difference from the normal development is visible when the outgrowing wing pouch is subdivided by a groove. The pouch does not attain the length of a normal one. A recently everted pupa wing of l3d is obviously different from a wild pupa wing of the same stage.
- 22.) In vestigial, the difference from wild type is already visible in the mature larva.

The circular portion which will invaginate to form the wing pouch has a markedly smaller area than in wild type. From the outgrowing wing pouch, a small part, the future distal portion of the wing, is constricted off by a fold. In the course of the subsequent development, this portion is obliterated. The remaining proximal portion of the wing pouch develops into the adult vestigial wing.

23.) In dumpy the difference from wild type is not yet visible in the recently everted pupa wing.

The deviation occurs later in pupal life, presumably through obliteration of marginal parts.

24.) 17b(unequal wings) has a temperature-effective period shortly before or at the time of pupation. Flies which have spent this period at low temperature do not manifest the mutant character at all.

25.) The bearing of these observations on the two problems: the time of action of the mutant gene on development-, and the process of gradual determination of the anlagen of legs and wings - are discussed.

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Explanation of figures.

1. The following magnifications have been used:

40 for the photographs of adult wings

110 " " " " pupa wings

70 " " " " sections

about 65 for the drawings of total preparations of pupa wings

" 750 for the drawings of histological preparations of the first instar

" 600 for the drawings of histological preparations of the second instar

" 300 for the drawings of histological preparations of the third instar and for figs. 45 and 17b (second instar).

2: All the histological preparations, unless otherwise stated, are cross-sections. It must be noted that the expressions cross- and longitudinal section refer to the direction of the plane of section relative to the larval body. Since the direction of the imaginal buds, especially of the lateral group, does not coincide with that of the body, a cross-section through the body does not necessarily mean one through the bud. A slightly oblique cross-section through a mature larva, e.g., may well be an almost longitudinal section through the dorsal mesothoracic bud.

3. Where there are several cross-sections through the same bud they are given in the anterior-posterior order. The distance between two consecutive ones is ^{indicated} by the figures in brackets behind the letter of the section.

4. On all the cross-sections, left and right are interchanged because of the orientation of the animal.

List of abbreviations.

l. and r.	in connection with the abbreviation for an organ mean right and left
I, II, III	first, second, third thoracic segment
1A	first abdominal segment
A	wing forming portion of the dorsal mesothoracic bud
a.sp.	anterior spiracle
b.a.	basal articulation of wing
b.m.	basal membrane
br.	brain
c.	connection of bud with hypoderm
ca.	circular area of wing disc
c.c.	cephalic complex
con.	connection of the dorsal mesothoracic and the ventral metathoracic bud
cr.	cross ridges on the wing disc
cut. (I, II, III)	cuticula (first, second, third instar)
cx.	coxa
d.ms.	dorsal mesothoracic bud
d.mt.	dorsal metathoracic bud
d.s.g.	duct of salivary glands
d. tr.	dorsal tracheal trunk
di.	distal part of wing pouch
e.d.	eye disc
f.	fat body
f.v.	fold in the wing pouch of the vestigial wing
g.	groove in the outgrowing wing pouch

g.c.	gastric coecum
h.	haltere
h.d.	halter disc proper
h.f.	hypodermal fold
hs.	"horseshoe" of the dorsal mesothoracic bud
i.h.	imaginal hypoderm
k.o.	Keilin's sensory organ
l.	lumen of bud
l.d.	leg disc proper
l.h.	larval hypoderm
lat. hy.	lateral hypoderm
lg.	leg
m.	muscle
m.l.	median line
m.r.	marginal ridge of wing pouch
n	nerve
n. pr.	neural process
p.b.	peripodial sac
p.c.	peropodial cavity
p.cs.	pupa case
p.s.	pupa sheath
p.w.	pupa wing
ph.	pharynx
ph.m.	pharyngeal muscles
ph.w.	wall of pharynx
pr.	proximal part of wing pouch
o.p.	obliterating portion of vg wing
r.f.	ring fold (circular fold)
s.g.	salivary gland
st.	stalk of bud
tr.	trachea
tr.pr.	tracheal process
trl.	tracheole

v.g.	ventral ganglion
v. hy.	ventral hypoderm
v.ms.	ventral mesothoracic bud
v.mt.	ventral metathoracic bud
v.pr.	ventral prothoracic bud
w.d.	wing disc proper
w.p.	wing pouch

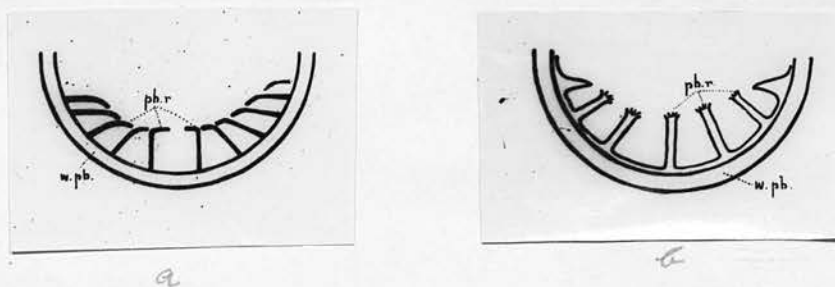


Fig.1 diagram of cross-section of the larval pharynx

a first instar

b second instar

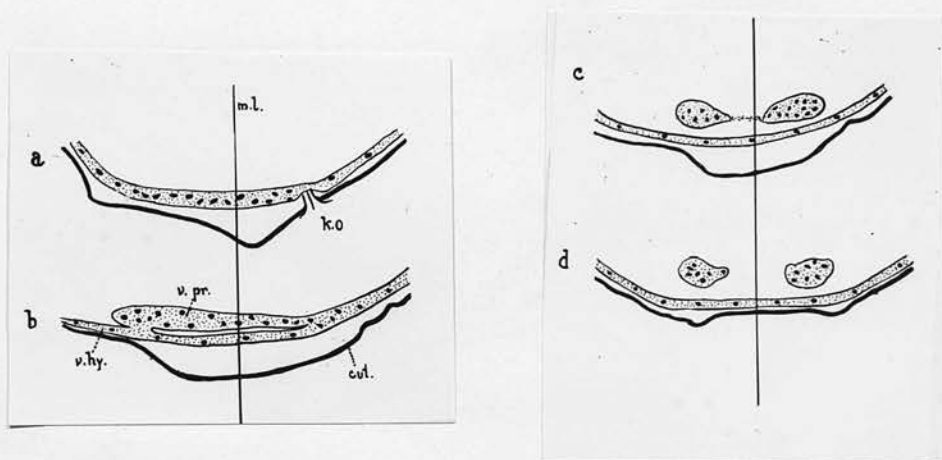


Fig.2 ventral prothoracic buds of a first instar, 2 hours after hatching

a a midventral thickening of the one-layered hypoderm. On the left, Keilin's sensory organ and the underlying depression of the hypoderm are present. Those on the right had been seen in the preceding section.

b (5) the common anlage of the two prothoracic buds separating from the hypoderm

c(5) the buds separating from each other, at the same time giving rise to the lateral processes

d the buds are completely separated; on the following sections they are drawn out into the thin neural processes



Fig. 3

Left ventral mesothoracic bud of the same larva as in fig. 2

a a thickening of the ventral hypoderm near the median line posterior to Keilin's sensory organ

b the bud is separated from the hypoderm, it gives rise to the tracheal process. On the following sections, the bud becomes drawn out into the neural process

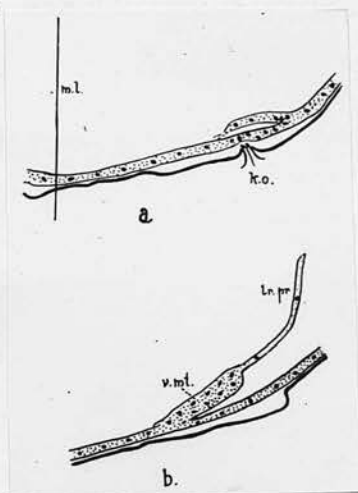


Fig. 4 Left ventral metathoracic bud of the same larva as in fig. 2

a the ventro-lateral hypoderm forms the bud under Keilin's sensory organ

b (5) the bud gives rise to the lateral process

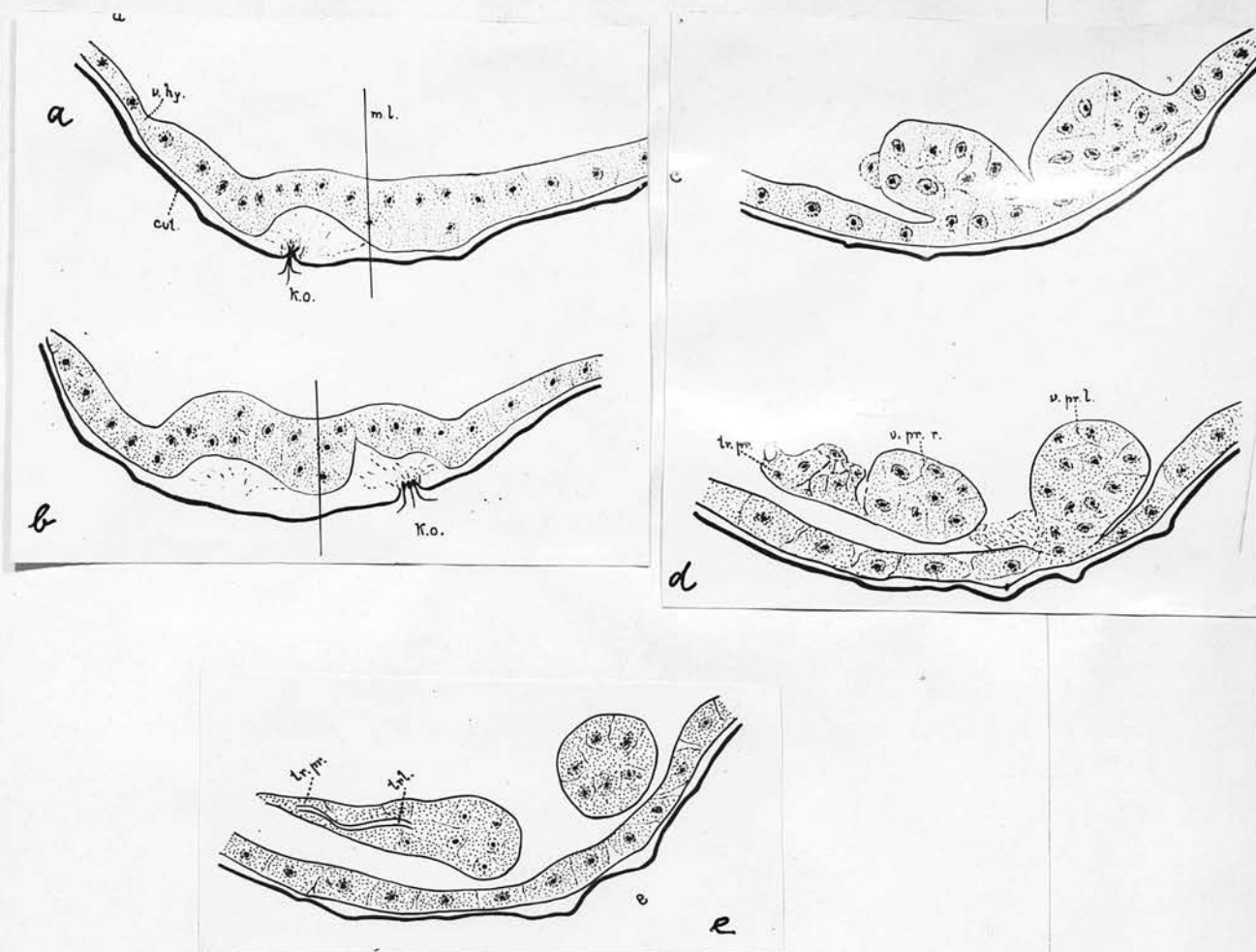


Fig. 5 Ventral prothoracic buds of a newly hatched *Calliphora* larva, at the same magnification as in figs. 2-4.

a sensory organ and hypodermal depression of the right bud

b the same of the left bud (5)

c(5) both buds visible; they are connected in the median line

d (5) the buds are separated from each other; the right one gives rise to the tracheal process

e(5) the buds are separated from the hypoderm; on the following sections they are drawn out into the neural processes. The tracheal process of the right bud with a tracheole in it is visible.

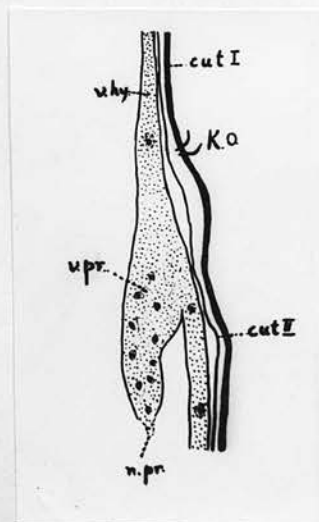


Fig. 6 Longitudinal section through one of the prothoracic buds of a first instar of 18 hours. On the following sections the neural process grows out to meet the ventral ganglion.

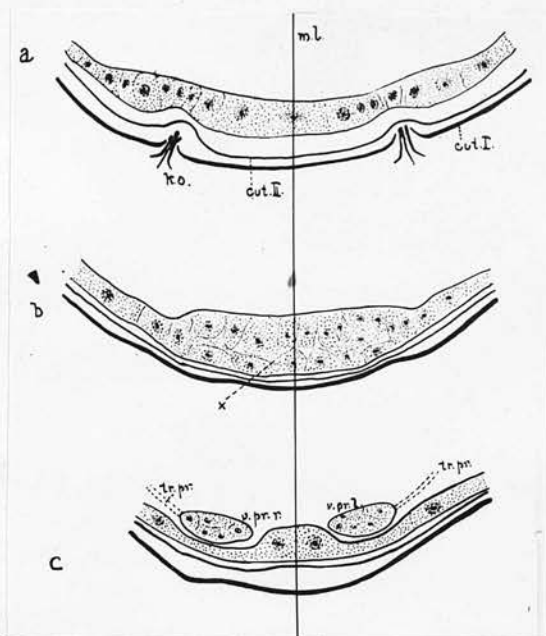


Fig.7 Ventral prothoracic buds of a late first instar (29hours), shortly before the first ecdysis

a both sensory organs and the underlying hypodermal depressions visible. The difference between the elongated imaginal cells and the large cells of the larval hypoderm is apparent.

b(10) at x fissure between the common anlage of the buds and the ventral hypoderm

c(10) the buds are separated from the hypoderm. The tracheal processes are shown diagrammatically, on the evidence of the following sections

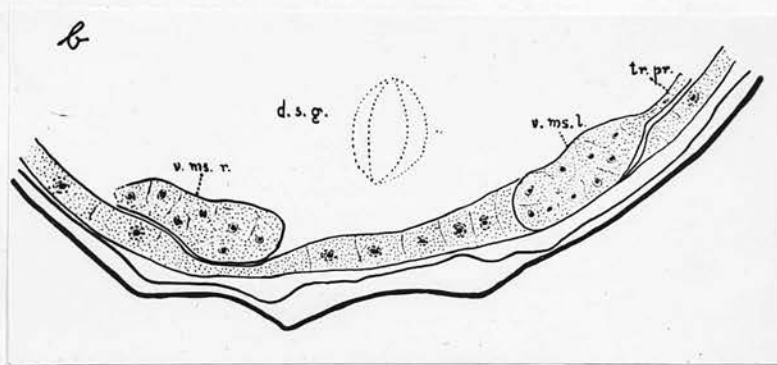
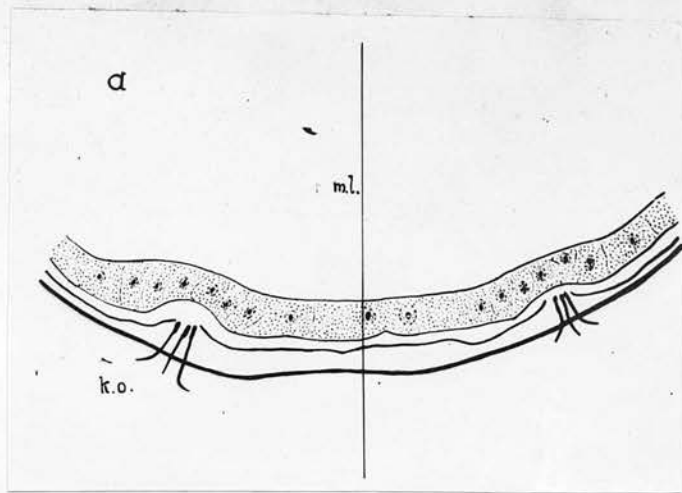


Fig. 8 Ventral mesothoracic buds of the same larva as in fig. 7

a both sensory organs and underlying hypodermal depressions are visible

b (10) the lateral processes are given off, while the buds grow out of the hypoderm

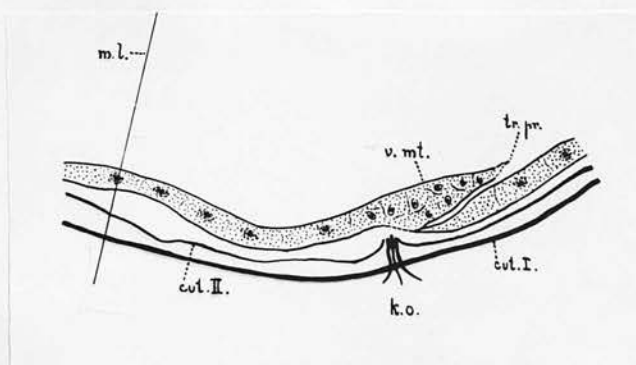


Fig. 9 Left ventral metathoracic bud of the same larva as in fig. 7

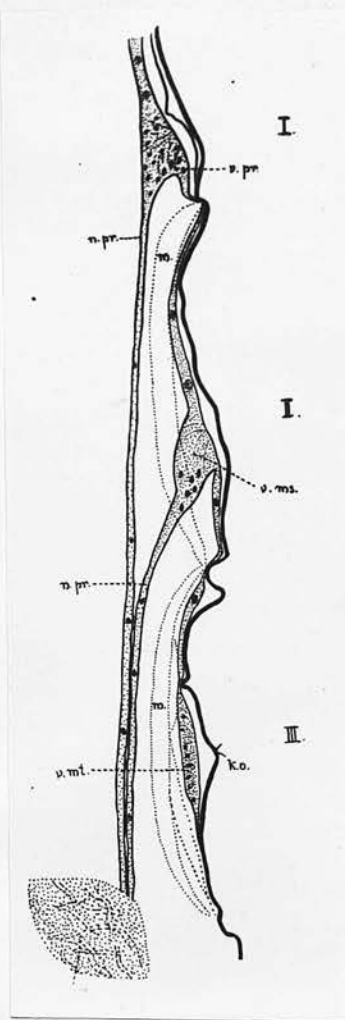


Fig. 10 Longitudinal view, reconstructed from several consecutive sections, of the three ventral buds of a larva about the middle of the second instar. The neural connections of the two anterior buds can be followed to the ventral ganglion. Keilin's sensory organ is visible over the metathoracic bud.

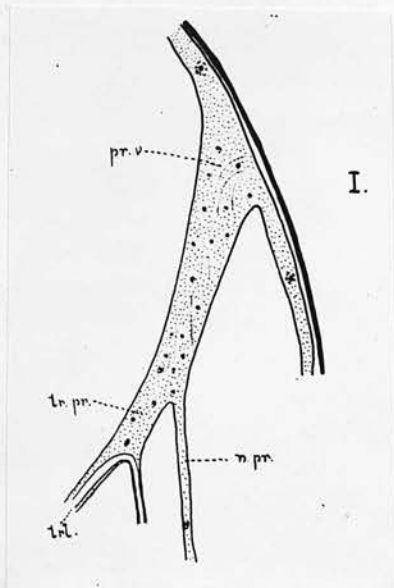


Fig. 11 Longitudinal section of a prothoracic bud of a larva of 32 hours, second instar. The tracheal process is seen to divide into two shortly before joining a branch of the dorsal tracheal trunk.

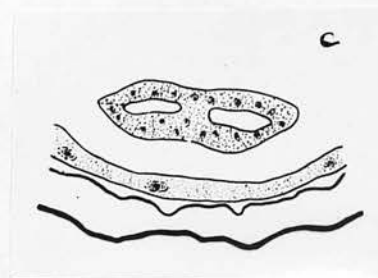
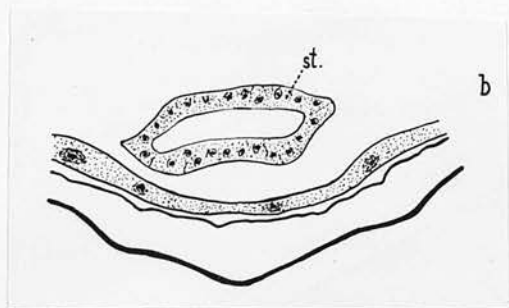
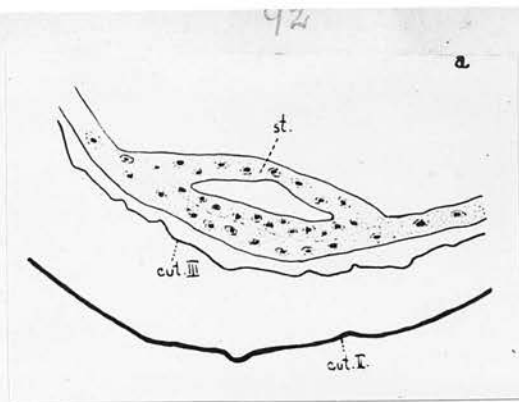


Fig. 12 Ventral prothoracic buds of a second instar shortly before the second ecdysis (46 hours).

a the midventral hypoderm gives off the common stalk of the buds

b(10) the stalk is separated from the hypoderm, it is very broad and short with a distinct lumen

c(5) the stalk divides into the two buds. No indication of the leg anlagen is visible on the following sections

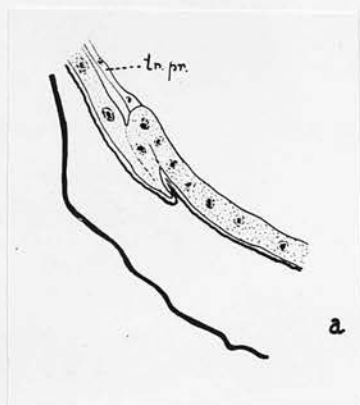
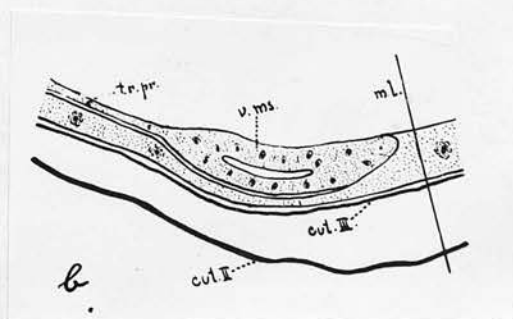
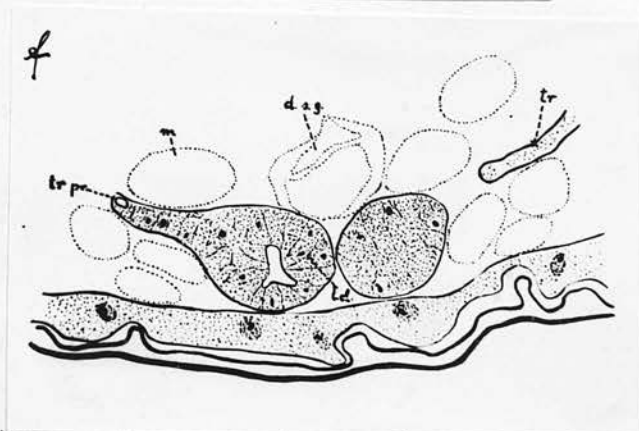
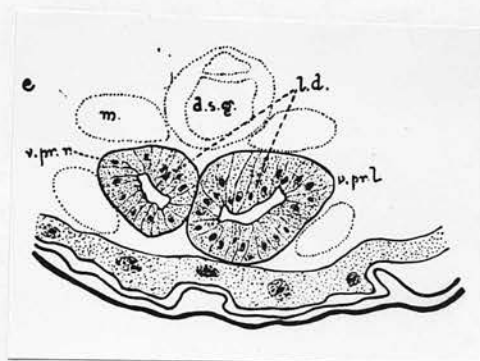
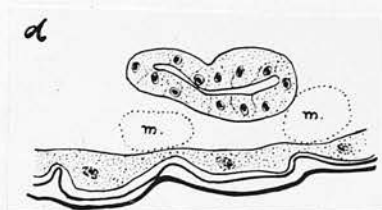
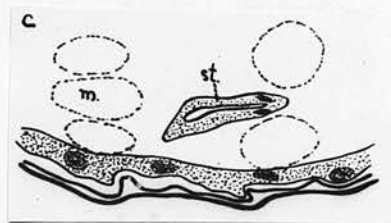
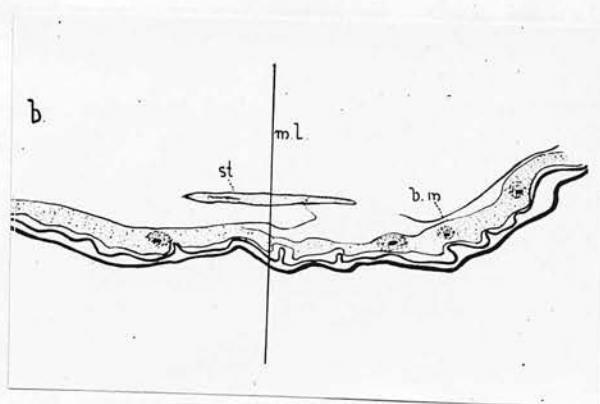
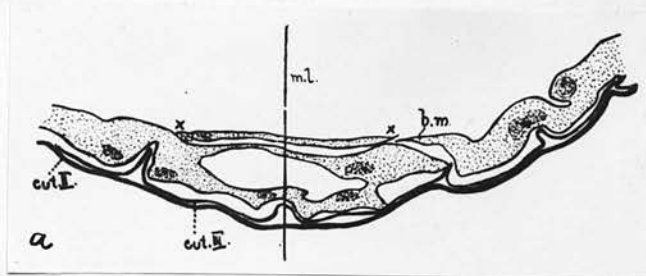


Fig. 13 Right mesothoracic bud of the same larva as in fig. 12

a the beginning invagination of the bud is visible

b(10) the bud is separated from the hypoderm, but still lying close to it. A lumen is developed, but no indication of the leg anlage





(Legend on next page)

Fig. 14

Fig. 14, legend (figure on preceding page)
 Ventral prothoracic buds of a larva of the same age as in fig. 13, but a little more advanced in development

a from x to x the common stalk of the buds is seen to separate from the hypoderm posterior to Keilin's sensory organs. The basal membrane of the hypoderm is torn open to give way to the protruding stalk

b (20) the stalk is clear of the hypoderm, the basal membrane is still open. The lumen of the stalk is reduced to an almost invisible fissure

c (40) the lumen in the stalk is clearly visible

d (20) the stalk begins to divide into the two buds, its wall has become thicker, it consists of a regular layer of imaginal cells.

e (35) the two buds adjoin in the median line. Their medio-dorsal wall shows a thickening, the first indication of the leg anlage

f (30) the right bud is drawn out into the tracheal process. A tracheal branch approaches the left bud

Fig. 15 legend, (figures on following page)
 Ventral mesothoracic buds of the same larva as in fig. 14

a Keilin's sensory organ at the beginning of the left bud

b (10) the beginning invagination of the left bud becomes visible

c (45) the stalk of the left bud is still attached to the hypoderm by a fragment of the torn basal membrane. Its lumen is too narrow to be visible

d (45) a tracheal branch approaches the stalk of the left bud

e (45) signs of a differentiation into the disc proper and the peropodial sac are seen in both buds, particularly in the left one

f (20) in both buds the disc proper has become differentiated

g (30) the right bud has thinned down to the neural process

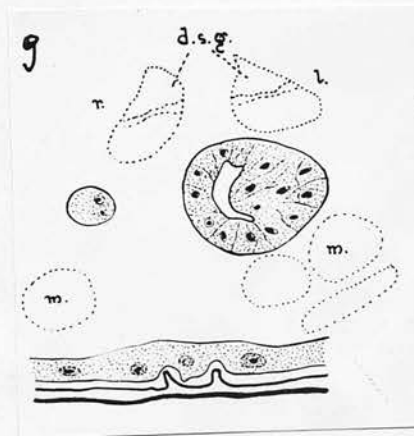
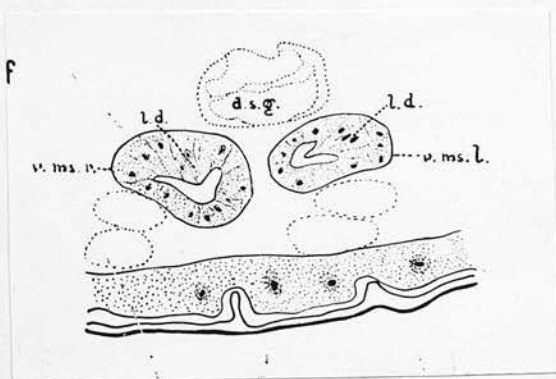
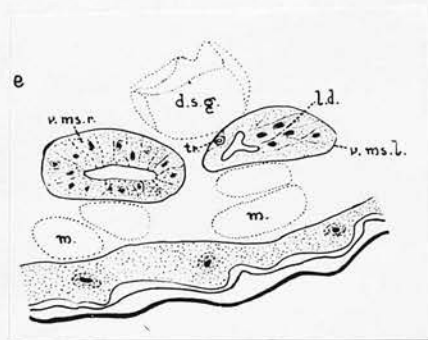
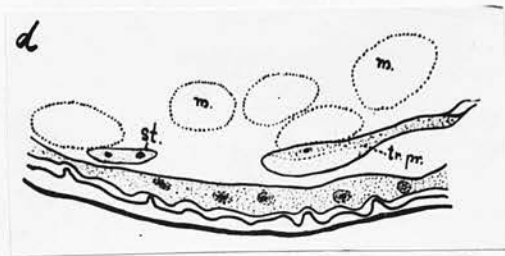
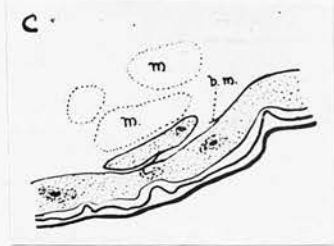
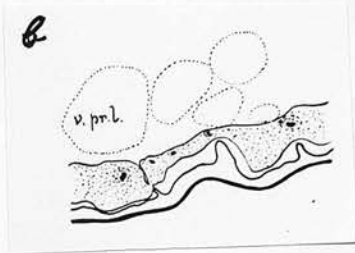
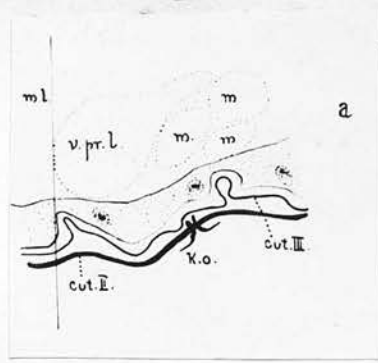


Fig.15
(Legend on preceding page)

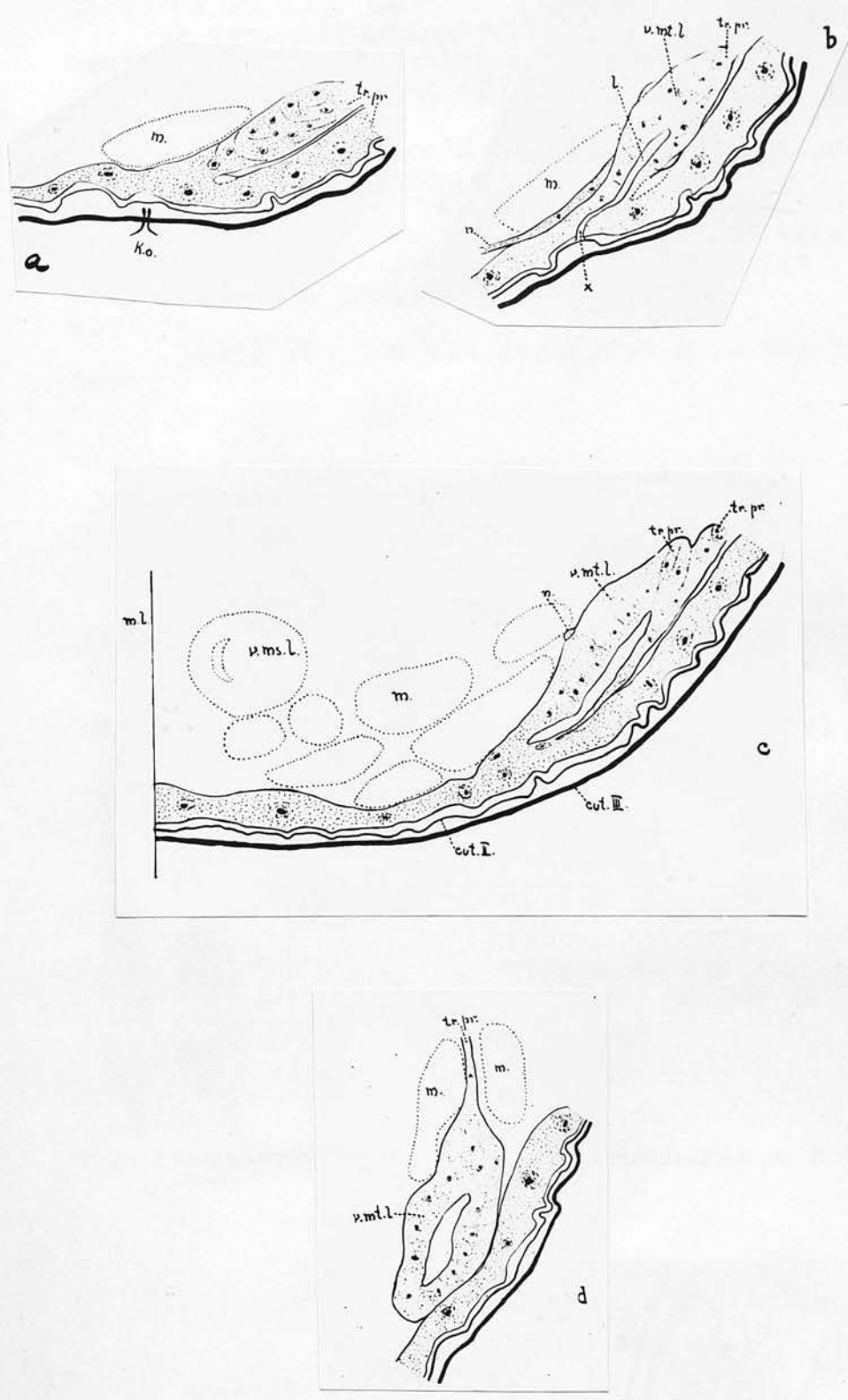


Fig.16

(Legend on following page)

Fig. 16 legend (figure on preceding page)
Left ventral metathoracic bud of the same
larva as in fig. 14

a reconstruction from two consecutive
sections, showing Keilin's sensory organ, the
underlying depression of the hypoderm and the
lateral process

b(5) at x invagination, forming the lumen.
A nerve seems to enter the bud from its
median side (see p. 29)

c (10) the bud gives off a second tracheal
process. The differentiation into the disc
proper and the peripodial sac is not yet
visible

d(25) the bud has separated from the hypo-
derm

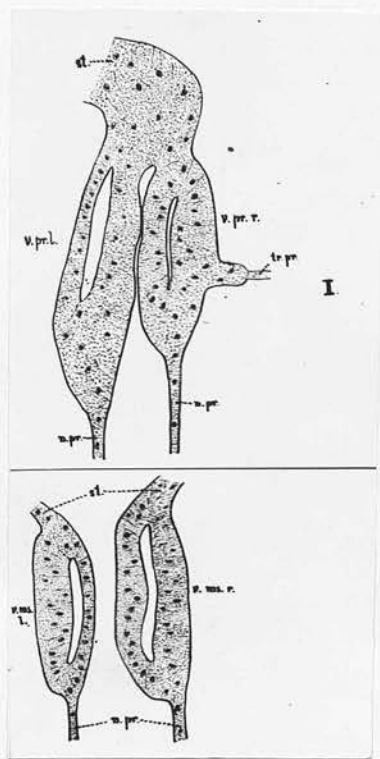
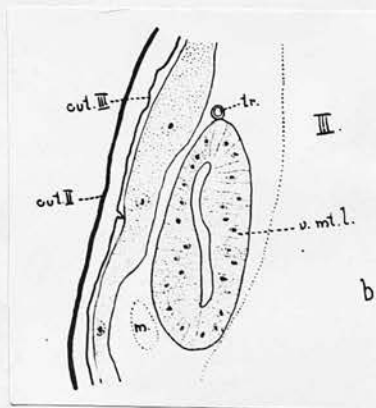


Fig. 17 Frontal sections
through the ventral
buds of a larva of the
same stage as fig. 14

a reconstruction from
three consecutive
sections, showing the
two anterior pairs of
buds. The tracheal pro-
cess is visible in only
one prothoracic bud. The
neural processes can be
traced to the ventral
ganglion on the following
sections

b metathoracic bud



T

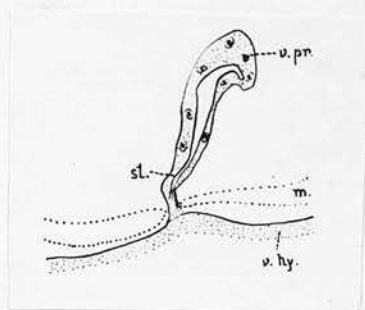


Fig. 18 Section through the common stalk of the ventral prothoracic buds of a larva of the same age as in fig. 13, but farther advanced in development, having entered the second ecdysis. The stalk is becoming drawn out into a thin tube (comp. fig. 13). On the following sections it divides into the two buds in the same way as in fig. 14

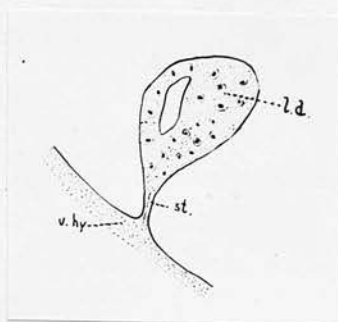
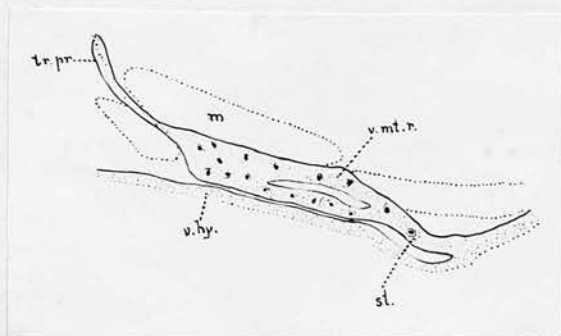


Fig. 19 Left mesothoracic bud of the same larva as in fig. 18, reconstructed from several consecutive sections. The attachment of the bud to the hypoderm by a thin stalk is visible. The differentiation of the bud into the disc and the peripodial sac becomes much more distinct on the following sections

Fig. 20 Right metathoracic bud of the same larva as in fig. 18. The stalk is drawn out into a thin tube (comp. fig. 16).



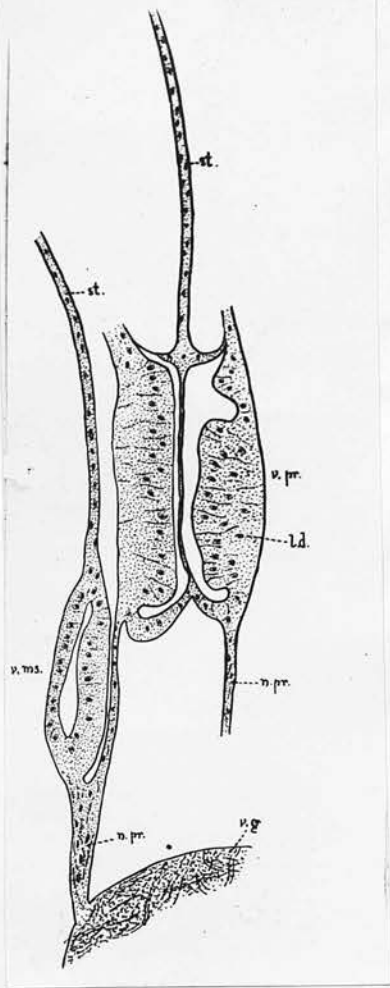


Fig. 21 Frontal section, reconstructed from several consecutive sections, through the ventral prothoracic and mesothoracic buds of a middle third instar (65 hours). One of the mesothoracic buds is not visible, the sections not being exactly frontal. These stalks have become long and thin, the neural connections correspondingly short, (comp. figs. 10 and 17). The anlagen of the legs are visible in the prothoracic buds, while in the mesothoracic they are outside the plane of the section.

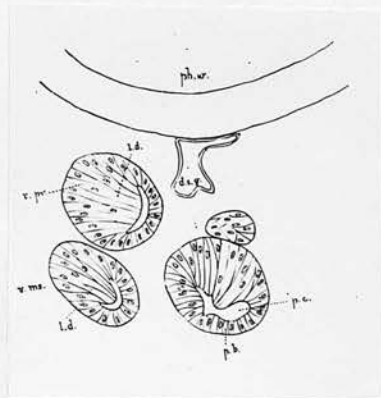


Fig. 22

The ventral prothoracic and mesothoracic buds of a larva immediately after the second ecdysis. The disc proper is protruding far into the peripodial cavity, the remaining ^{portion} of the wall of the bud forming the peripodial membrane. Of the left mesothoracic bud only the neural process is seen.

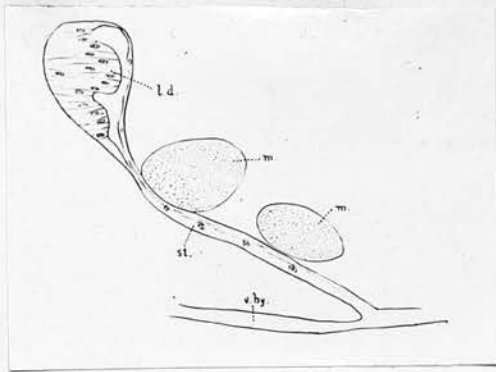


Fig. 23 Ventral metathoracic bud of the same larva as in fig. 22. The anlage of the leg is visible.

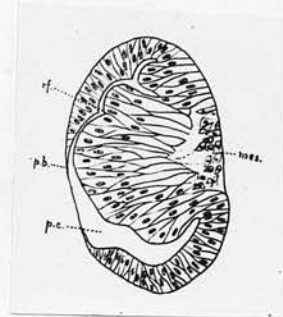
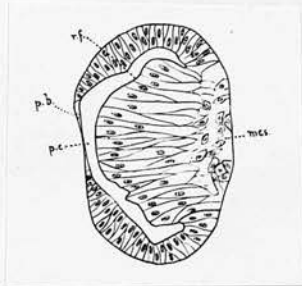


Fig. 24 Ventral mesothoracic bud of a middle third instar (65 hours). The disc proper has differentiated into the thoracic protion and the anlage of the leg protruding into the peripodial cavity. The first indication of an oval annular fold on the surface of the anlage of the leg becomes visible. Mesenchyme is seen to migrate into the cavity of the leg.

Fig. 25 Ventral mesothoracic bud of a 72 hours old larva. The differentiation of the disc by annular folds is more advanced than in fig. 24

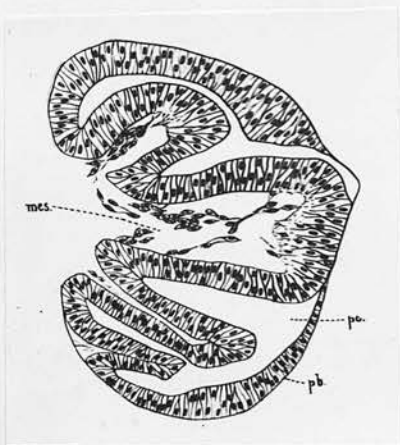


Fig. 26 Ventral metathoracic bud of a larva of 81 hours. The protruding leg disc fills the peripodial cavity. Its wall is formed by a single layer of cells, while its lumen is filled with the body fluid containing mesenchyme cells. Differentiation by means of concentric annular folds is advanced.

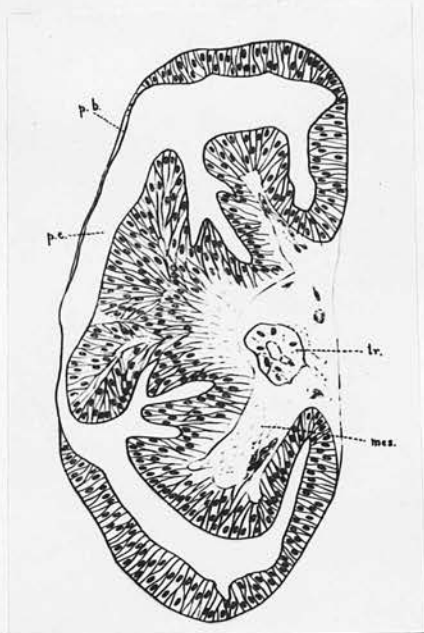


Fig. 27 Ventral meso-thoracic bud of a late third instar (96 hours). The folding is nearly completed. A trachea has entered the lumen of the anlage of the leg.

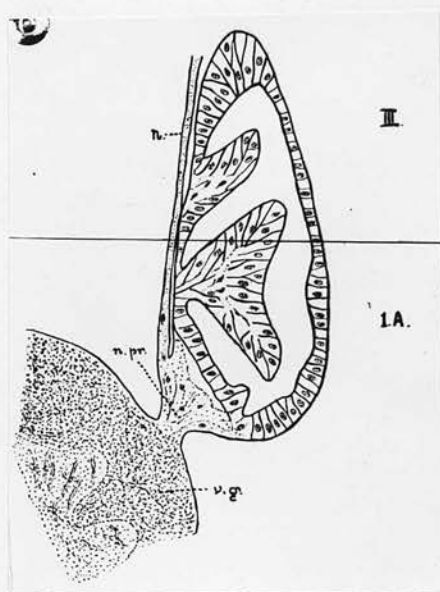


Fig. 28 Longitudinal section through the ventral mesothoracic bud of a larva of the same age as in fig. 27. The connection of the bud with the ventral ganglion has become quite short.

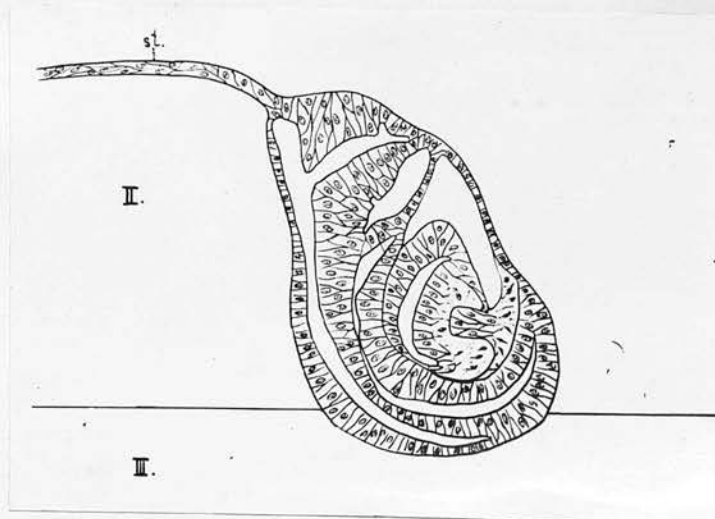


Fig. 29 Longitudinal section through the ventral mesothoracic bud of a larva of the same age as in fig. 27, showing the stalk to the hypoderm of the second thoracic segment.

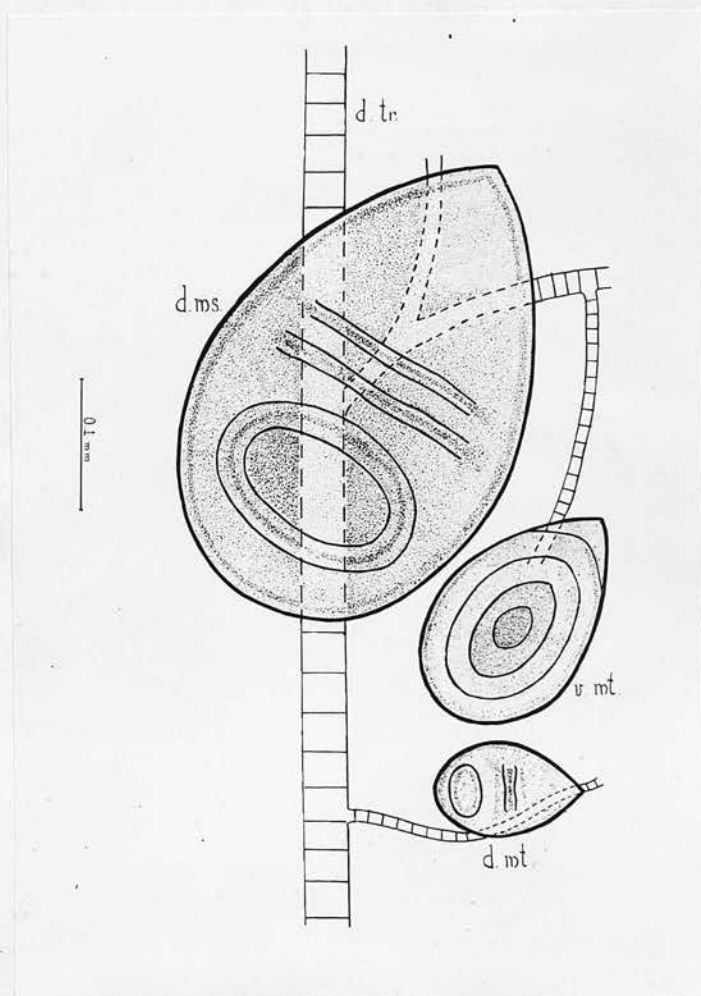


Fig. 31 (fig. 30 see following page)
Diagram of the lateral group of the thoracic buds of a mature larva.

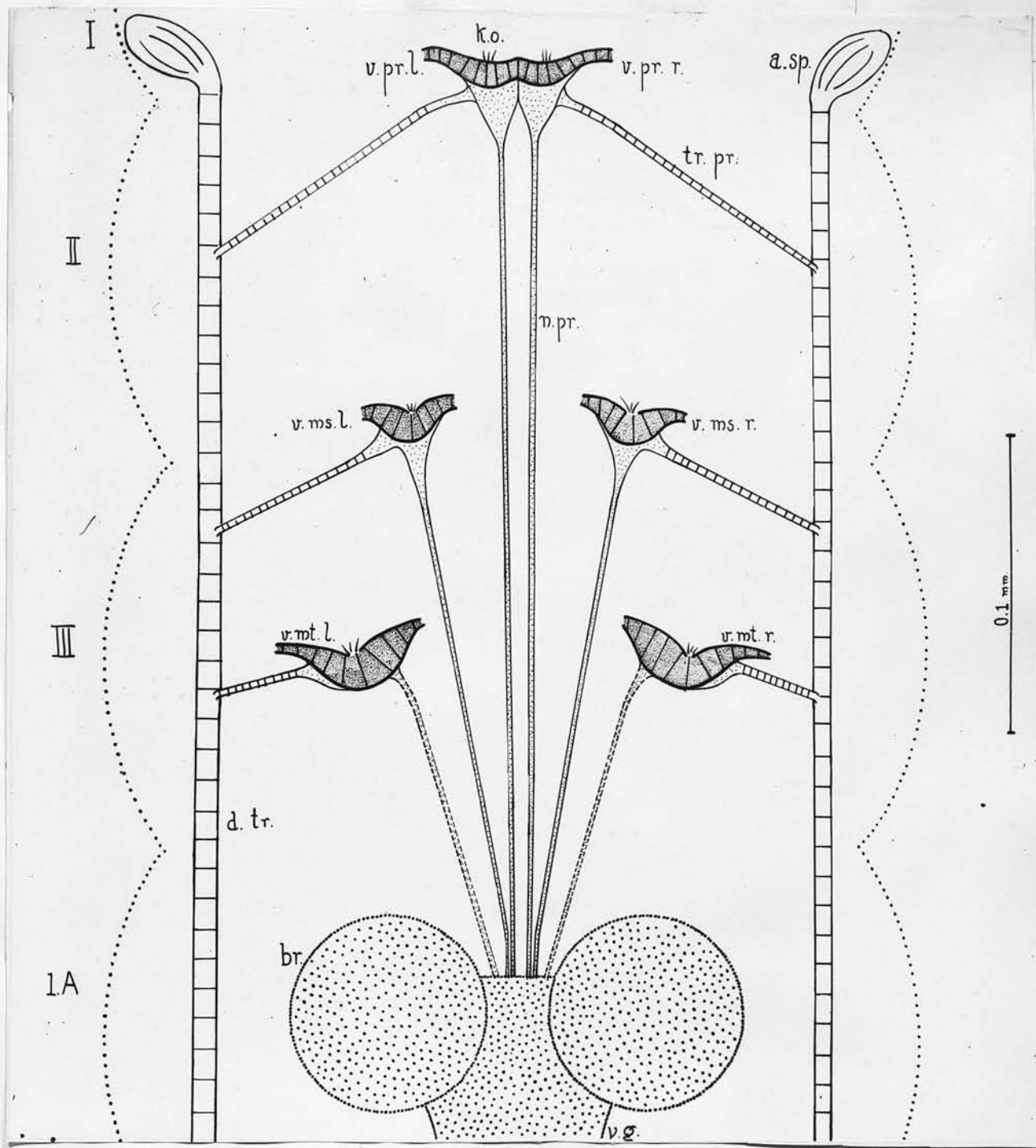
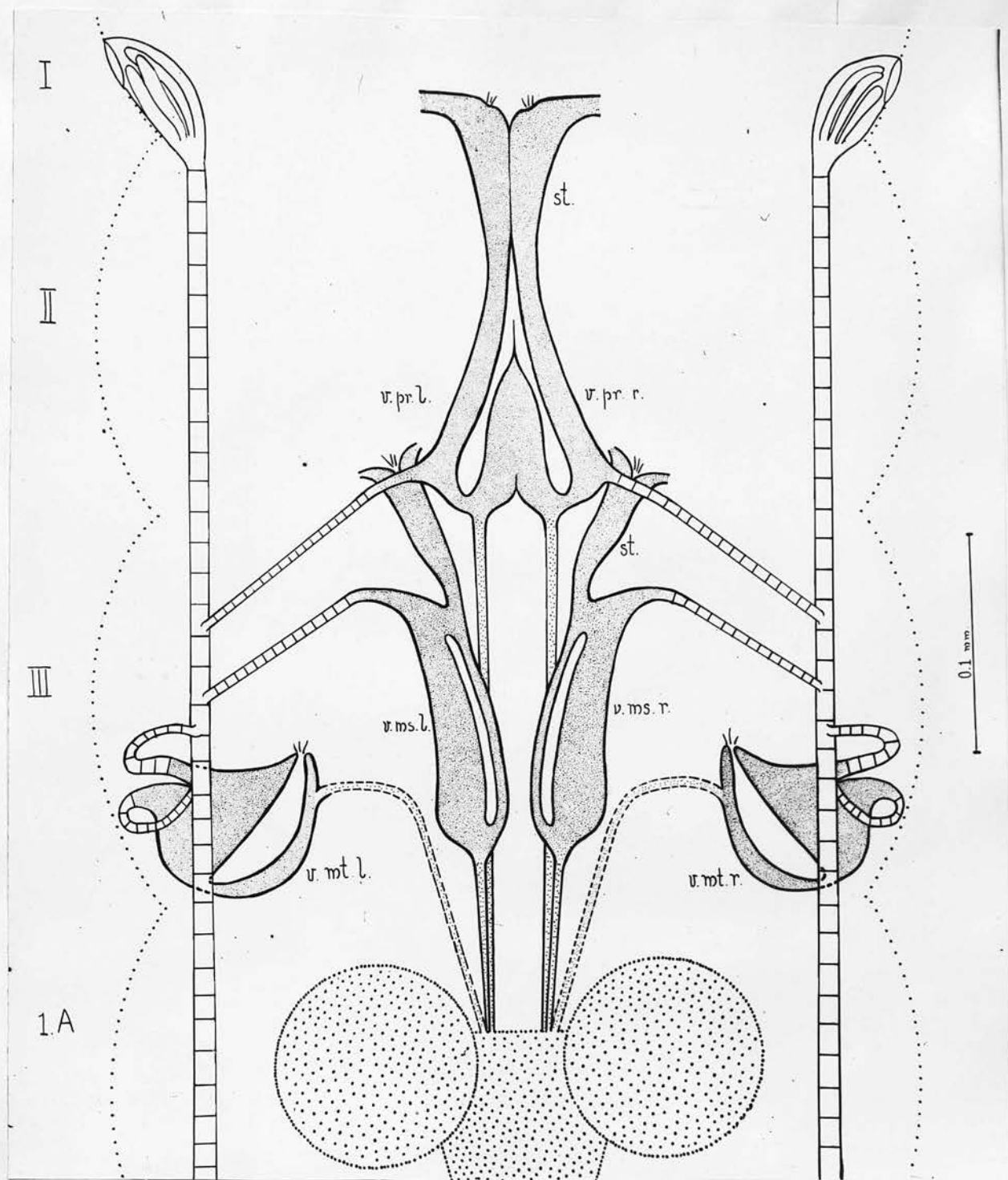
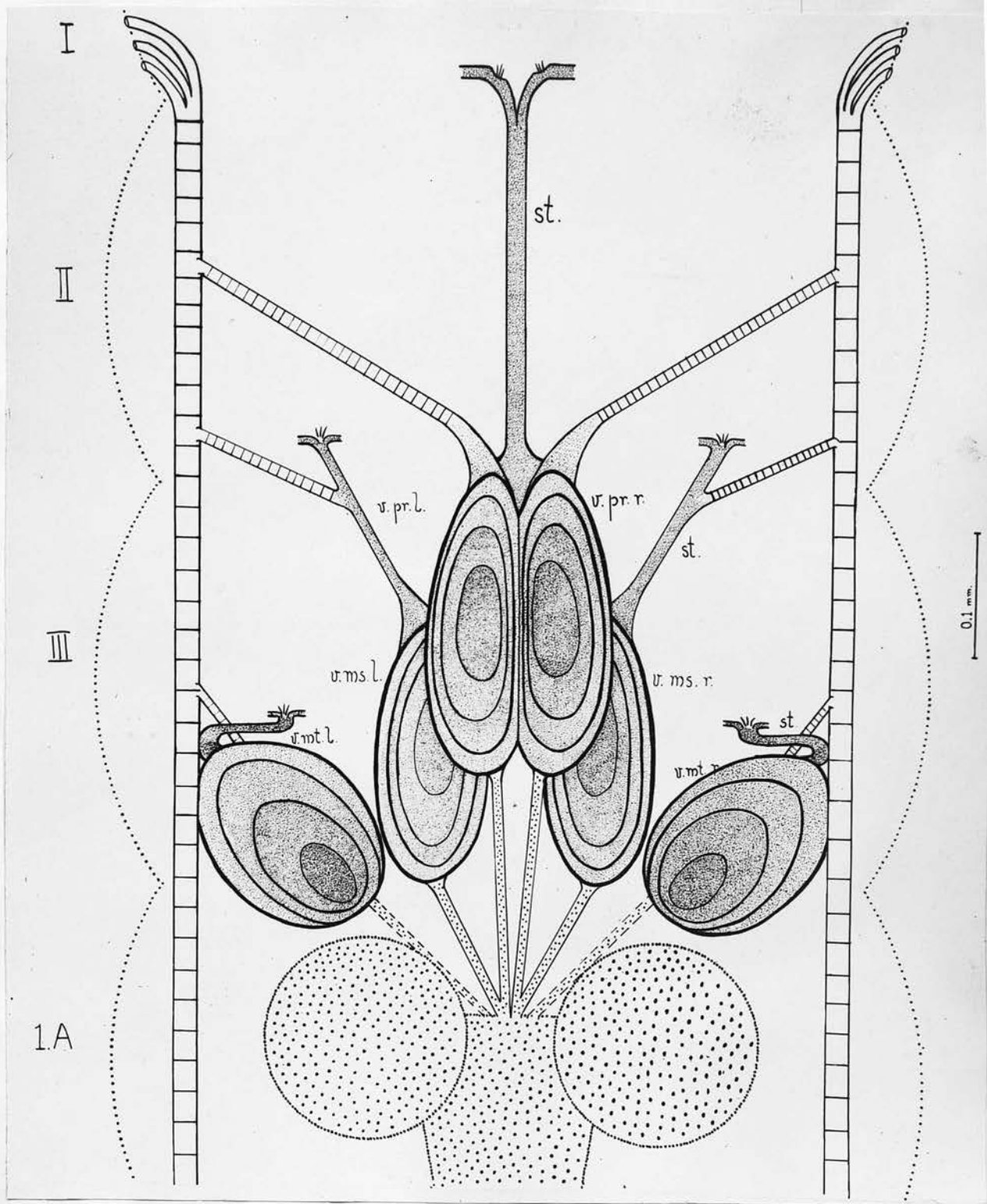


Fig. 30 Diagrams of the development of the legs during the three larval instars. (see p)

A first instar. The buds are seen as thickenings of the ventral hypoderm of the three thoracic segments. The subsequent invagination is indicated only by a shallow depression of the hypoderm underneath Keilin's sensory organ. The neural connections are long.



B late second instar. The buds, by invagination into the body cavity, have acquired a stalk connecting them with the hypoderm, and a lumen. The differentiation of the bud into the disc proper and the peripodial sac has started.



C third instar, mature larva. The stalks of the buds have become long and thin, the neural connections short. The disc is differentiated by a number of concentric oval folds.

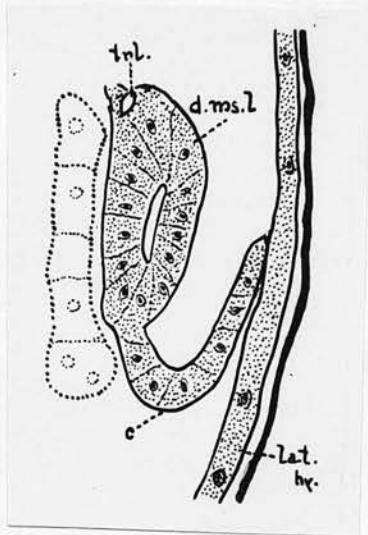


Fig. 32 Dorsal meso-thoracic bud of a newly hatched *Calliphora* larva. The bud is already invaginated into the body cavity, a lumen is visible. The connection c with the hypoderm is presumably not the stalk of the bud.

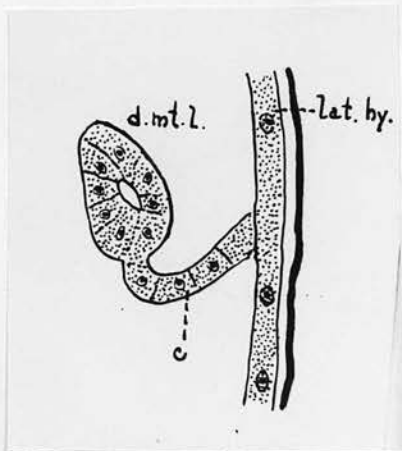


Fig. 33 Dorsal metathoracic bud of the same larva as in fig. 32. The invagination and the lumen are visible. c is presumably not the stalk of the bud.

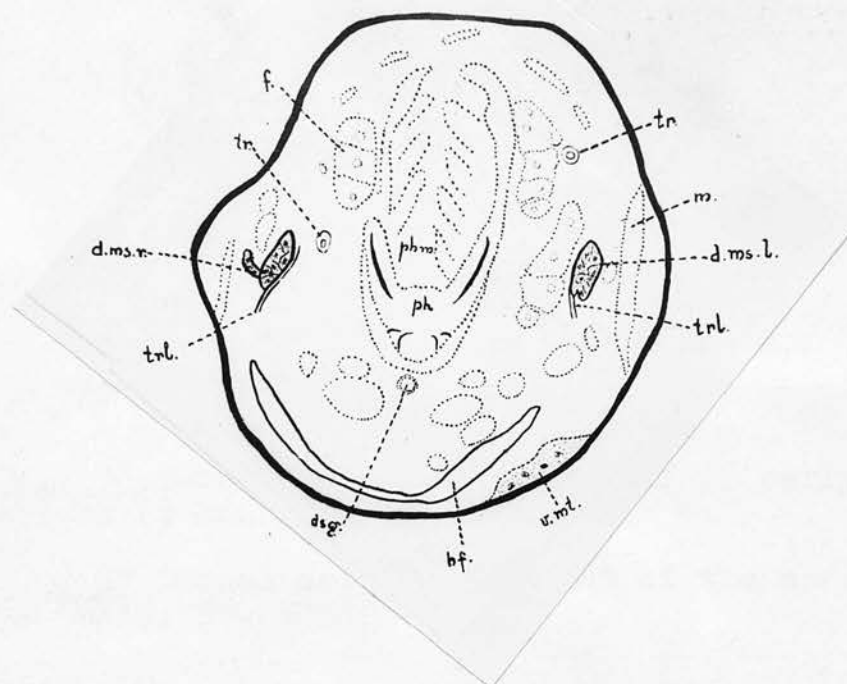


Fig. 34 Cross-section through the second thoracic segment of a newly hatched *Drosophila* larva with the dorsal mesothoracic buds. The buds are already invaginated into the body cavity, a lumen is not discernible.

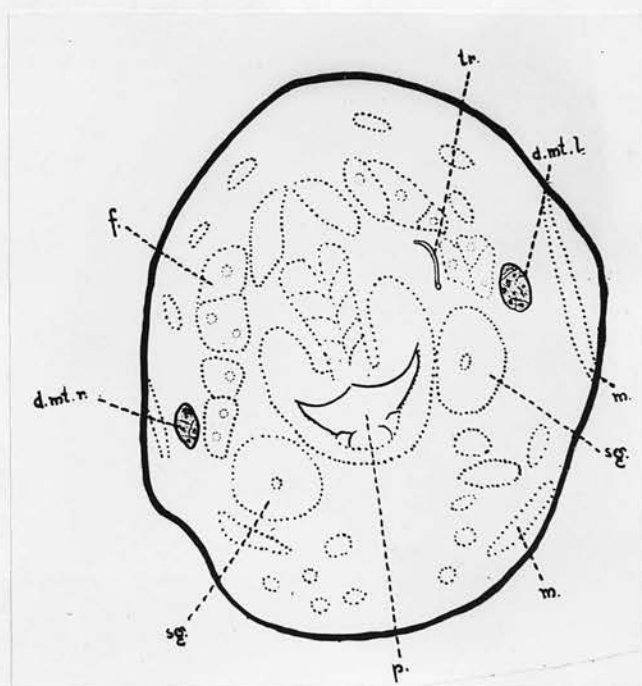


Fig. 35. Cross-section through the third thoracic segment of the same larva as in fig. 33. with the dorsal metathoracic buds. The buds have invaginated, a lumen is not discernible.

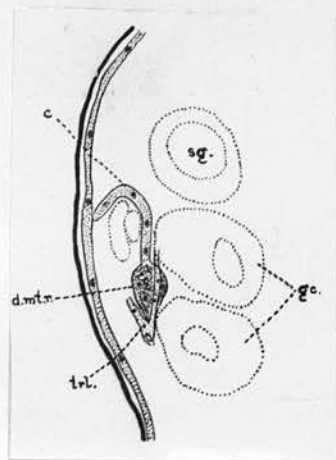
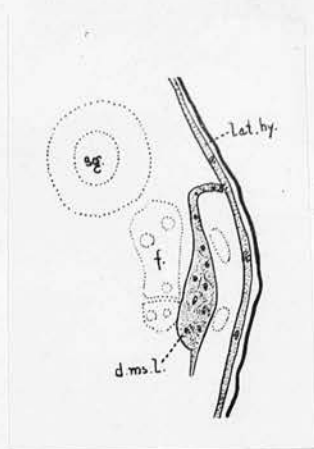


Fig. 36. Dorsal mesothoracic bud of an early first instar (6 hours).

Fig. 37 Dorsal metathoracic bud of the same larva as in Fig. 36.

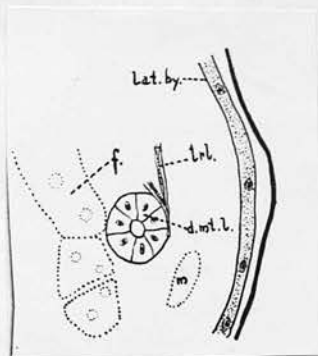
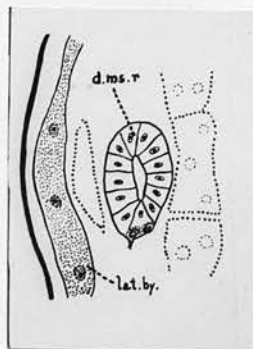


Fig. 38 Dorsal mesothoracic bud of a middle first instar (15 hours). A distinct lumen has become visible.

Fig. 39 Dorsal metathoracic bud of the same larva as in Fig. 38. A distinct lumen is visible.

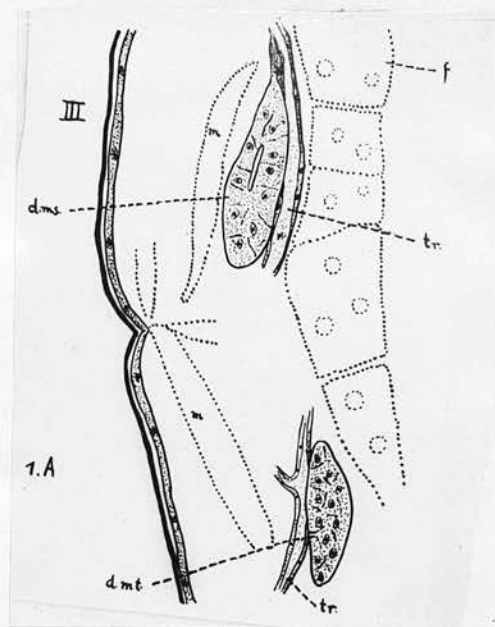


Fig. 40 Longitudinal section through the dorsal mesothoracic and metathoracic buds of a late first instar. (18 hours). Both buds are attached to tracheal branches.

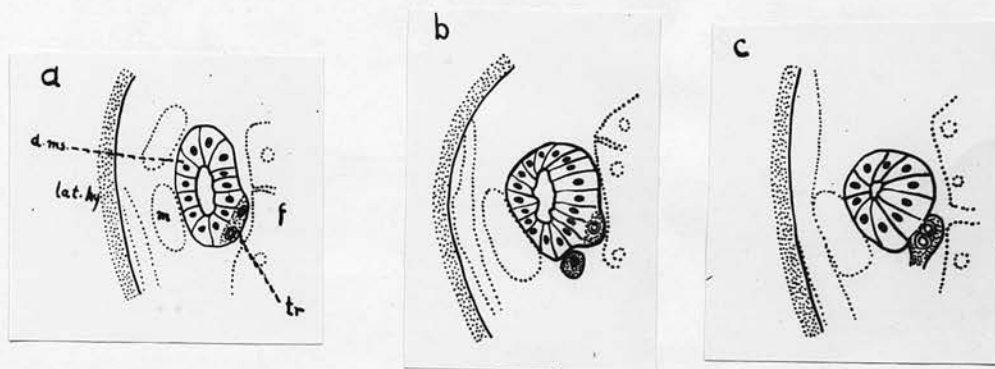


Fig. 41 Dorsal mesothoracic bud of an early second instar.

a the bud at its anterior end is attached to a tracheal branch.

b(15) the tracheal branch is separating from the bud. The differentiation into the disc proper and the peripodial sac is beginning

c posterior part of the bud. The tracheal branch is completely separated from it.

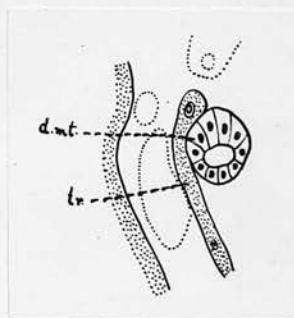


Fig. 42 Dorsal metathoracic bud of the same larva as in fig. 41. The differentiation of the bud into the disc proper and the peripodial sac is beginning.

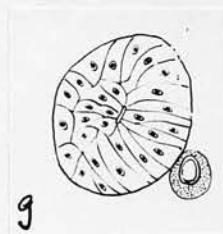
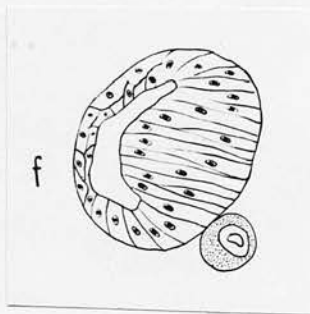
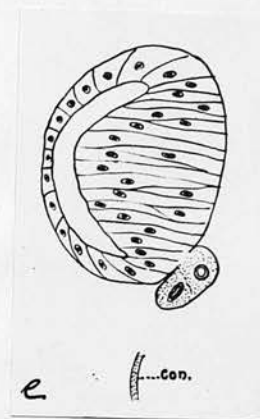
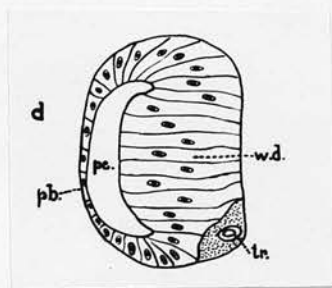
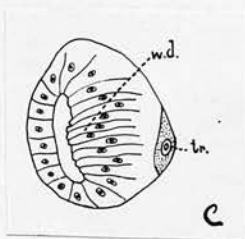
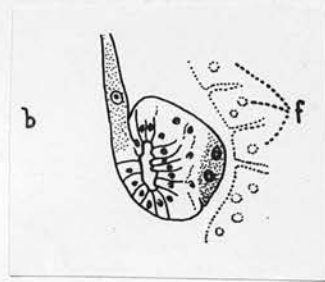
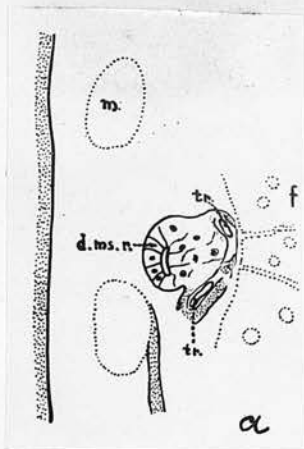


Fig. 43 Dorsal mesothoracic bud of a middle second instar (42 hours). The differentiation into the peripodial sac and the disc proper has become very distinct. The disc is protruding far into the peripodial cavity. In its anterior part it is attached to the tracheal branch. The distances between the sections are: a-b 15μ , the rest 10μ .

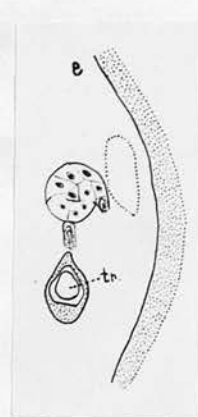
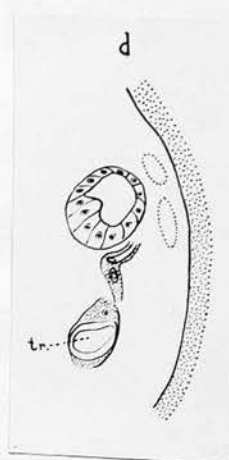
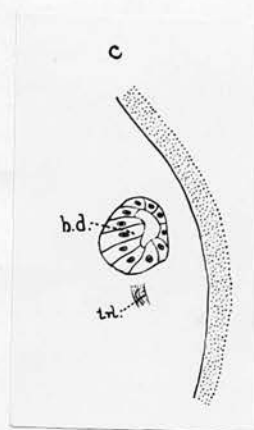
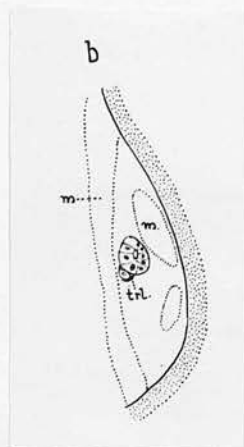
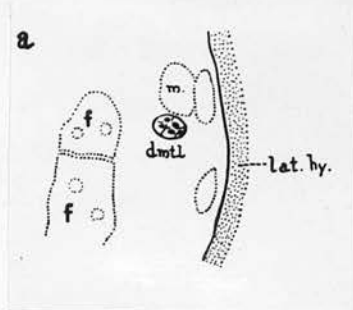


Fig. 44 Dorsal metathoracic bud of the same larva as in fig. 43. The differentiation into the disc proper and the peripodial sac is plainly visible, but not as pronounced as in the dorsal mesothoracic bud.

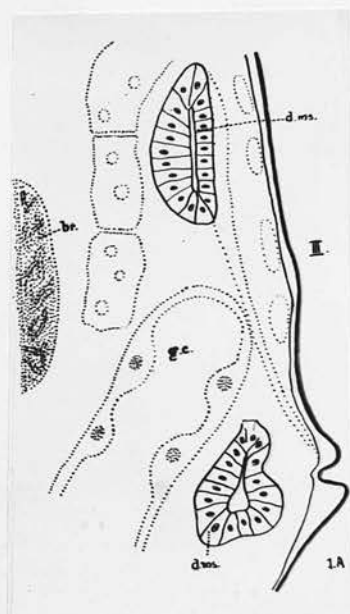


Fig. 45 Longitudinal section through the dorsal mesothoracic and metathoracic buds of a larva of the same age as in fig. 44. The mesothoracic bud has shifted to the third thoracic segment, the metathoracic to the margin between the third thoracic and the first abdominal segment. At the anterior end of the metathoracic bud the place of insertion of the stalk is visible.

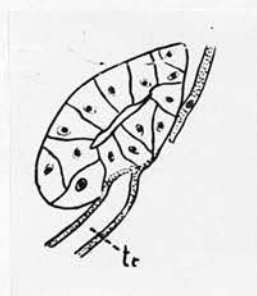
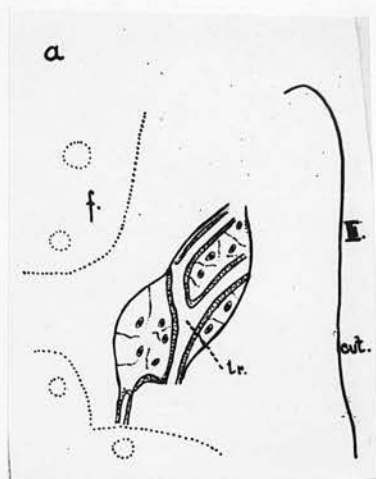


Fig. 46 Longitudinal sections through the dorsal mesothoracic bud of a middle second instar (32 hours), to show the connection of the bud with the tracheal branch. The sections follow one another in ventro-dorsal direction, their distances are 5μ .

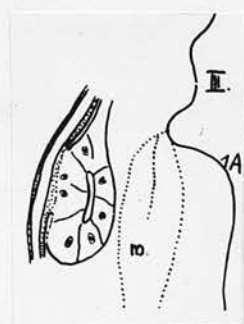


Fig. 47 Longitudinal section through the dorsal metathoracic bud of the same larva as in Fig. 46, showing its connection with the tracheal branch.

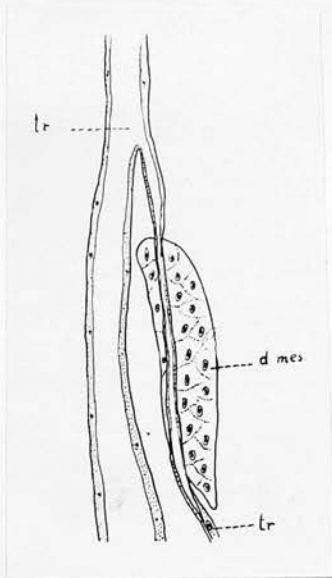


Fig. 48 Longitudinal section through the dorsal mesothoracic bud of a late second instar, showing its connection with the dorsal tracheal trunk.

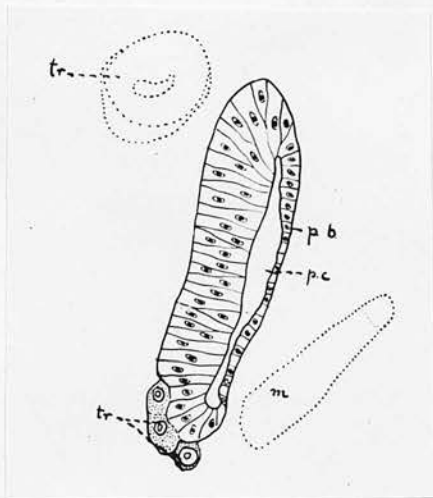
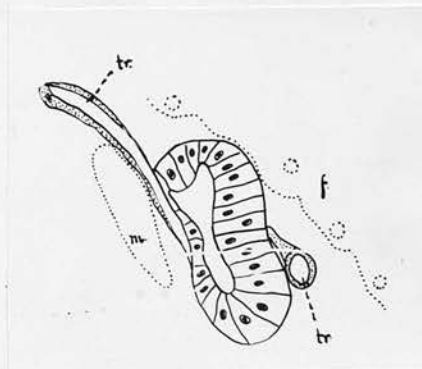
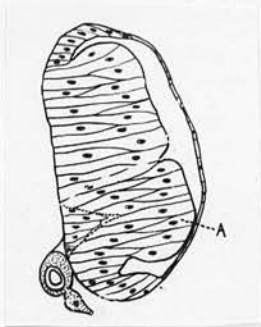


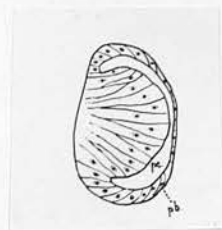
Fig. 49 Dorsal mesothoracic bud of a larva of 54 hours, in the second ecdysis.

Fig. 50 Dorsal metathoracic bud of the same larva as in fig. 49





51



52

Fig. 51 Dorsal meaothoracic bud of an early third instar (41 hours). The first cross ridge has appeared, separating the disc into the posterior wing-forming portion, and the anterior thorax-forming portion.

Fig. 52 Dorsal metathoracic bud of the same larva as in fig. 51. No differentiation of the disc is as yet visible.

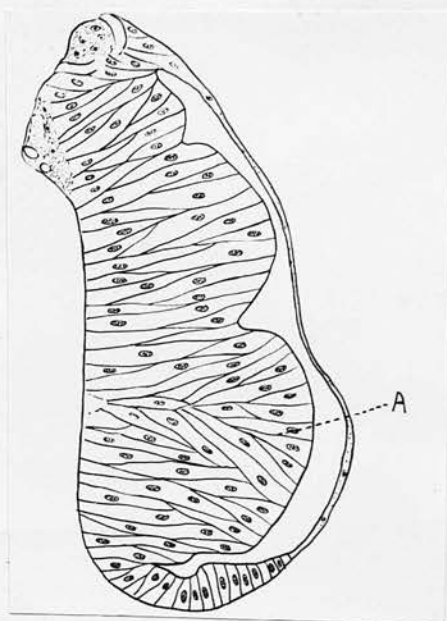


Fig. 53 Dorsal mesothoracic bud of a more advanced third instar (57 hours). The second cross ridge has appeared.

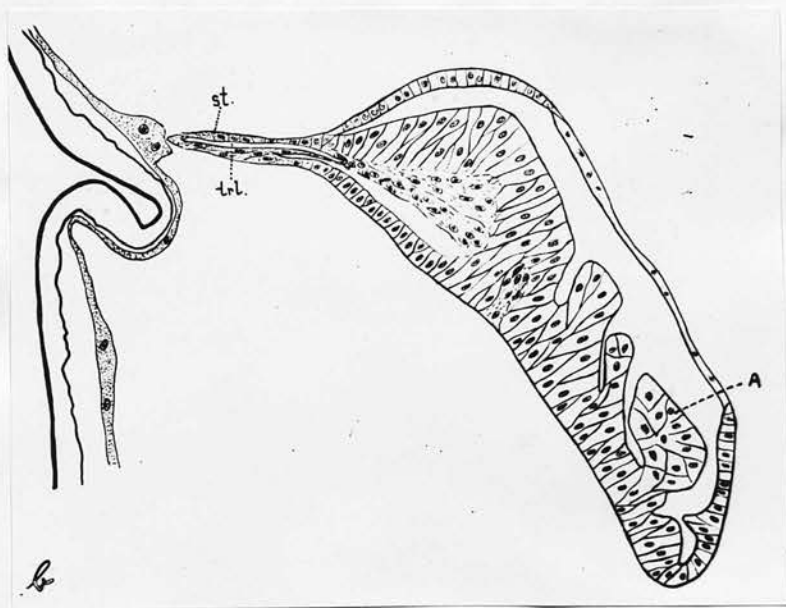
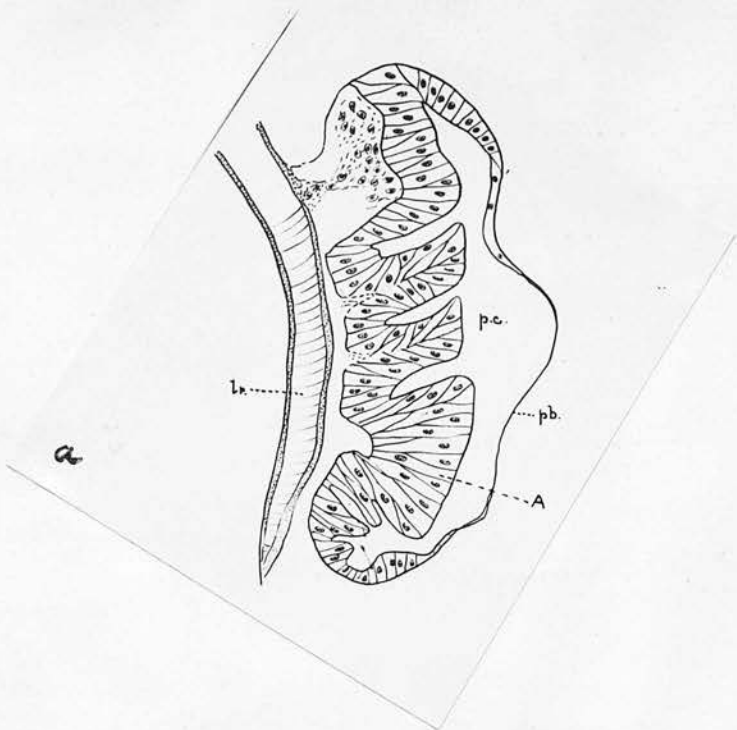


Fig. 54 Longitudinal sections through the dorsal mesothoracic bud of a late third instar (96 hours).

a the development of the transverse and circular ridges is completed. The connection with the tracheal branch is visible.

b the stalk with a tracheole inside is visible.

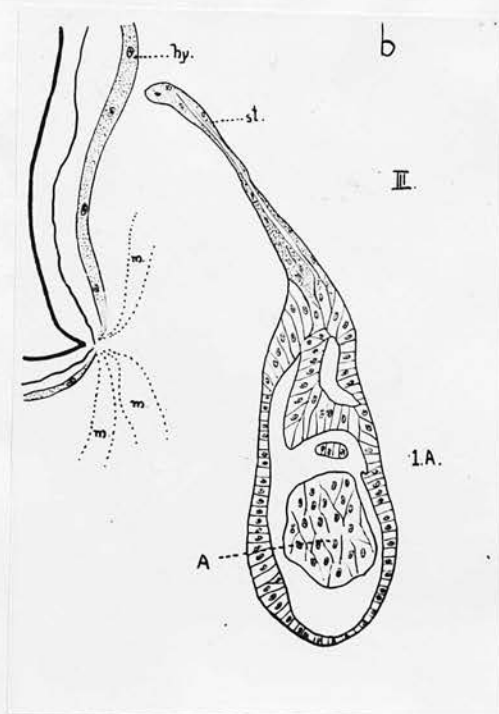
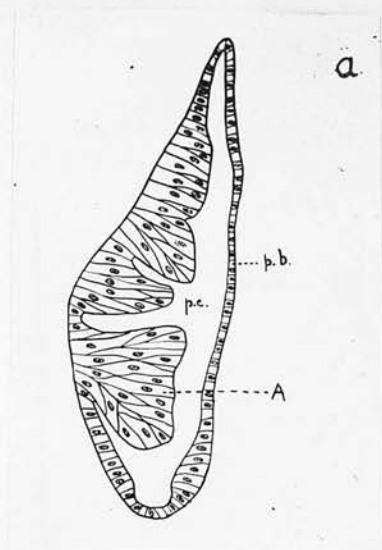


Fig. 55 Longitudinal sections through the dorsal metathoracic bud of a larva of the same age as in fig, 54

a the folding of the disc has been completed. It is similar to, but less complex than that of the mesothoracic bud

b the stalk is visible

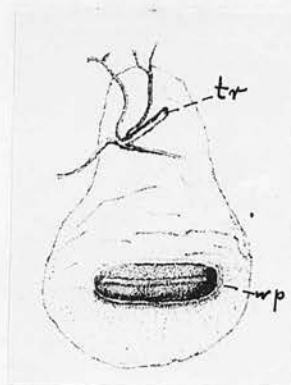


fig. 56

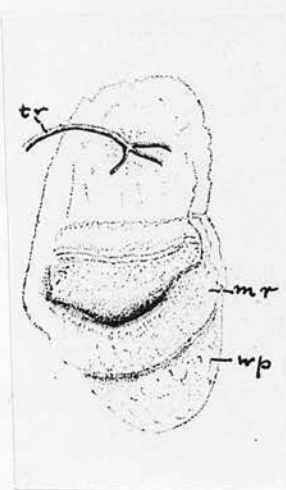


fig. 57

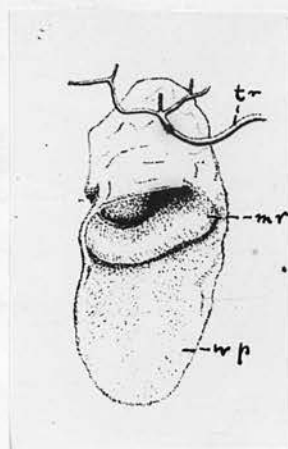


fig. 58

figs. 56, 57, 58 Semidiagrammatic representation of the growing-out of the wing pouch from the circular area of the dorsal mesothoracic bud.
 fig. 56 Dorsal mesothoracic bud of a mature larva
 fig. 57 " " " about 2 hours after pupation
 fig. 58 " " " about 4 hours after pupation

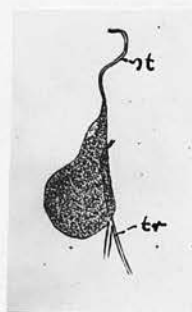


fig. 59

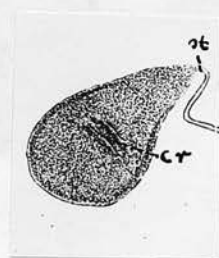


fig. 60



fig. 61



fig. 62

figs. 59-62. Four stages in the development of the dorsal mesothoracic bud in the third larval instar.
 fig. 59 Early third instar (about 55 hours after hatching of the egg)
 fig. 60 About 60 hours after hatching of the egg
 fig. 61 " 65 " " " " "
 fig. 62 Mature larva shortly before pupation



Fig. 63



Fig. 64



Fig. 65



Fig. 66



Fig. 67



Fig. 68a

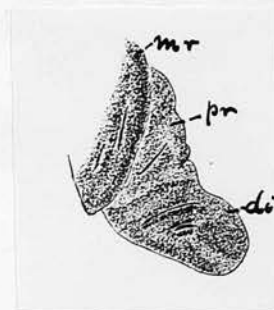


Fig. 68b

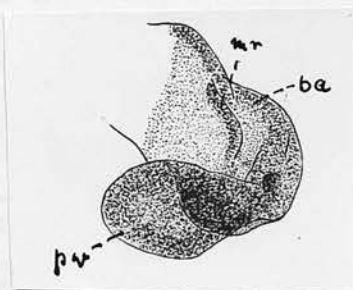


Fig. 69



Fig. 70

Figs. 63-70 (Legends on following page)

Legends to figs. 63-70 (on preceding page.)

Nine stages in the development of the wing in the prepupa of wild type.

fig. 63. 1 hour after pupation. The circular area begins to invaginate.

fig. 64. 2 hours after pupation. The "horseshoe" is formed.

fig. 65. 3 hours after pupation. The outgrowing wing pouch is subdivided by folds.

fig. 66. 4 hours after pupation. The wing pouch has grown out farther. The groove separating the distal from the proximal part is distinct.

fig. 67. 5 hours after pupation. The outline of the wing pouch has become square.

figs. 68a and b. The two dorsal mesothoracic buds of a prepupa 5-6 hours after pupation. The wing pouch is in the process of stretching and rotation.

fig. 69. 6 hours after pupation. The young pupa wing is everting from the peripodial sac and bending over to acquire its final position in the pupa.

fig. 70. 7 hours after pupation. A recently everted pupa wing. Venation is indicated by light lines.

Legends to figs. 71-78 (on following page.)

Eight stages in the development of the wing in the prepupa of l3d.

fig. 71. 1 hour after pupation. The "horseshoe" is formed.

fig. 72. 2 hours after pupation. The wing pouch is growing out. The groove separating the distal from the proximal part is visible.

fig. 73. 3 hours after pupation. The wing pouch has grown out farther.

fig. 74. 4 hours after pupation. The wing pouch has reached its maximal length.

fig. 75. 5 hours after pupation. The process of stretching of the wing pouch has started.

fig. 76. The young pupa wing is everting from the peripodial sac and bending over to acquire its final position in the pupa. 6 hours after pupation.

fig. 77. 7 hours after pupation. Eversion of the pupa wing is nearly finished.

fig. 78. A recently everted pupa wing.



fig.71



fig.72



fig.73



fig.74



fig.75

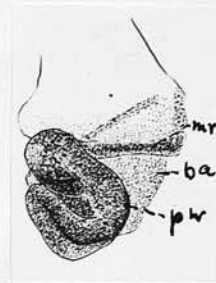


fig.76



fig.77

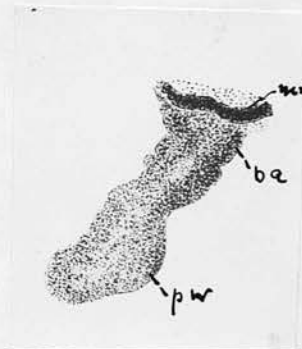


fig.78

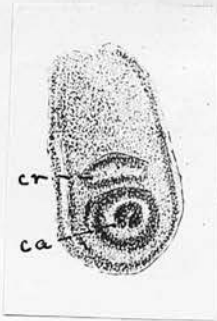


fig.79



fig.80



fig.81



fig.82



fig. 83



fig.84



fig.85



fig.86

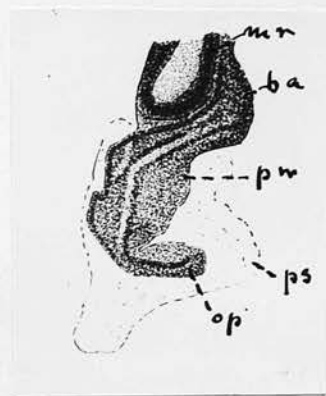


fig.87

Figs. 79-87. Nine stages in the development of the wing in the prepupa of vestigial.
 fig. 79. The dorsal mesothoracic bud of a mature larva shortly before pupation.
 fig. 80. 1 hour after pupation. The wing pouch is growing out. A small part of it is constricted off by a fold.
 figs. 81,82. 2-5 hours after pupation. The wing pouch is growing out farther. The proximal and distal part are separated by a groove.
 figs. 83-85. 5-7 hours after pupation. Stretching and eversion of the pupa wing.
 fig. 86. A recently everted pupa wing.
 fig. 87. 10 hours after pupation. The pupa sheath is formed. It is torn open in the preparation.

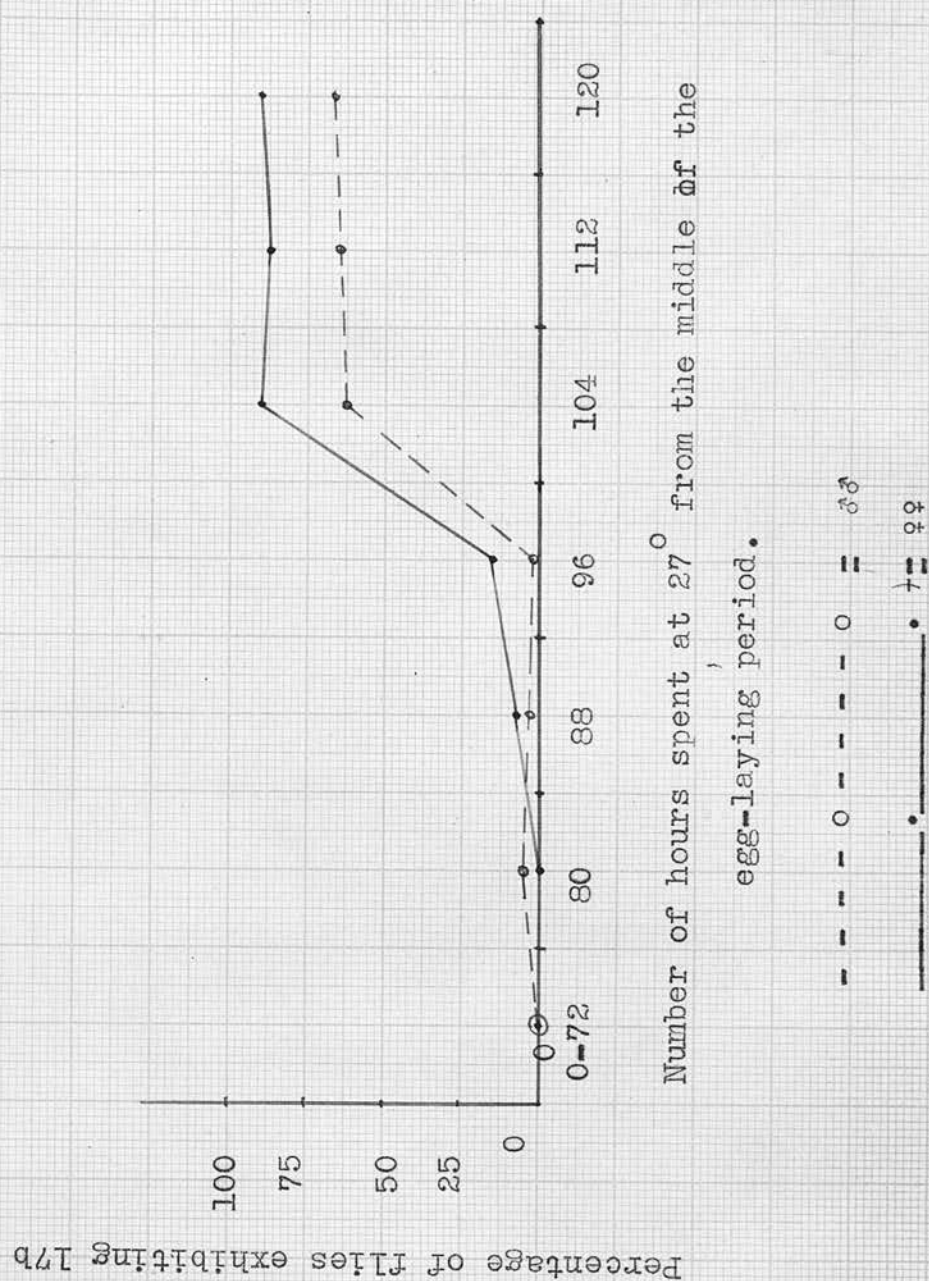
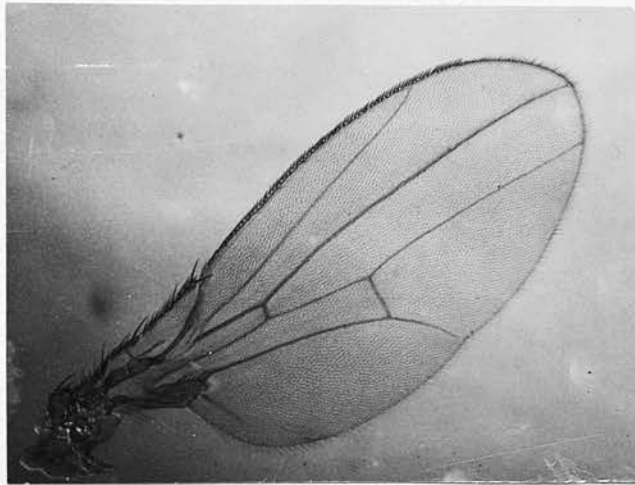
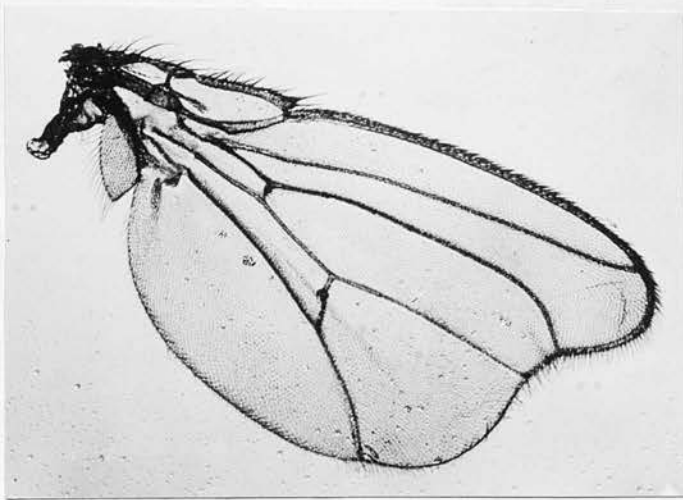


Fig. 88. Graph representing the influence of temperature on the manifestation of 17b.

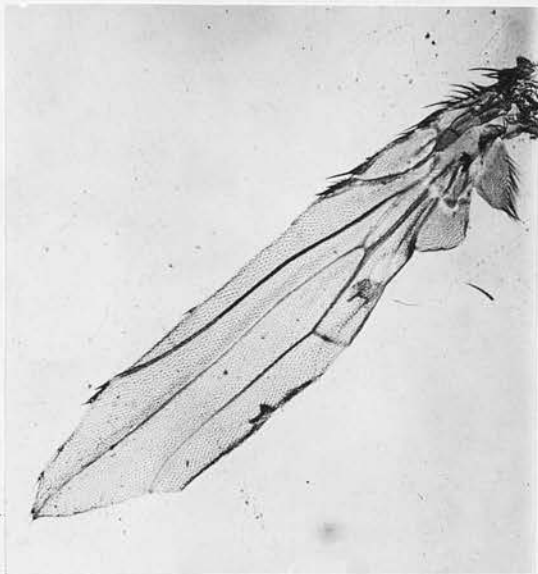
Photos. 1-4. Adult wings of wild type and mutant.



Phot. 1. wild type



Phot. 2. dumpy



Phot. 3. l3d.

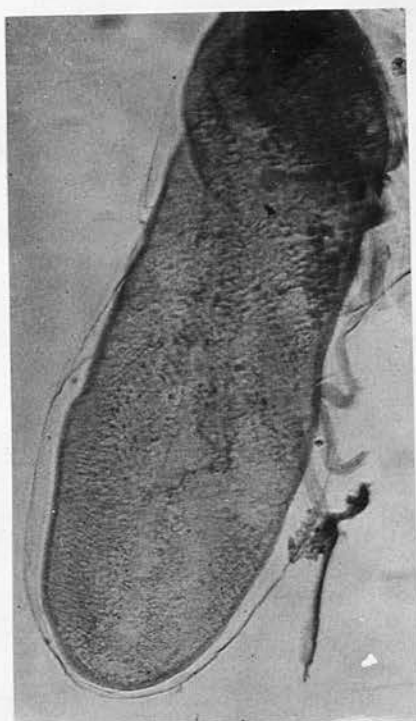


Phot. 4. vestigial

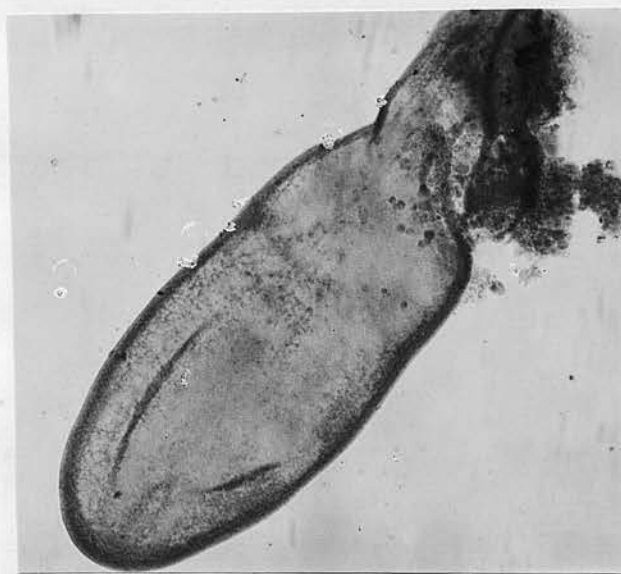
Phots. 5-7. Pupa wings of wild type.



Phot. 5. 5.8 hours
after pupation.



Phot. 6. 15 hours
after pupation.



Phot. 7. About 20 hours
after pupation.

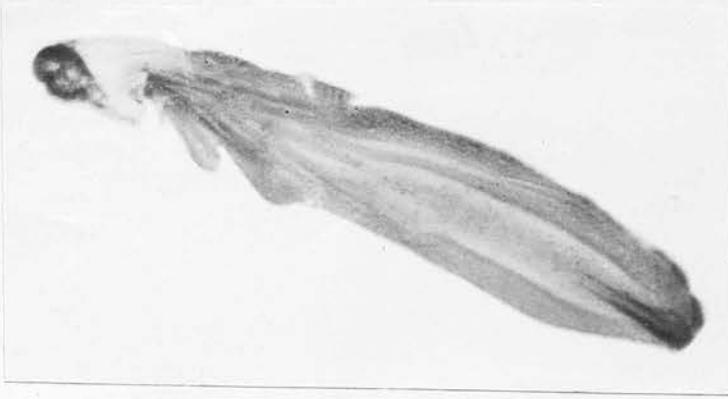
Phots.8 and 9. Pupa wings of wild type.



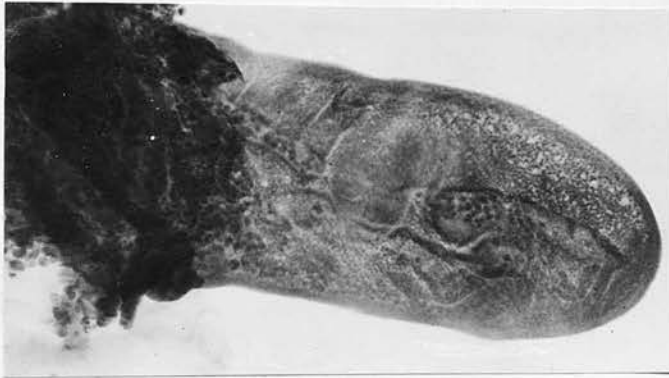
Phot. 8.
About 24 hours after pupation.



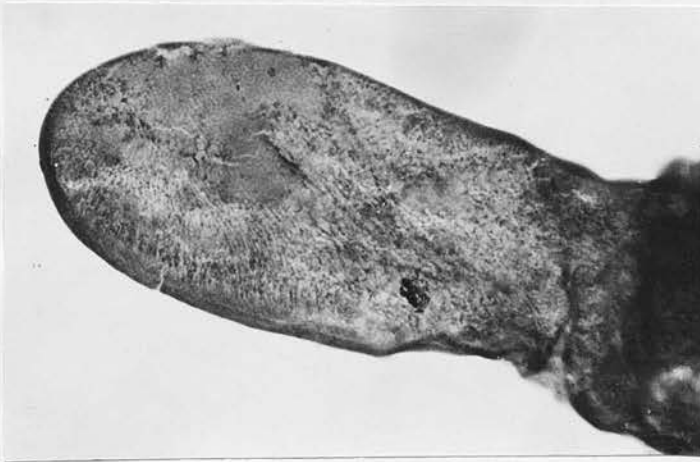
Phot. 9.
About 30 hours after pupation.



Phot. 10. Pupa wing of l3d,
about 24 hours after pupation.

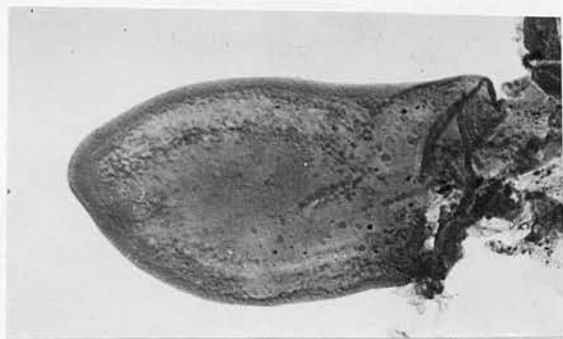


Phot. 11. Pupa wing of dumpy,
8 hours after pupation.

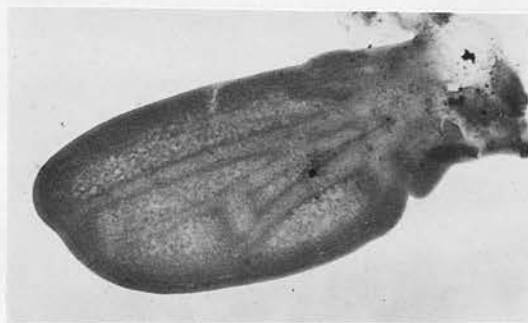


Phot. 12. Pupa wing of dumpy,
15 hours after pupation.

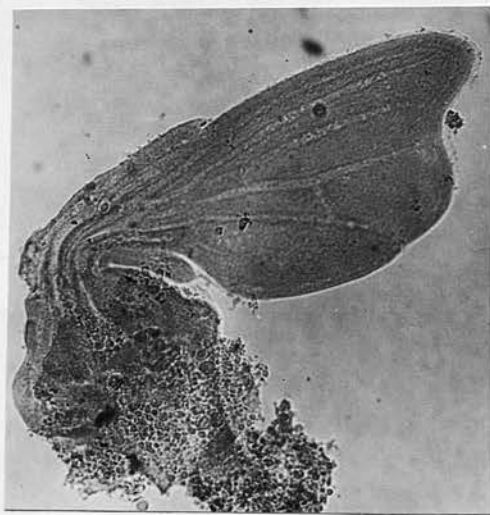
Phots. 13-15. Pupa wings of dumpy.



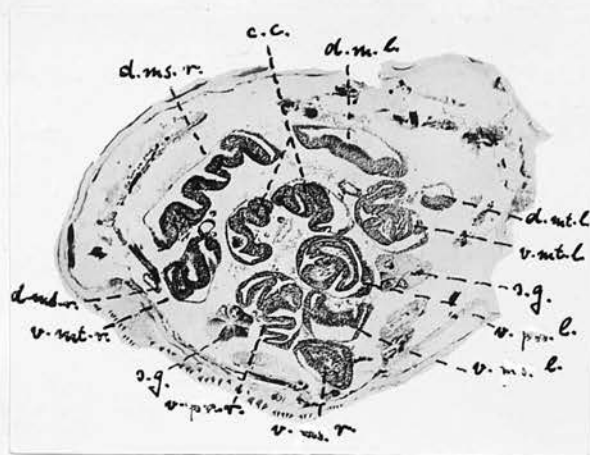
Phot. 13. About 20 hours
after pupation



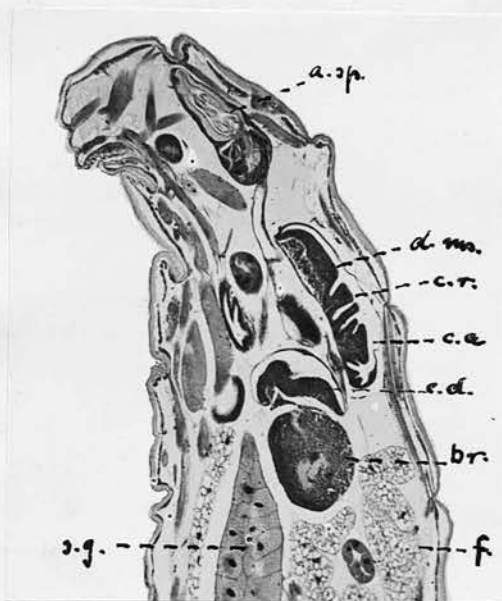
Phot. 14. About 30 hours after
pupation.



Phot. 15. About 35 hours
after pupation.

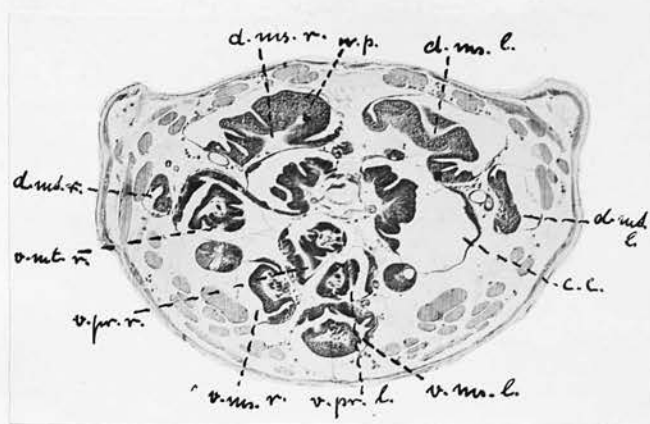


Phot. 16. Cross-section through mature larva of wild-type. All the thoracic buds are visible.

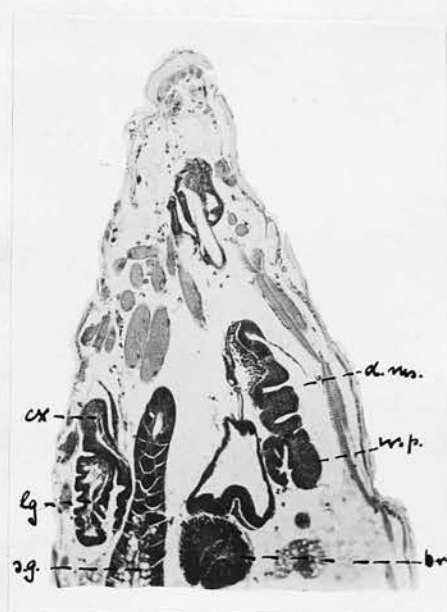


Phot. 17. Longitudinal section through the anterior part of a mature larva of wild type.

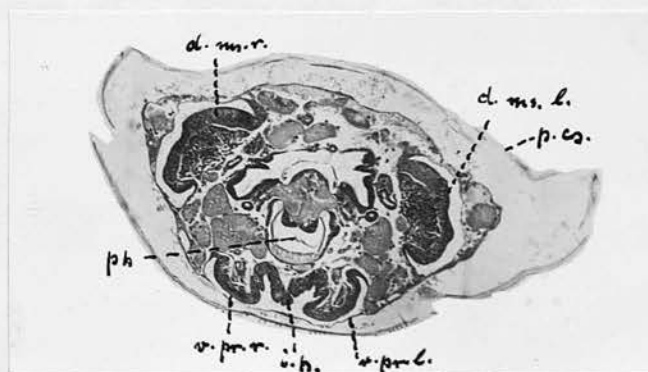
Phots. 18-20. Sections through young pupae of wild type. Explanation see p.



Phot. 18. 2 hours after pupation.

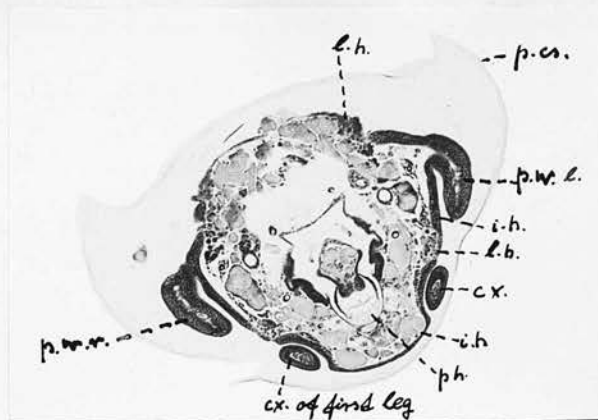


Phot. 19. Longitudinal section, 2 hours after pupation.



Phot. 20. 4 hours after pupation.

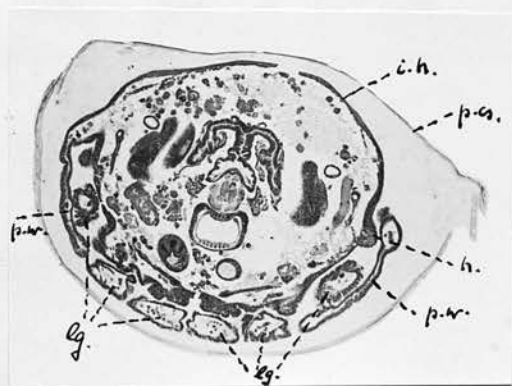
— Photos. 21-23. Sections through young pupae of wild type. Explanation see p.



Phot. 21. 7 hour after pupation.



Phot. 22. More posterior cross-section through the same larva as in phot. 21.



Phot. 23. 10 hours after pupation.