CHROMOSOMAL DEFICIENCIES AND EMBRYONIC DEVELOPMENT

Thesis by

Donald F. Poulson

In Partial Fulfillment

of the

Requirements for the Degree of

Doctor of Philosophy

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

1936

Foreword

The investigation of eggs carrying homozygous deficiencies was undertaken at the suggestion of Prof. A. H. Sturtevant. In the course of the work it became essential to obtain further information concerning the normal embryology of Drosophila. The result was that fewer deficiencies were investigated than originally planned. However, with the normal embryology known, further investigations of the action of deficiencies can proceed with a minimum of difficulties.

The author wishes to make acknowledgment to Profs. A. H. Sturtevant and Th. Dobzhansky for their many criticisms and suggestions throughout the course of the work. Likewise, he wishes to acknowledge many valuable discussions with Drs. Albert Tyler, Jack Schultz, and many other members of the department of biology.

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INTRODUCTION

The progress of genetics has been so rapid in a number of respects that many fields of inquiry have been entirely neglected or left for a future in which fields now so fertile may be exhausted. Much of this progress has been concerned with the relations between genes or groups of genes and the chromosomes bearing them, i.e., with the mechanism of transmission of characters. The problems of what the gene itself does in development and the manner in which it acts have received recent attention (Schultz 1935), but are still completely open.

The relative roles of gene and cytoplasm have long been known. An egg is incapable of further development without a nucleus, although some division is not impossible in the absence of the latter. That at least one set of chromosomes is required for development and two for complete normal development was found in early experiments with Echinoderm eggs. That the character of the embryo is determined by the genes carried in its chromosomes has also long been known. A certain amount of information is available concerning what happens when extra chromosomes or sets of chromosomes are added to the normal diploid number - upset genic balance. Likewise the exaggeration effects of heterozygous small deficiencies have been studied. One approach has only recently been made use of: the behavior of lethal factors and of homozygous deficiencies. Lethal types have been studied in some forms, e.g., the mouse and the fowl. But in only one case in the mouse is it very definitely known that the lethal is a deficiency

(Snell and others - 1934).   Nearly all homozygous deficiencies have
been shown to be lethal at earlier or later stages.   The most favorable
material for such studies exists in Drosophila melanogaster.   The
behavior of many small deficiencies has been studied there by Demerec
(1934) using stocks of flies which give a high frequency of chromosome
elimination and thus patches of "deficient tissue".   No studies have
been made on the whole organism homozygous for deficiencies.   It was
proposed, therefore, to determine what happens to such "deficient" eggs.
This seemed not impossible, as techniques for observing living eggs through-
out development had just been introduced by Huettner and his students.
Such studies proved not too difficult in cases where normal development
ceased very early, but in cases where development became abnormal in
the later stages, the situation was more complex.   This was principally
because the embryology of Drosophila beyond the time of inclusion of
the pole cells was unknown.   In the developmental studies which had
been made, especially those by Sturtevant (1929) on gynandromorphs,
the processes had been assumed to be similar to those of the related
Muscids.   Although the validity of this assumption has since been
shown, it was essential that a careful study of embryonic development
be made not only as a standard of comparison for "deficient" types,
but also to give detailed information concerning the origin of the
various larval organs and the anlagen of the adult, the imaginal discs.

The results of these investigations are embodied in the
present work, the first section of which consists of a survey of the
literature on insect embryology, particularly that of the Diptera.
This is essential as a background for the account of Drosophila
embryology to follow.   Later sections deal with the literature on
deficiencies and the behavior of a number of the deficiencies studied.

LITERATURE ON THE EMBRYOLOGY OF INSECTS

- PARTICULARLY DIPTERA

The early literature on the embryology of insects is scattered and difficult of access. The errors of observation were many, partly because of inadequate methods, and range from Aristotle's mistaking the pupa for the egg to Kölliker's (1842) and Robin's (1862) believing the blastoderm to be several layered. Many other erroneous observations have at times confused the issue and will be mentioned at appropriate points.

One of the earlier and more fundamental observations on the development of insects was that of Leuckart, who discovered the micropyle and its function of admitting the sperm to the egg. Leuckart (1858) also made rather careful observations on the development of the pupiparous Diptera, a subject more fully investigated later by Pratt (1900) and still more récently by Hardenburg (1929).

The study of insect embryology really begins with the classic (and at the same time modern) paper of Weismann (1863) on Chironomus and Musca vomitoria (Calliphora). This paper, which can be consulted and read today with considerable profit and pleasure, is a model of scientific method and exposition. Weismann's observations were mostly on whole and living eggs, for the method of sectioning by microtome was not introduced until 1870 by His. Practically the whole of Weismann's accounts were verified by subsequent investigators using sections. The works of Kowalevsky (1871, 1886) and Noack (1901) are really monuments to Weismann's scientific abilities.

The Pole Cells and their Significance

The pole cells of insects were first observed by Robin (1862) who thought them to be polar bodies and believed that they, as well as the blastodermal cells, were formed by gemmation. That they were true cells which take part in the later development of the embryo was pointed out by Weismann (1863) although he failed to grasp their real significance. This was first comprehended by Leuckart (1865) and especially by Metschnikoff (1866) who followed their fate to the gonad in Miastor. Grimm (1870) found the same thing in Chironomus. However, Weismann was loath to accept this interpretation until the very thorough work of Balbiani (1885) on Chironomus. Then it was doubtless a notable factor in the formation of the "continuity theory" and especially of the concept of the separation of germinal and somatic tissues. Further work on the pole cells of Chironomus was done by Ritter (1890), and even as late as 1911 they were the object of a careful study by Hasper who made more detailed observations on the development of the sex organs.

The presence in the posterior polar plasm of granules (polar granules) which are always included in the cytoplasm of the pole cells, but never in that of the blastodermal cells, was demonstrated in Chironomus by Ritter (1890). Among other things he showed that these granules are distinct from the yolk. These deeply staining granules had been seen in pole cells by Weismann and Metschnikoff, but these authors had never observed them before their inclusion. Hasper (1911) has given a detailed account of their behavior preceding and throughout the formation of the pole cells of Chironomus.

Similar phenomena have been described in the Hymenoptera by

Sylvestri (1906-1908) and especially in the Coleoptera by Hegner (1908, 1909,a, b) who tried to remove them by various mechanical means: pricking, centrifuging, cautery. In a few cases he obtained adults without gonads. Hegner made a great deal of the "germ cell determinants", as he called them.

The formation of the pole cells of Drosophila melanogaster has been investigated in some detail by Huettner (1923) who found the polar granules to accompany them. Their fate has been followed externally, up to the time of their disappearance in the dorsal invagination, by Child and Howland (1933). Beyond this there are no published observations, although Geigy (1931) was able to castrate flies effectively by destruction of the pole cells through the action of ultra-violet radiation.

Some changes in the development of the germ cells in Sciara have been studied by DuBois (1932) who observed their origin in that form and their inclusion within the embryo.

Cleavage

Cleavage nuclei are the products of the division of the zygote nucleus. Each becomes surrounded by a mass of protoplasm which is distributed to the daughter nuclei at the succeeding divisions. Additional protoplasm is taken up between divisions from the mixture of yolk and protoplasm which makes up the larger part of the egg. Thus the cleavage nuclei are always surrounded by clear islands of protoplasm. Blochmann (1887), who was among the first to investigate cleavage, described the formation of the polar bodies in the egg of Calliphora and called attention to the fact that the cleavage nuclei

and the protoplasm surrounding them make up a syncytium, and that the use of the term cleavage cell is incorrect.   Cells do not form until after the migration of the nuclei to the surface to form the blastoderm. Heider (1889), Heymons (1895), and Lecaillon (1897) also held this point of view.   Cleavage nuclei, which may remain in the yolk after the migration, form the primary yolk nuclei, while secondary yolk cells may appear later as a result of migration of cells from certain regions of the blastoderm.   In several orders of insects (Orthoptera, Lepidoptera, and Coleoptera) the yolk undergoes special cleavage forming polyhedral masses of yolk, each including one or more yolk nuclei.

The Blastoderm and the Germ Band

The first observations on the early embryology of Chironomus were made by Kölliker (1842).   He observed the blastoderm, but thought it to become many-layered before further development took place.   Robin (1862) also described the formation of the blastoderm in Chironomus believing that it arose, as he believed the pole cells to arise, by gemmation of the protoplasm.   In both Chironomus and Musca vomitoria (Calliphora) Weismann (1863) carefully observed the migration of the nuclei to the surface, the formation of cell walls between them, and the growth of the blastodermal cells by the absorption of the inner blastema (inner cortical layer of protoplasm).   He found the ventral wall of the blastoderm to become somewhat thicker than the dorsal, and later observed the "gastrular furrow" along the ventral mid-line. Weismann's observations on the formation of the blastoderm (Keimhaut) of Calliphora were so complete that subsequent observers could only confirm them (Kowalevsky 1871, 1886; Noack 1901).

Before the appearance of the furrow along which the formation
of the germ layers takes place Weismann found the anterior one-quarter
of the blastoderm to be separated from the rest by a furrow around the
whole egg (analogous to that shown in Figs. 7, 8 and 10). This he
termed the "vordere Falte". The ventral blastoderm was then found to
extend around the pole of the egg dorsally, at which time the pole cells
moved from their posterior position into a dorsal depression which proved
to be the first sign of the proctodaeum. By the time that the pole
cells disappeared into this invagination the "gastrular groove" had
formed and extended from this point around the pole and along the
ventral mid-line nearly to the anterior end of the egg. Weismann
showed, by dissection of eggs in fixative and subsequent examination
under high powers of the microscope, that this represented the origin
of the germ layers. He found Chironomus and Calliphora differed in
that the "unterblatte" in the former was formed chiefly at the two ends
of the ventral furrow while in the latter formation occurred along the
whole length of the furrow. With the methods at his disposal, it was
impossible to settle the details of such processes. Kowalevsky, who
was the first person to apply paraffin sections to the study of insect
eggs, in 1871 verified Weismann's observations by means of sections.
Graber (1889) described "gastrulation" from transverse sections of the
eggs of Calliphora, but, according to Noack (1901), did so incorrectly.
Noack gave what is probably the true picture. He found endoderm to be
formed centrally at the two ends of the furrow and the mesoderm "budded
off" laterally along the length between. The cells which gave rise
to endoderm and mesoderm were found to lie along the ventral mid-line
and nearly always included some small yolk spheres in their cytoplasm.

This enabled them to be followed for some time at least.

The picture thus given of gastrulation in Calliphora is in accord with that found by Wheeler (1889) for Doryphora (Leptinotarsa) in which he considers the gastrular groove as an elongate blastopore with the endoderm formed only at the two ends and the mesoderm along the remainder of the length. In Blatta, Wheeler (1889) found the endoderm to be cut off only at the posterior end, and this he considered to be the primitive condition for insects. Lecaillon (1898) has devoted a great amount of study to the origin of the germ layers in the Coleoptera. Much of the huge literature which exists on the formation of the germ layers has been discussed by Nelson (1915) and especially by Eastham (1930). A great deal of this is controversial and has little interest today.

In all groups of insects the thickened ventral blastoderm with the underlying mesoderm and endoderm is referred to as the germ band (Keimstreif) or ventral plate. Less frequently it is called the embryonic plate. It represents that portion of the blastoderm which will form the embryo proper. The other portions usually go to form the embryonic membranes.

The germ band may be invaginated as in the Libellulids and Rhynchota, or grown over by the membranes as in most other insects, except the Coleoptera which are intermediate in condition (Korschelt-Heider 1899). In the Diptera the germ band is superficial. The embryonic membranes are complete in the case of the lower Diptera (Chironomus) and nearly absent in the higher Diptera (Calliphora, Drosophila, Melophagus). In the honey bee (Nelson 1915) the distinction between the parts of the blastoderm appears very early. Much of the dorsal blastoderm goes to form the amnion and serosa.

Soon after its establishment the Dipteran germ band elongates and extends along the dorsal side. The space necessary for this is made by the invagination of the proctodaeum and by the growth of the embryonic membranes which are usually tenuous sheets of cells. During this period of maximum extension most of the principal organs are laid down and the period of development following its contraction is chiefly one of differentiation.

Embryonic Membranes

Shortly after the establishment of the ventral plate the thinner dorsal blastoderm begins to extend and folds of it grow over the lateral and terminal portions of the ventral plate. These are the amnion folds. Eventually, in most insects, the folds fuse along the mid-ventral line leaving the germ band covered on the ventral side by an inner (amnion) and an outer (serosa) membrane. The classical cases of this are given for insects with complete membranes by Kowalevsky and by Tichomiroff for Lepidoptera, by Kowalevsky and by Graber for the Hymenoptera (Korschelt-Heider 1899), by Wheeler (1893) for Orthoptera, and by Weismann (1863) for Chironomus.

In Chironomus Weismann found the posterior end of the germ band to extend around on the dorsal side so that the two ends nearly meet. The caudal fold of the embryonic membranes first arises and begins to grow posteriorly along the dorsal side. By the time that the posterior end of the germ band has reached its most anterior position (comparable to Fig. 11), the caudal fold (or tail fold) has extended around to the ventral side. The cephalic fold (which is much smaller) begins to grow over the anterior end at about the same time.

The membranes grow laterally as well, and the embryo is soon enclosed. The stomodaeal and proctodaeal invaginations occur in the germ band beneath the overgrowing membranes. The same process has been described by Ritter (1890) from longitudinal sections of Chironomus eggs.

Normally the membranes are lost at the time of hatching, although in some forms, notably Chironomus, the serosa is absorbed into the yolk on the dorsal side. Only the amnion then remains.

However, in the Muscidae and other Brachycerous Diptera the membranes are quite incomplete, and in the Pupipara they are very rudimentary (Pratt 1900). Weismann (1863) observed signs of these folds in Calliphora and thought that the membranes became complete though exceedingly thin. Kowalevsky (1871, 1886) could find no such thing; neither could Graber (1889) nor Noack (1901). The caudal fold was found to form and later simply to flatten out and take part in the formation of the dorsal integument. Lowne (1890-92), who followed other authors too assiduously and his own sections not enough, misinterpreted the case and gives figures of embryos with complete membranes. In most respects his accounts of the embryology of Calliphora are completely misleading.

The Gut and its Derivatives

The fore- and hind-guts are formed by invaginations of esto-derm as the proctodaeum and the stomodaeum. The former usually occurs first and the latter soon follows. All investigators from Weismann (1863) on agree on the mode of formation. Noack (1901) gives excellent figures of longitudinal sections showing the origin and completion of the gut in Calliphora. He found, as Graber (1889) had found, that the

Malpighian tubes are formed from proctodaeal cells and obtain their lumen very early. Their formation is similar to that shown in Fig. 13. Pratt (1900) found this to be the case in Melophagus (Pupipara).

The mid-gut has been the subject of much controversy. Graber (1889) derived it directly from proctodaeum and stomodaeum, as did Heymons (1897) and Lacaillon (1898). The latter authors even went so far as to say that endoderm is entirely wanting in the adults of the higher insects. Others derive the mid-gut from the anterior and posterior rudiments of endoderm which grow toward one another and fuse. This was found by Wheeler (1889) to be the case in Doryphora.

In Calliphora Weismann (1863) derived the mid-gut from the "Unterblatte" in which he was unable to distinguish endoderm from mesoderm. He believed the yolk to become enclosed in a sac formed of cells derived from the under layer. This communicated at the two ends with the proctodaeum and the stomodaeum. Kowalevsky (1886) found the mid-gut to arise from two endoderm rudiments, an anterior and a posterior one. According to Noack (1901) the nature of the mid-gut and its origin were correctly inferred by Butschli (1888). Noack found the mid-gut to result from the fusion of anterior and posterior rudiments of endoderm. However, he did not give the details of the dorsal enclosure of the yolk by the mid-gut wall. Likewise in the Pupipara, Pratt (1900) found two rudiments of endoderm fusing to form the mid-gut.

It is worth noting the fact that those who espoused the ectodermal origin of the mid-gut have limited their studies to the Lepidoptera, Coleoptera, and Orthoptera while the proponents of the "bipolar" theory have worked chiefly with Lepidoptera, Diptera, and

Hymenoptera. An exception to this is Wheeler (1889, 1893) whose work on both Orthoptera and Coleoptera has substantiated the view of the latter.

The fore-gut is formed entirely from the stomodaeal invagination, from the anterior portion of which the salivary glands later arise. In some forms (Apis) the invaginations occur in the surface ectoderm which is later drawn in to form the anterior part of the pharynx (Nelson 1915). In the Muscids and the Pupipara the salivary glands form after the ectoderm has become a part of the ventral pharyngeal wall. The two glands in Muscids take their departure from the pharynx in a common tube forming two branches which course posteriorly on either side beneath the nervous system. In the embryonic stages their nuclei do not differ much from those of other cells of the fore-gut.

Balbiani (1881) first noticed the remarkable structure of the nuclei of the larval salivary glands of Chironomus. Between his time and the later careful studies of Heitz and Bauer (1933) there was considerable controversy as to whether the coiled bodies within salivary gland nuclei really were chromosomes. The latter authors have found that practically all the cells of the gut tissue, including the Malpighian tubes, of many Diptera have similar nuclear structure.

The Nervous System

All parts of the nervous system are of ectodermal origin. The first signs are usually two parallel swellings running along the mid-ventral line sometime after the establishment of the germ band. These extend from behind the stomodaeum to the edge of the proctodaeum and are known as neural ridges or primitive swellings. Usually there

is more or less of a groove between them, the neural groove, and from the bottom of this the neuroblasts of the median cord are formed. The neuroblasts of the lateral cords were found by Wheeler (1893) to arise in the Orthoptera by lamination of the cells in the neural ridges. The rudiments of one pair of ganglia appear in each of the segments of the embryo. In later stages fusion may occur and bring about an apparent reduction in their number.

The sub-oesophageal ganglion arises from the broadened parts of the neural ridges just posterior to the stomodaeum. The large lobes of the supra-oesophageal ganglion are formed from swellings on the anterior portion of the dorsal side.

The only extensive work on the Dipteran nervous system has been that of Escherisch (1902) on Musca. He gave essentially the same picture as that of Wheeler and described the considerable concentration of ventral cord by the fusion of the consecutive pairs of ganglia. The nervous tissue, first laid down during the extended stage of the germ band, was found to become more concentrated on the ventral side as shortening occurred.

The Tracheae

Also ectodermal in origin are the tracheae. These arise rather early in development as paired lateral invaginations in each segment. Later the invaginations widen at their bases and fuse longitudinally to form the tracheal trunks; the original apertures are retained as the stigmatic openings. At the same time the side branches begin to extend. Only very late in embryonic development is the chitinous lining secreted. This is the condition in the honey bee as

described by Nelson (1915).   Pratt (1900) and Hardenburg (1929) have given accounts for the Pupipara essentially similar to this.

In the Muscids the situation is somewhat different, for there exist but four spiracles of which only the two posterior are functional. Through these the whole tracheal system is fed with air.   Weismann (1863) observed the trachea to form only from the posterior segment at the positions of the hind-spiracles.   Weismann first made the observation that the tracheae of Calliphora become filled with air, released from the tissues, several hours before the larva hatches.

Mesodermal Organs

The mesoderm which in early development formed a somewhat flattened plate between the ventral ectoderm and mid-gut wall extends laterally and around to the dorsal side.   Graber (1889) found the mesoderm to remain solid in the muscids and to show no sign of coelomic sacs such as found in Donacia by Hirschler (1909) or of confluent lateral tubes which Nelson (1915) described for the honey bee.   Segmentation does not become marked in the muscids until the contraction of the germ band.   The mesoderm is then constricted transversely into segments which correspond to those of the ectoderm and are known as mesoblastic somites.   Only after this is mesoderm differentiated into muscles, both longitudinal and circular.   Two strands of mesoderm which form the heart unite along the dorsal mid-line, as the lateral portions of the extended mesoderm fuse on the dorsal side.   The fat body arises from cells of the dorsal portions of the mesoderm.

Imaginal Discs

Anlagen of the adult organs make their appearance in the larvae of holometabolous insects. These were first observed by Swammerdam (1737-38) who described the thoracic limb and wing buds of the butterfly and the honey bee in the larvae of those insects. Other later investigators also correctly described them, but it remained for Weismann (1863, 1864, 1866) to lay the foundations of our present knowledge of the imaginal discs in the Diptera. In his paper on the embryology of Calliphora (1863) he described the presence of six pairs of disc-like bodies within the thorax as well as another pair closely applied to the "brain". He found them to give rise to adult organs and so termed them imaginal discs. In his later investigations (1864, 1866) on further development and metamorphosis he found that most of the larval tissues disintegrate, while the imaginal discs form the greater part of the adult. To the process of disintegration he applied the term "histolysis".

Numerous investigators took up the study of the imaginal discs. One of the best accounts is that of Pratt (1900) who has described their origin in the embryo of the Pupiparan, Melophagus. Pratt found the discs to be formed as ectodermal invaginations in the cephalic and thoracic regions at a time when the mesoderm had not yet extended to the dorsal side, i.e., before the middle of embryonic development. Not all of the discs were laid down so early - although most of the thoracic and all of the cephalic complex were found to originate then - the discs of the external genitalia and those of the internal organs and of the abdominal hypoderm did not appear during

embryonic life.   Nerves, mesoderm, and tracheae were found not to
enter the discs until the larval stage.

The invaginated discs of the appendages are usually broad
at the base and become narrowly constricted at the point of attachment
to the hypoderm.   In later stages they become lined with cuticle which
is shed at each moult.

The need for detailed knowledge of the imaginal discs in
Drosophila is even more pressing since the introduction of the technique
for their transplantation by Ephrussi and Beadle (1935).   An account
of their growth from early larval stages on has been given by Chen (1929)
and their history has been extended somewhat earlier by Medvedev (1935).
In neither case was an histological study made.   No account of their
origin exists.   E. Strasburger (1935) in his book on Drosophila melano-
gaster figures an embryo in which can be seen the invagination to form
the frontal sac.   There is nothing beyond a simple reference to the
figure in the meager text.

Segmentation and External Form

The larvae of Muscids and of the related higher Diptera are
acephalic and apodous.   The freshly hatched larva has twelve segments,
of which eight are abdominal, three thoracic, and one cephalic.   The
cephalic segment is largely involuted before hatching so that only the
"neck" portion is external.   In the early stages of embryonic develop-
ment of Calliphora, Weismann (1863) observed the appearance of the
rudiments of mouth parts homologous to those of other insects.   By the
time that the thoracic and abdominal segments had become complete, these
transitory parts had entirely disappeared.   No signs of other segmental

appendages were found. The question of the homology of these parts with those of other insects is hardly a burning problem of modern biology and is best dealt with by those whom it does interest.

Stages of Development

In Calliphora, Weismann (1863) has referred to the period of development up to the completion of the germ band as the "Erste Entwicklungsperiode" (0-6 hours) and the subsequent period up to the beginning of the dorsal closure as the "Zweite Entwicklungsperiode" (6-11 hours). The remainder of development up to the time of hatching he termed the "Dritte Entwicklungsperiode" (11-24 hours). In the subsequent literature on Muscids and related Diptera these terms have always been used.

Experimental Embryology of Insects

The results obtained by Hegner (1909) from injuries to the pole cells of the Coleopteran Calligrapha have already been described. In addition Hegner found that injury to any part of an egg leads to failure of development of the organs normally derived from that region. Even anterior or posterior injuries before cleavage result in defects in those parts of the embryo.

Somewhat similar results were obtained by Reith (1925) working with the eggs of Musca domestica. When eggs were cauterized before cleavage, the nuclei later failed to enter the injured region, accumulated near its boundary, usually failed to divide, and certainly did not take a further part in development. If the nuclei themselves were injured

in the earliest stages, no development occurred at all. Anterior
injuries always gave anterior defects, and posterior injuries, posterior
defects. The uninjured parts developed normally. There were never
any extra segments or organs. No gonads were ever found in any embryos
in which the posterior tip had been cauterized in early stages. Ab-
normal processes seldom occurred in operated eggs. No evidence could
be found for regeneration or regulation.

Pauli (1927) followed the effects of constricting and centri-
fuging the eggs of Calliphora and Musca domestica. The fragments
obtained by constriction showed the typical partial development found by
Reith, thus demonstrating that in Reith's case the results were not in-
fluenced by the presence of dead material. By means of centrifugation
it was possible to concentrate the cleavage nuclei in one zone. After-
wards, when migration occurred, most of the nuclei would go to different
regions of the "Keimhautblastem" than normally. No really abnormal
development was obtained in these cases, although frequently the larvae
would be too weak to escape the egg membrane. In cases of strong
centrifugation, in which the "Keimhautblastem" was stratified, develop-
ment was largely abnormal.

Geigy (1931 a, b) used ultra-violet radiation to injure
different regions of the eggs of Drosophila melanogaster. Not only
was he able to castrate individuals by treatment of the pole cells (as
discussed elsewhere), but, by irradiation of different parts of embryos,
was able to produce imaginal defects. Irradiation in the early stages
up to the maximum extension of the germ band (1-6 hours of development)
gave a high egg mortality, but no imaginal defects were produced.
After this time irradiated eggs showed a low mortality, and the adults

arising from them had various defects in organs of ectodermal origin, but few or none in other organs. By irradiating different portions of the dorsal and ventral sides at different stages Geigy was able to show that the anlagen of the imaginal organs are shifted positionally from anterior to posterior along the dorsal side. This coincides with the shortening of the germ band described in a later part of this work. These results then indicate that larval determination occurs in the earliest stages (cf. Reith and Pauli) and that imaginal determination occurs during the period of maximum extension of the germ band (and certainly before its contraction).

It would be interesting to know what happened to the embryos which did not survive, but unfortunately Geigy made no sections of eggs. He does not mention whether he examined Malpighian tubes or salivary glands - both of which are of ectodermal origin - very likely he did not.

Howland and Child (1935) also obtained imaginal defects in Drosophila melanogaster by removal of small amounts of embryonic material by means of micropuncture. The defects recorded were all in ectodermal structures. Although punctures were made on embryos of different ages and at different positions, no data was given on the frequency with which defects occurred in such cases. The earliest stage at which they obtained imaginal defects was four hours after fertilization (i.e., at the time of formation of the germ band). Recently Howland and Sonnenblick (1936) have published a case of what they believe to be regulation in the early egg. When small amounts of the cortical ooplasm (Keimhautblastem) were removed by micropuncture, the blastoderm was found to form around the injured region. Only a small proportion

of such eggs came through as adults, and these showed no defects. However, the later embryonic stages were not studied for abnormalities. It is possible that no defect would be noticed because of the small amount of material lost. They found that when a larger amount of material is removed there are practically no survivors. Another source of difficulty which these authors did not consider arises from the fact that they punctured eggs both from normal wild type females and from attached-X $(\hat{X}XY)$ females and present this data indiscriminately lumped. It has long been known that one-fourth of the eggs of the latter type of females die in the early stages of development (Li 1927). The behavior of such "deficient" eggs is described in a later section of this paper.

A very different picture of determination in a Libellulid, Platycnemis pennipes, has been found by Seidel (1926, 1928, 1929 a, b). Using the methods of constriction and cautery he demonstrated that a particular zone in the posterior portion of the egg is essential for the formation of the blastoderm (Bildungszentrum). If a constriction was made anterior to this region after cleavage had begun, the blastoderm formed only in the posterior portion and a diminutive insect obtained from it. In cases where the constriction was incomplete (so that diffusion could occur) blastoderm was laid down in both parts. In order to make his experiments more exact Seidel divided the egg arbitrarily into forty units and with the aid of microcautery destroyed various small regions. In this way the "Bildungszentrum" was accurately located.

In later stages of development this center was found to move anteriorly, so that larger and larger portions of the posterior region could be destroyed without interfering with development. It was found

necessary for cleavage nuclei to reach this region before the effect was obtained. Constrictions just tight enough to prevent nuclei from reaching this region stopped all development. This center has nothing to do with specific determination or differentiation and so differs from an "organiser".

A center necessary for differentiation (Differenzierungszentrum) also has been localized by Seidel (1934). This appears much later in the anterior·portion of the egg. In the stages following this it has been possible to map out the limits of the various regions of the larva. Seidel (1935) has recently published a large paper describing the "Anlagenplan" of the Libellulid egg.

The egg of Camponotus, an ant, has been found by Reith (1931, 1932) to be capable of regulation in the early cleavage stages and to become mosaically determined at the time of the visible differentiation of the blastema into regions.

* * * * * * * * * *

This completes a brief survey of some of the more important points in insect embryology. The survey has been incomplete, and a great deal of literature had to be neglected. What has been given should be sufficient for orientation.

THE EMBRYONIC DEVELOPMENT OF DROSOPHILA MELANOGASTER

Materials and Methods

A wild strain of Drosophila melanogaster known as Oregon R was used as a standard. Two strains of attached-X females, one homozygous for forked (f), the other for yellow-2 (y^2), vermilion (v), forked (f), and carnation (cr) were used to obtain eggs lacking X-chromosomes. In order to obtain eggs deficient for halves of the X-chromosome, females of the latter type were crossed to males bearing an X-IV translocation known as T1,4-CRB. For studies on Notch-8, females of the constitution Notch-8/yellow, Hairy-wing, delta-49 were used. Details concerning these and the other deficiencies studied are given in the sections dealing with the respective deficiencies.

For the purpose of obtaining eggs vigorous young females were mated in vials with appropriate males. A single female and two males were placed in each vial. Food was supplied and eggs collected on special metal trays, constructed by Dr. G. W. Beadle and used through his kindness. These trays were filled evenly with ordinary culture medium, to which fine charcoal had been added (to make the eggs readily visible on the surface) and a portion of the surface liberally supplied with yeast. When laying began the males were usually removed. The trays were changed every two to four hours and eggs of a known age so obtained. These were then allowed to develop to the desired stage.

The work was done at room temperature, 22°-23° C., a thermometer being kept close to the vials and the microscope at which observations were made. For these investigations any more precise control of temperature was unnecessary.

Normal and "deficient" embryos were studied from living eggs and from sections.   For observations on living eggs the technique of Huettner and Rabinowitz (1933) and Child and Howland (1933) was used. The egg of Drosophila is covered by an opaque chorion which must be removed by needle before observations can be made.   Such a procedure (it had been used by Weismann in 1863) nearly always injures the egg so much that it begins to develop abnormally.   The technique above mentioned consists chiefly of refinements and involves fastening the egg to a slide and removing the chorion by careful manipulation of fine needles.   Huettner's technique is elaborate and time consuming. A short cut was sought.   It was found that eggs could be effectively fastened to a slide by removing with them, from the food on which they had been deposited, a small amount of the yeast, which quickly hardens holding the egg firmly. .   The eggs must not be allowed to stand more than a minute or two this way, as too much drying makes the chorion brittle so that the egg will be damaged.   Instead of using sharp pointed needles for dechorionation, it was found that a needle somewhat rounded at the tip was best.   With this a gentle lateral pressure was applied to the egg.   Usually the chorion split immediately and with pressure at the proper point the egg would "pop out".   If this did not happen immediately, a gentle stroking along the sides and top of the egg was usually effective.   The egg was then quickly placed with others in a hanging drop of salt solution (.75%).   The eggs were first examined to see whether they were injured.   If so, Brownian movement could generally be detected at once in or about the region of the micropyle or along the edges of a median optical section.   The eggs were then examined at intervals.   Usually 90-100% hatching resulted.   Because of

heat effects, it was found desirable not to examine the eggs under a strong light for too long a time at one observation. Also it was found essential to seal the hanging drop slide to prevent evaporation and concentration of the salt solution. Many abnormalities resulted from failure to seal the cover slip.

For study of internal structure (difficult because of the opacity of yolk) eggs were mounted on an ordinary slide under a slightly raised cover slip. Thus it was possible to use higher magnifications. Eggs cannot be observed for more than an hour or so in this way without replenishing the salt solution to avoid concentration.

Although many observations were made on living eggs using a modified Huettner technique, it was absolutely essential to study the material carefully by means of sections. Eggs of known age, obtained as described above, were dechorionated, carefully pricked in the fixative with a very fine needle, and generally allowed to remain for twenty-four hours. Fixatives would not work properly without the pricking. Even heat treatment was unsatisfactory, obliterating many of the details and making good staining difficult. Pricking must be done with extreme care as the contents of the egg tend to extrude from the puncture and so distort the orientation of the cellular material within the egg. Care had to be taken to make the puncture in such a portion of the egg as would not interfere with the details being studied. For instance, in order to get a clear picture of the dorsal extension of the germ band and the attendant growth of the hind-gut and its diverticula, it was necessary to make ventral punctures, and vice versa for studies of the ventral side.

A number of fixatives were employed, the most satisfactory

being a formol-alcohol-acetic acid mixture (5:15:1) similar to that
used by Huettner (1913). This was made up freshly each time it was
to be used, for it quickly loses its effectiveness. Eggs can be left
in this fixative for a considerable time, if necessary, as the alcoholic
concentration is approximately 70%. This fixative (F.A.A.) has the
advantage of a low surface tension so that eggs may readily be wet
by it and the process of pricking carried out more rapidly. Also,
it is possible to proceed directly to 70% alcohol.

Navashin's fluid (1% chromic acid, 10 parts; formalin, 5 parts;
glacial acetic acid, 1 part) was employed to some extent. The time of
fixation here was usually 8 to 12 hours, after which the eggs were
washed in distilled water for nearly as long. The chief difficulty
encountered with this fluid (aside from the fact that tedious washing
was involved) was its high surface tension which made wetting of the
egg surface very troublesome, interfering a great deal with the process
of pricking.

Carnoy fluid (glacial acetic, 1 part; absolute alcohol, 6
parts; chloroform, 3 parts) was tried. The time required for fixation
after proper pricking was found to be short (1 to 2 hours), but a good
deal of shrinkage and distortion was encountered. The even more violent
Gilson-Carnoy fluid (the above saturated with $Hg\ Cl_2$) was tried with
less success.

The fixed eggs were run through the alcohol and xylol into
paraffin on a schedule which had been used and was suggested by Prof.
B. P. Kaufmann, to whom the author is indebted for much information con-
cerning histological technique.

The thinnest sections cut (7μ) were of the early stages; for

later stages, sections were cut at 10 μ. The total number of eggs cut was somewhat more than 2000.

A number of stains were employed. Haematoxylin (Heidenhain's) gave good results - but had the slight disadvantage of staining yolk and cytoplasm somewhat more heavily than desirable. Gentian violet was used a good deal and was especially useful in studying the early stages of blastoderm and germ band formation. The most generally useful and satisfactory stain was found to be Grenacher''s Borax-carmine, long the stand-by of the older embryologists and especially applicable to insect embryos. Staining was done on sections rather than in toto before embedding.

The figures, except where otherwise indicated, are camera lucida drawings with 10x objective and 20x ocular at table level. In preparation of the reproductions they have been so reduced as to be almost exactly 200x.

The Structure of the Unfertilized Egg

In the eggs of most insects there are distinctions between the anterior and posterior poles which bear a definite relation to the position of the future embryo. The eggs lie in the ovarioles in such a position that the cephalic pole is directed toward the head of the mother; the dorsal and ventral sides also correspond. That the axes of the egg thus correspond to those of the parent was first demonstrated by Hallez (1886). Nonidez (1920) has shown that the egg of Drosophila, which is elongate, has this position and that during fertilization it lies in the vagina, dorsal side to dorsal wall. This side of the egg is somewhat flatter than the ventral and bears the filaments which are

processes of the chorion near the anterior end.   Fertilization occurs in the vagina.   The egg is so held that the micropyle lies against the opening of the ventral receptacle from which the sperm enter.   The egg is always deposited posterior end first.   Eggs may not be deposited immediately after fertilization except under favorable conditions. Frequently larvae nearly ready to hatch may be found on examination of newly laid eggs.

Externally the egg is covered by the chorion, which is formed of chitin-like material.   Within this is the vitelline membrane which is rather closely applied to the contents of the egg.   In sections there is some shrinkage and this membrane appears sharply separated from another, the plasma-membrane, which marks the boundary of the protoplasm.   Whether this is a real membrane or only a product of fixation need not concern us.   The extra-nuclear contents of the egg are made up of two parts: protoplasm and yolk.   The former fills much of the space and the yolk appears embedded in it.   The most exterior portion of the protoplasm is quite clear and free of yolk material forming a boundary layer which is variously known as the periplasm, cortical ooplasm, or the Keimhautblastem (cf. Weismann 1863).

The yolk spheres are of several sizes although apparently all of the same kind.   These stain nearly black with haematoxylin and take gentian violet well, though the latter tends to wash out. When this happens partially, structures can be made out within the spheres.   These structures can also be noted in the living egg and as soon as the egg is injured many of them begin to dance about as though taking part in Brownian movement.   In sections a clear area is usually noted around each sphere.   This is probably a result of

shrinkage. Numerous apparent vacuoles of about the same size as the yolk spheres are noted, some of which are doubtless the result of oblique cutting, but others by examination of several sections appear to be real vacuoles. These are to be noted at the periphery of the yolk and especially in the posterior region near the polar granules. These may be the result of fixation, but they were noted with all the fixatives used.

The cortical ooplasm is considerably thicker at the poles of the egg. The anterior polar plasm penetrates the micropylar cone. The posterior polar plasm is even thicker than the anterior. At the external edge of the posterior polar plasm are to be found the polar granules which are included when the germ cells are cut off. Early studies in the polar granules have been referred to elsewhere. Certainly in all cases studied they are included in the cytoplasm of the pole cells, never in somatic cells. Their behavior in Drosophila has been followed in considerable detail by Huettner (1923).

There are to be noted in the protoplasm other small granules, somewhat larger than the polar granules, which are scattered about, especially in the anterior portions of the egg. These stain very dark with gentian violet and are usually somewhat elongate.

Maturation, Fertilization, and Early Cleavage

The earliest stages in the development of the Drosophila egg have been described in considerable detail by Huettner (1923, 1924). There is nothing to add to his account. The writer's own observations have been less extensive and only a few cases of first and second cleavages have been observed. Huettner found polyspermy to be the

prevalent condition in Drosophila, although only one sperm fuses with the female pronucleus. The first polar body spindle is in metaphase at the time the sperm enters. The sperm activates the formation of the polar bodies. The first polar body consists of eight chromosomes which divide into two groups of four at the same time that the second polar body is formed. The polar body groups then enter the resting stage while the pronuclei approach one another (Figs. 1-a,b; 2-a). Both the latter become resting nuclei. Polar body nuclei and the two pronuclei resolve themselves then into haploid chromosome groups. The chromosome groups of the pronuclei go into the first cleavage spindle as separate groups and the pairing of homologous chromosomes occurs for the first time at the second cleavage.

The first cleavage spindle lies anteriorly in a clear proto-plasmic area about one-third of the length of the egg from the micropyle and near the dorsal side. This area remains recognizable during the early part of cleavage. The nuclei resulting from the first cleavage divide together, the products of their division divide synchronously, and so on. The chromosome groups of the polar bodies come together at the time of the first cleavage and form a circular arrangement with the smallest chromosome pairs in the center. This arrangement prevails up to the blastoderm stage when the groups become indistinct and eventually disintegrate.

The fusion of the sperm head with the female pronucleus and the disposition of the polar bodies at that time are shown in Figs. 1-a,b; 2-a. The chromatin appears here in the vesicular stage which precedes the formation of the more condensed metaphase chromosomes.
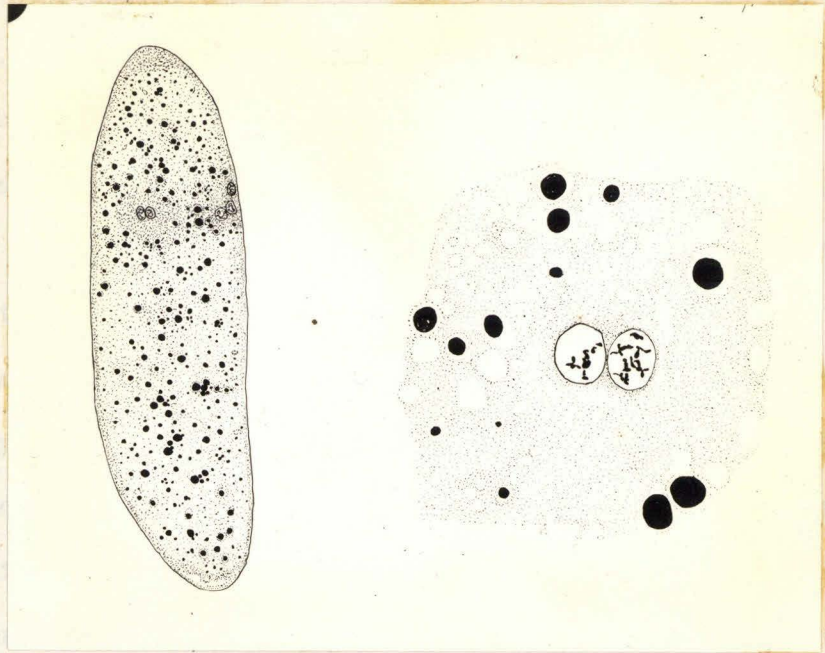
Figure 1.   (a) Section showing fusion of the pronuclei
            and the position of the polar bodies (200x).
            (b) Detail of pronuclei (1130x).
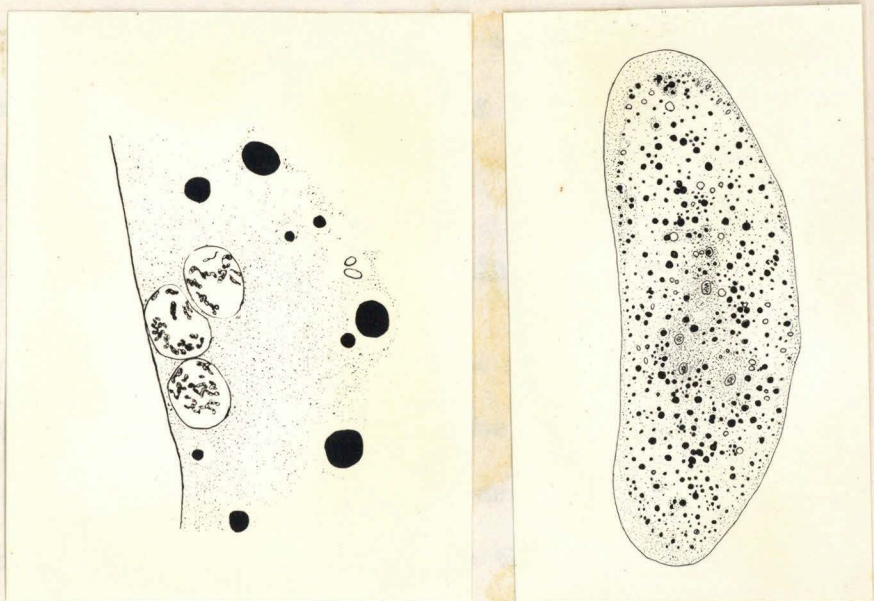

Figure 2.   (a) Detail of polar bodies of Fig. 1-a. (1130x).
            (b) Section showing nuclei between third and fourth
            cleavages (200x).

The first polar spindle lies in an island of clear protoplasm free of vacuoles, yolk spheres, granules, and other bodies such as fat, etc. Many of the larger spheres stain, as previously described, with gentian violet, although they fail to stain with borax-carmine. Others take no stain at all. These may be noted in the figures just referred to. Following the maturation divisions the polar bodies are found to lie in such a clear protoplasmic island close to the periphery of the egg. Presumably some of this material has been carried with the polar bodies as they move away from the female pronucleus. Fertilization takes place within the large island, whose size has been materially increased by the protoplasmic material which accumulates about the sperm nucleus as it approaches the female pronucleus.

During the next few cleavages the nuclei do not move far from the original position of the first cleavage spindle. Such movement as there is, is to the central portion of the egg. Each cleavage nucleus gathers an island of protoplasm about it. In early cleavage eggs, nuclei are most easily located by first looking for the clear islands (Fig. 2-b).

Later Cleavage, Migration of Nuclei, and Pole Cell Formation

By the end of the fourth cleavage the nuclei lie uniformly distributed throughout the central region of the egg. With increase in the number of cleavages, the nuclei move somewhat peripherally, though by no means uniformly. It must not be thought from the above that the nuclei form any regular figure or move with any precision and regularity in this early stage. The positions of the nuclei are by no means so fixed as in the egg of the honey bee (Nelson 1915) where

they form a "cleavage cone", nor do they move as regularly when migration begins as shown in the classic figures of Hydrophilus to be found in Korschelt-Heider (1899).

The egg at this stage is essentially a syncytium with many naked nuclei dividing synchronously. At the temperature at which the studies were made (22° to 23° C.) migration of the nuclei to the surface begins about the end of the first hour after fertilization. The nuclei are uniformly distributed throughout the egg at the 128-nuclei stage and begin migration at the 256-nuclei stage. The nuclei which penetrate the polar region reach the periphery of the egg first, though shortly thereafter nuclei appear at the surface in all portions of the egg.

These polar nuclei, of which there may be 3 to 5, are now partners in a remarkable behavior. The posterior polar plasm containing the polar granules pinches off in pockets, each including a nucleus (Fig. 3). These, the future germ cells, when first observed in Chironomus by Robin (1862) were called "globules polaires" under the delusion that they were polar bodies. Weismann (1863) made a considerable point of demonstrating that these were true cells and that they entered the blastoderm to take a part in the future embryo. A discussion of this early work is given in the section on the literature of insect embryology. Huettner (1923) also gives an extended discussion.

The nuclei lying in the center of the polar plasm take up more material than those at the edge and so contain more granules. However, all of the cells containing granules stay together in a packet at the pole until the time of migration (q.v.). As shown by Huettner, the pole cells are not all derived from any one particular
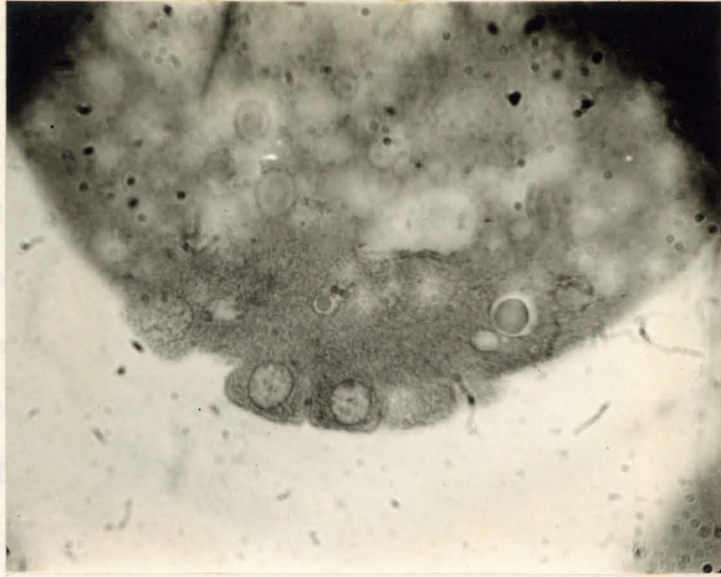
Figure 3.   Section showing formation of the pole cells,

four of which appear in the photomicrograph

(904x).

Figure 4.   Section of dorsal blastoderm as it nears

completion.   Vitelline membrane, elongate

nuclei, cell walls, inner cortical layer, and

yolk spheres.   Photomicrograph (904x).

nucleus, but from the first 2 to 5 nuclei which get into the polar plasm. A nucleus may divide so that one daughter will lie in the polar plasm, the other outside it. The former will become a germ cell, the latter an ordinary blastodermal cell.

The pole cells are the first to depart from the synchrony of division which has obtained to this point. While the remainder, the somatic nuclei, undergo the next division together, the pole cells are out of step and divide much more slowly. They remain large and have the shape of oblate spheroids at a time at which the blastoderm cells are becoming elongate and quite columnar. The total number of cells in this group is about 32 at the time of migration of the pole cells following completion of the blastoderm.

By the close of the 256-nuclei stage (the end of the first hour of development at 22°-23° C.) most of the nuclei are at the surface of the egg and an incipient blastoderm is present. Shortly after reaching the surface, the nuclei undergo a division, rest and grow a bit, and then divide again synchronously. In the sections this behavior of the nuclei is very striking and, with so many nuclei in the same stage, it is always possible to find some good figures.

After the tenth cleavage the nuclei grow considerably and in surface view are seen to pack more tightly together so that many appear nearly hexagonal. At the same time they grow in length and become columnar. Meanwhile cell limits have begun to appear and extend inward toward the yolk; these include with the nuclei material from the inner cortical layer, the "innere blastema" of Weismann; but these new cells remain open on the yolk side for some time. The inner cell limits are the last to form. Fig. 4 shows a stage in the

formation of the blastoderm with the elongation of the nuclei, the extension of the cell limits and the inclusion of the inner cortical material.

The yolk nuclei which take part in the digestion of the yolk are, according to Huettner, formed by those nuclei which are left behind during migration. However, in sections studied, very few of them have been found - though they are admittedly harder to see than the blastoderm nuclei. The evidence points to their formation at a later stage.

Inclusion of the Pole Cells and Formation of the Germ Band

The cell limits separating the nuclei of the blastoderm continue to grow and include in each cell a portion of the inner blastema (cf. Weismann 1863) as shown in Fig. 4. With the absorption of blastema material the cells attain their maximal size. The cells on the ventral side are considerably larger than those on the dorsal side and remain so to a much later stage of development. This is especially true of the longer dimension, so that the ventral portion of the blastoderm is thicker than the dorsal (Figs. 7 and 12). In Calliphora, Noack (1901) found that small yolk spheres were included in most of the ventral cells, especially those near the mid-line. This does not appear to be so in Drosophila, and no sign of yolk was found in sections. The ventral blastoderm then soon becomes the germ band from which much of the embryo will subsequently be formed.

There follows an active period of growth and division, the chief results of which are the formation of the cephalic groove (a transverse furrow) and a longitudinal ventral furrow (nearly at right

angles to the former), from the inner edge of which the mesoderm and
endoderm form; and a growth and extension of the germ band around the
pole to the drosal side of the egg.   These can be seen in Fig. 10.
There is also a folding of the dorsal blastoderm as though it were too
long for the space in which it must lie (Fig. 7).

Intimately associated with the lengthening of the ventral
blastoderm is the migration of the germ cells, the external phenomena
of which have been described by Child and Howland (1935).   During the
third hour of development these cells rather suddenly begin to move
anteriorly along the dorsal side to a point about one-fifth of the
length of the egg from the pole.   A depression which has been formed
beneath the pole cells deepens and receives them.   This can be seen in
Fig. 6, which was drawn from a living egg.   Within 20 to 30 minutes
after the first movement they have disappeared into the invagination.
The closure is not complete on the surface and this point can be fol-
lowed as it moves forward along the dorsal side.   In sections the
posterior (caudal) lip of this invagination shows numerous mitoses.
In Figs. 7 and 8 this and two other folds can be seen in the thin dorsal
blastoderm.   One is immediately anterior to the invagination, the other
represents the dorsal portion of the cephalic groove.   Germ cells can
be seen in the invagination.   With the establishment and extension of
the germ band, this dorsal (proctodaeal) invagination moves anteriorly
along the dorsal side until the aperture reaches the region of the
cephalic groove.   This is the most anterior point reached by the
dorsally invaginated material and this stage represents the greatest
length of the germ band.   Coincident with this forward movement of the
dorsal invagination, which proves to be the proctodaeum, the embryonic

membranes begin to grow caudally from the anterior and lateral lips of
the invagination...
opening is still...
may cover about...

The...
vagination, which...
cells (Fig. 9)...
granules in their...
They continue to...
tion for some time...
section.
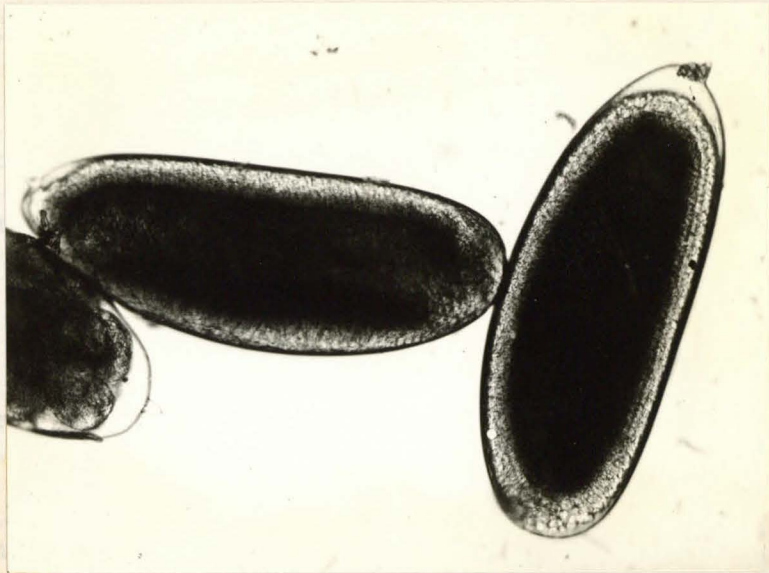
. Figure 5.   Photomicrograph of living eggs.   On the left the
              blastoderm is just forming.   The pole cells are
              hidden by the egg on the right which has a complete
              blastoderm and pole cells.   Micropyle is at the
              anterior end.   The flattened side is dorsal. (150x)

Figure 6.   Optical section of living egg (semi-schematic)
             showing beginning of dorsal invagination.   Pole
             cells entering invagination.   (440x)

membranes begin to grow caudally from the anterior and lateral lips of the invagination. By the eighth to tenth hours, when the prodtodaeal opening 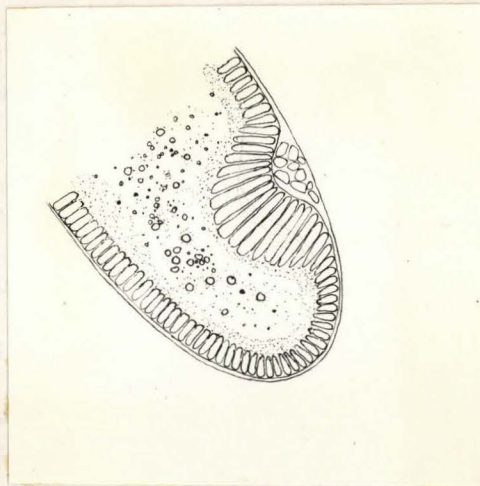is still at its most anterior position, the amnion and serosa may cover about one-fifth of the dorsal surface of the egg (Fig. 12).

The germ cells are to be found at the inner end of the invagination which sections show to consist of a single layer of columnar cells (Fig. 9). The germ cells are readily recognizable by the polar granules in their cytoplasm and form a compact though flexible group. They continue to lie in this position at the tip of the dorsal invagination for some time. Their further history will be taken up in another section.

By means of the movements just described the larger columnar cells of the ventral side are extended around and two-thirds of the distance along the dorsal side, and with them also is moved dorsally the underlying mesoderm, though to somewhat less extent, so that for the time a portion of the future ventral side of the larva lies on the dorsal side. (This is of interest in connection with Geigy's (1931) results on the irradiation of different parts of eggs with ultra-violet light.)

The movement of the germ cells is probably incorrectly referred to as "migration", for the germ cells do not move of their own accord, but doubtless as a result of the elongation of the thick ventral blastoderm which forces them along the line of least resistance. The phenomenon may be much more justly called the "inclusion" of the germ cells in the proctodaeum.

Origin of the Germ Layers

The external phenomena during the formation of the germ layers
have been briefly referred to above.   Just prior to and during the
movement of the pole cells the cephalic (anterior) groove appears and
becomes very marked.   Shortly afterward a longitudinal ventral furrow
(gastrular groove) is formed which intersects the former at right angles
and extends beyond it anteriorly as in Fig. 10.   This furrow really be-
gins on the dorsal side at the posterior lip of the proctodaeal invagina-
tion beneath which the posterior rudiment of endoderm is formed (Fig. 7).
Mesoderm is first formed near the posterior end and extends along the
bottom of the furrow to the point of intersection with the cephalic
furrow.   The remainder of the endoderm is formed anteriorly to the
cephalic furrow and so lies beneath the ectodermal cells which soon in-
vaginate to form the stomodaeum.   Although this point has not been
studied in as much detail as desirable, it is certain that both endoderm
and mesoderm are formed.   The mesoderm is distinctly separate from the
two rudiments of endoderm.

From the time of the invagination of the stomodaeum the
anterior endoderm is closely applied to its tip.   The endoderm grows
out in two strands coursing posteriorly along the ventral side above the
mesoderm which separates it from the ectoderm.   This is indicated rather
clearly, though schematically, in Fig. 23-c and d.   These strands which
will fuse subsequently to form much of the ventral wall of the mid-gut
are seen in transverse section in Fig. 13 and can be followed from the
point of union with the stomodaeum well into the mid-region of the egg.
The posterior rudiment of endoderm is not so prominent in the early
stages and the cells are frequently indistinguishable from the adjacent
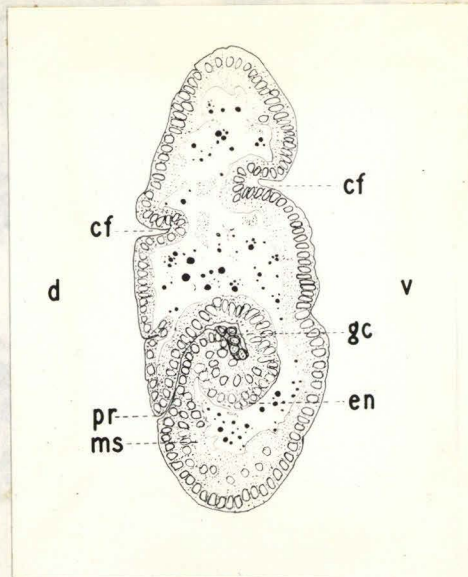
Figure 7.   Sagittal section showing dorsal invagination and
the beginning of germ layer formation. Cephalic
furrow (cf), endoderm (en), germ cells (gc), meso-
derm (ms), proctodaeum (pr).
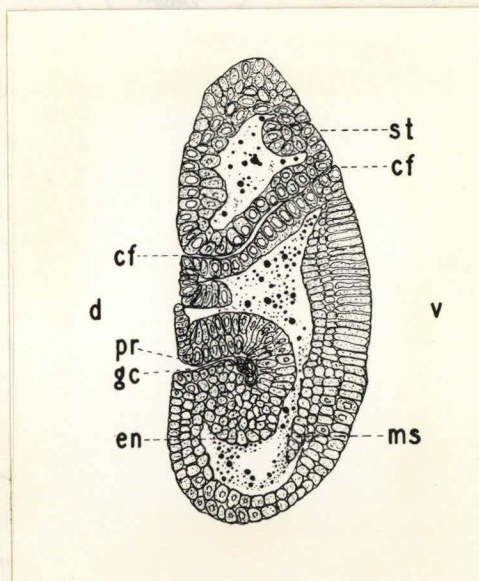
Figure 8.   Section (not quite sagittal) at a later stage than
Fig. 7 showing ventral mesoderm, the anlage of the
stomadaeum, and one side of the cephalic furrow.

Figure 9.   Photomicrograph of germ cells in the dorsal
             invagination at a stage comparable to Fig. 11.
             (904x)
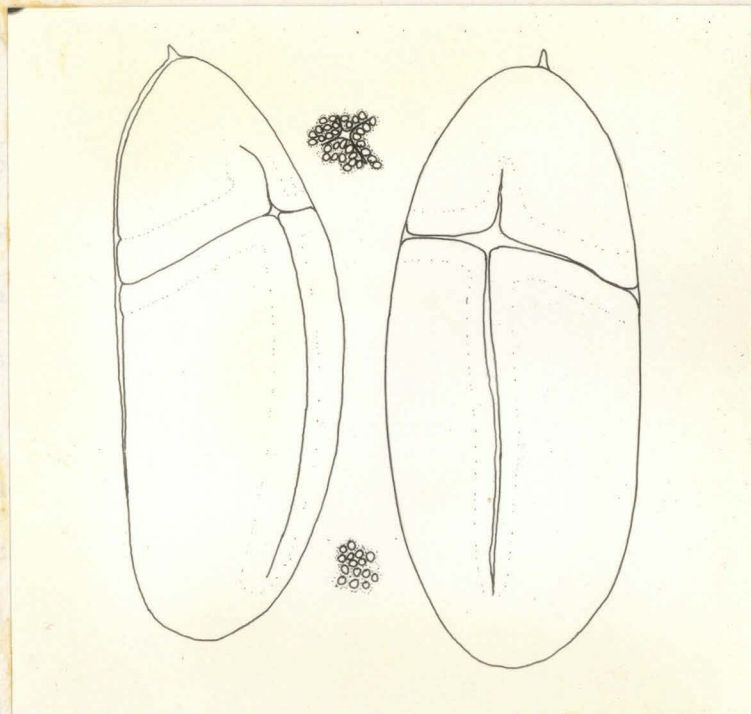
Figure 10. Views of ventral surfaces of eggs showing mid-
             ventral and cephalic furrows.   From the inner
             edges of the former, endoderm and mesoderm are
             formed.   A stage intermediate between Figs. 7
             and 8.   (440x)

cells of the hind-gut wall.   However, its extent becomes clearly

visible by the time of the shortening of the germ band, as shown in

Fig. 16.   The two rudiments complete the mid-gut by growing together

around the yolk.   Thus the condition in Drosophila is like that found

in Calliphora by Noack (1901) and in Leptinotarsa (Doryphora) by

Wheeler (1889).

The mesoderm formed along the ventral furrow lies as a

flattened mass beneath the ectoderm.   Whether in the early stages it

consists of two strands (each beneath and slightly lateral to the ventral

furrow) has not been determined.   The mesoderm is solid and no coelomic

sacs exist.   Segmentation does not begin until after the formation of

the nervous system.   The formation of the nervous system from swellings

of ectoderm along the mid-line of the germ band separates the mesoderm

into two equal lateral masses connected above the nervous material by

a thinner median part.   This may be seen in Fig. 13.   At the anterior

end in the neighborhood of the stomodaeum the mesoderm is split into

two distinct parts (Fig. 14).   The extent to which the mesoderm has

encroached laterally beneath the ectoderm may also be seen in the same

figures.   Longitudinally the mesoderm extends from just anterior to the

stomodaeum along the whole length of the ventral side and around the

dorsal side to the proctodaeum.

The extending ventral blastoderm and the underlying mesoderm

and endoderm will henceforth be referred to as the germ band.

Formation of the Stomodaeum: Growth of Proctodaeum

When the dorsally invaginated material is moving (as a whole)

anteriorly along the dorsal side, the first signs of the stomodaeal

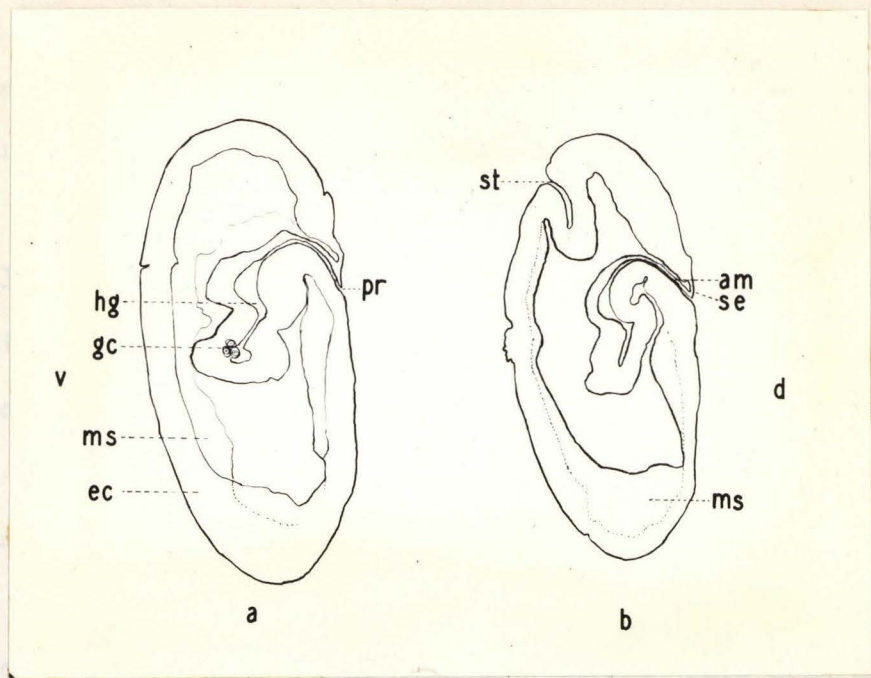Figure 11. Sections of an egg (5-6 hrs.) showing dorsal invagination (hind-gut), the newly formed stomadaeum (st), position of the mesoderm (ms), and the amnion and serosa (am, se).
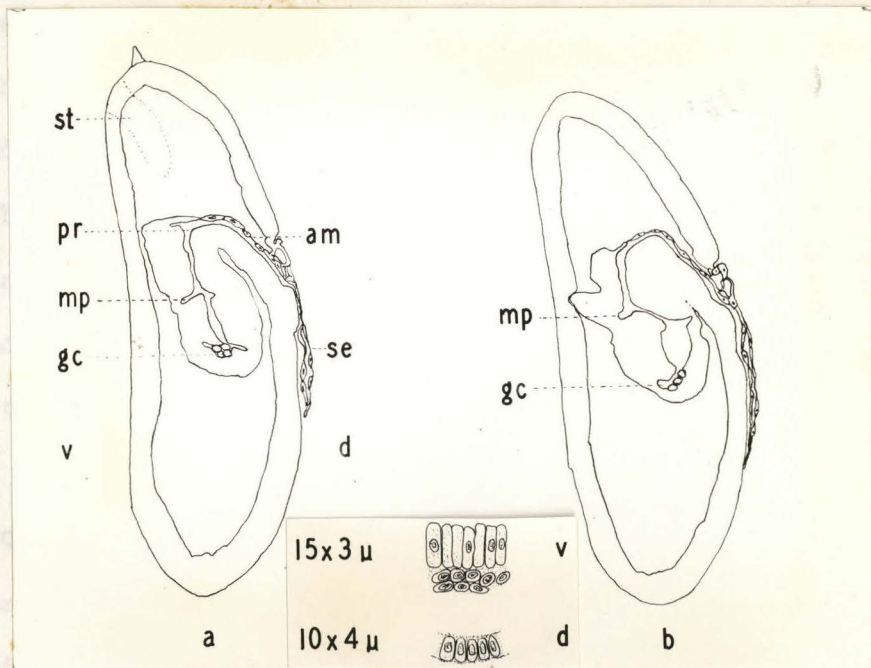

Figure 12. Later stage than Fig. 11 (7-8 hrs.), showing the greatest extent of the embryonic membranes. The anlagen of the Malpighian tubes are indicated by mp. For simplicity, endoderm and mesoderm are not shown.

invagination (Fig. 8) may be made out on the ventral side about one-eighth of the distance back from the micropylar cone. This inward growth continues while the material on the dorsal side is moving forward (Fig. 11). At its first appearance the stomodaeum is seen to be formed of ectodermal cells which turn inward. This region can be identified as one of rather large cells in eggs in which the germ cells have just begun to disappear. In the early stages of invagination many mitoses are observed in this region. Previous to this time these cells have undergone many fewer divisions than their sister cells of the ventral blastoderm. In point of fact all of the cells anterior to the cephalic groove have remained larger and embryonically more primitive than the other cells of the blastoderm and begin to take part in morphogenesis later than the cells of other regions. The stomodaeal invagination takes place quite as rapidly as the hind-gut invagination, and the stomodaeal cells are soon in close contact with those of the anterior portion of the endoderm with which they soon form a continuous mass (Figs. 13 and 14). Just posterior to the incipient stomodaeal invagination are to be found traces of folds which represent the first signs of the subsequent swellings to form the anterior portion of the nervous system. These become prominent by the time that the stomodaeum is confluent with the mid-gut anlage. Their future history will be described in the section on the brain and nervous system.

From the time that the germ cells first migrate the inturned ectodermal material increases tremendously in amount. The cells which have at first formed only a simple tube (Fig. 9) come to fill about one-fourth of the interior of the egg within a period of four hours (Fig. 16). A large part of this increase is the result of growth

and frequent mitoses, and to some extent the result of the movement (extension) of the germ band dorsally.   One of the consequences of the dorsal invagination and the growth of the embryonic membranes (q.v.) is a reduction in the thickness of the lateral walls joining the dorsal and ventral portions of the germ band.   In transverse section the embryonic walls appear as in Figs. 13, 14 and 15).   These lateral walls are in all appearances like the attenuated cells of the amnion.   Through them the yolk and the hind-gut can be rather clearly seen in the living egg.

When first turned in, the cells of the dorsally invaginated tube (the proctodaeum) are quite indistinguishable in size and form from their sister blastodermal cells.   They are somewhat columnar and remain so through much of their later history.   However, the adjacent cell sheet which forms at the lip of the dorsal invagination and makes up the embryonic membranes is notably different in structure. The cell bodies are strongly attenuated and the nuclei are somewhat flattened so that relatively few cells cover a large surface.

The posterior movement of the hind-gut and the posterior endoderm closely parallels the extension of the anterior mid-gut rudiment on the ventral side.

During the invagination and the anterior movement an uncommonly large number of mitoses are observed at the lip of the invagination.   There is a higher frequency here than in any other portion of the egg in this and the immediately following stages.
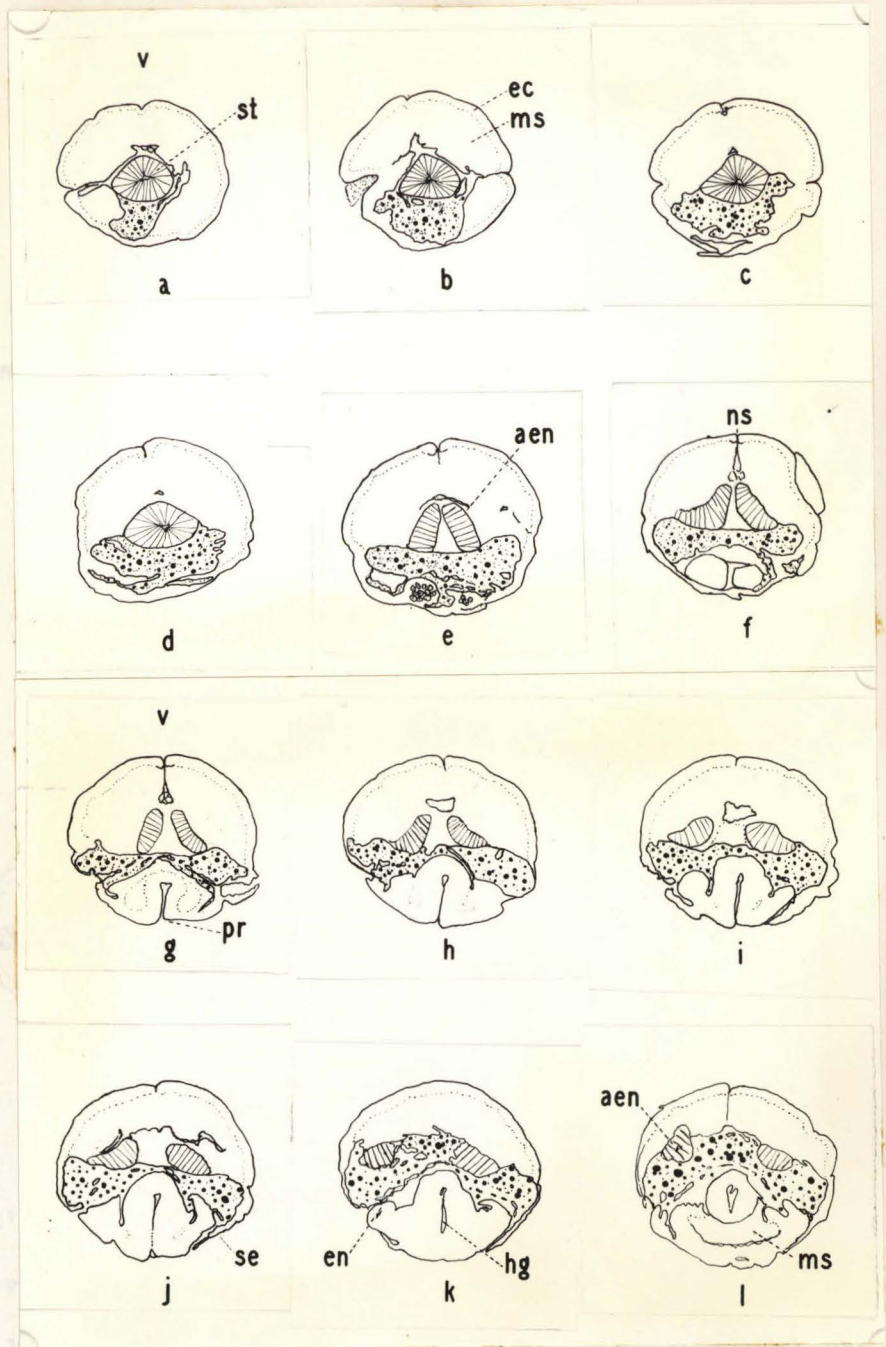
Figure 13. Series of transverse sections beginning near the
anterior end of an embryo 8 hrs. old. The stomadaeum
(st) can be followed to the point of fusion with the
anterior endoderm (aen) and the latter can be followed
well beyond the mid-region of the egg. The embryonic
membranes (am, se) and the Malpighian tubes (mp) are
shown in the more posterior sections on the following
page.
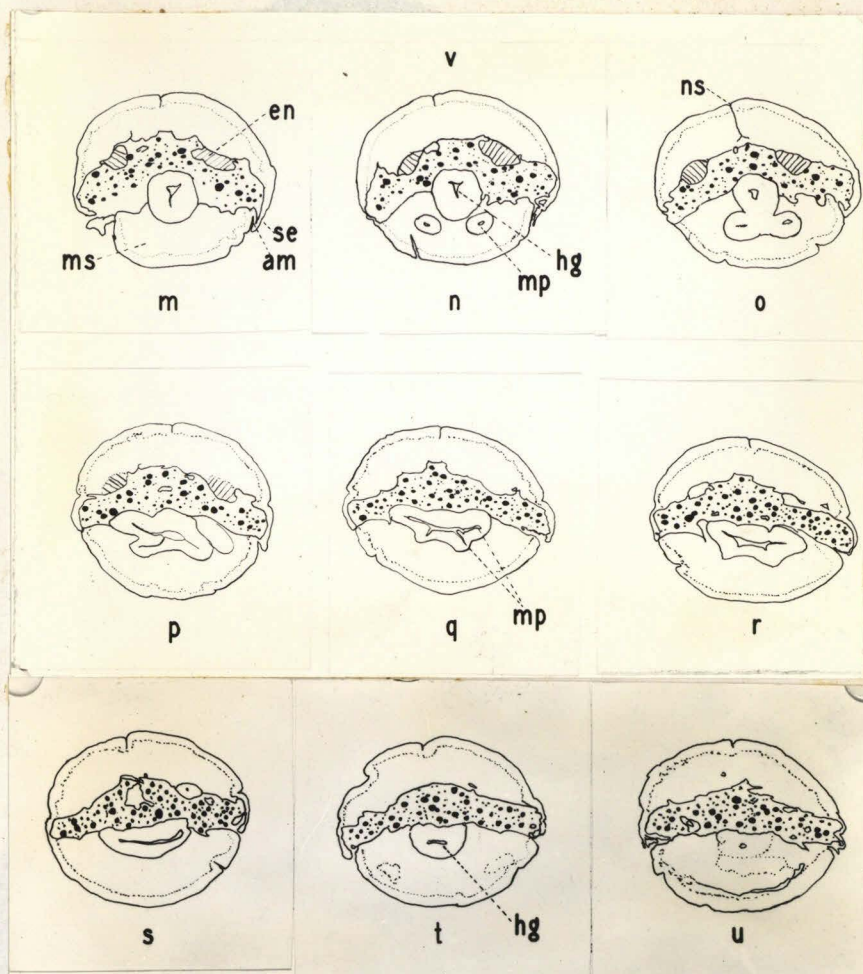
Figure 13 - Cont'd.   The first signs of amnion (**am**) and serosa (**se**)

are visible in "**m**".   The mid-gut rudiment (**en**) ends at

the level at which the Malpighian tubes leave the hind-gut.

The first signs of the nervous system (**ns**) can be followed

through the series.   Groups of large cells on either side

of the groove proliferate to form the nervous tissue.

Later they form a compact flattened rod.   The mesoderm (**ms**)

is entirely undifferentiated.
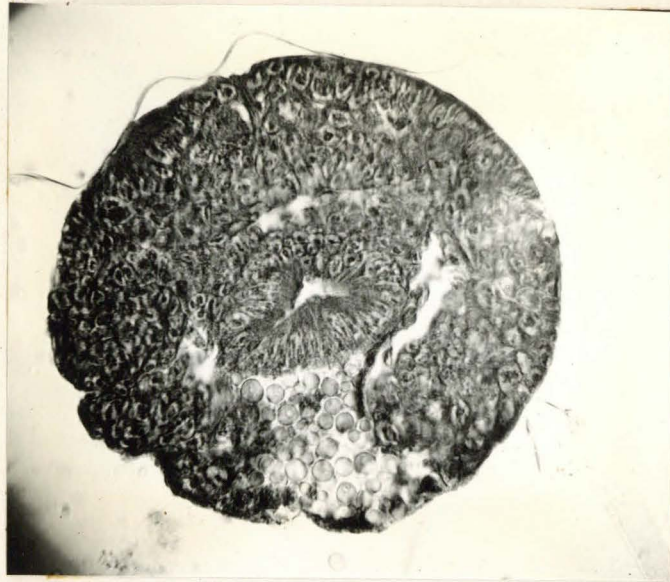
a
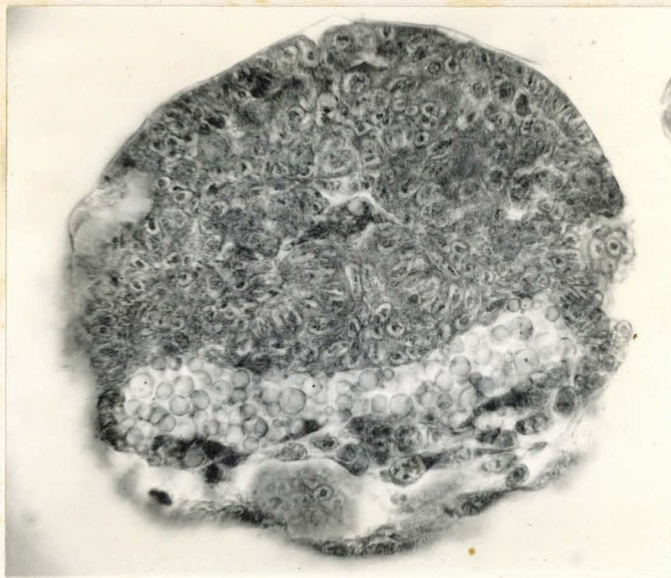
e

Figure 14. Photomicrographs of sections drawn in Fig.

13 - a, e, f, g. These show rather clearly

the appearance of the cells in the several

structures indicated previously. The nature

of the region of the proctodaeal invagination

is evident. (600x)
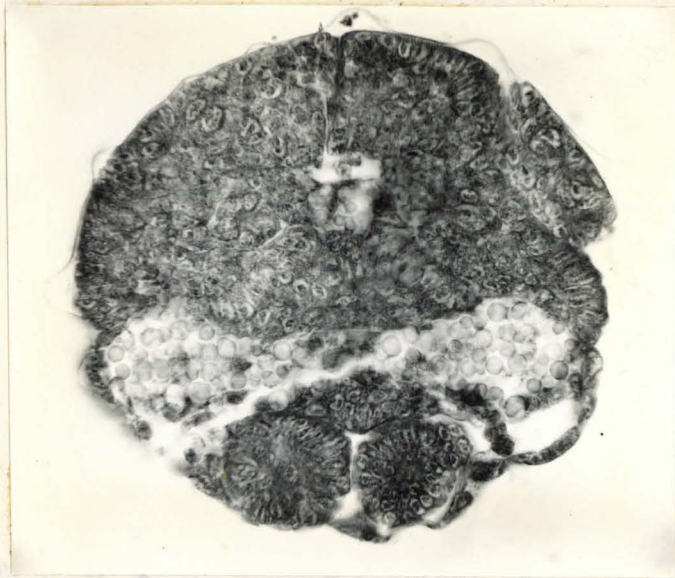
f
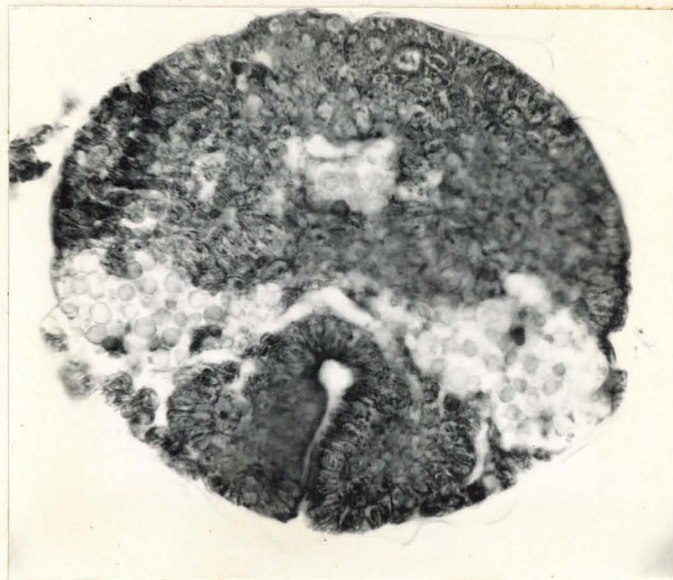
g

Figure 14 – Cont'd. Photomicrographs (600x) [of the posterior
end of the hind-gut (dorsal) invagination and proceeding
anteriorly to the point of invagination. The age is
between 9 and 12 hrs. The relation of the posterior
enenteron (mp) to the hind-gut (hg) is made clear. The
formation of the Malpighian tubes (mp) and the disposi-
tion of the adipose membrane are evident.]

Figure 15. Series of transverse sections beginning near posterior
end of the hind-gut (dorsal) invagination and proceeding
anteriorly to the point of invagination. The age is
between 8 and 10 hrs. The relation of the posterior
endoderm (pen) to the hind-gut (hg) is made clear. The
formation of the Malpighian tubes (mp) and the disposi-
tion of the embryonic membranes are evident.

Embryonic Membranes

In marked contrast with the Nematoceran Diptera in which
the embryonic membranes envelope the embryo completely (e.g. Chironomus)
the Brachycera possess only vestiges of such.   Kowalevsky and Noack
found them to be transitory in Calliphora and gave but little informa-
tion concerning their maximum extension.

The amnion fold first appears in the Drosophila embryo
shortly after the beginning of the proctodaeal invagination.   It
arises from cells of the anterior and lateral lips of the invagination.
These grow in the form of a double sheet (serosa external, amnion
internal) and extend caudally as the germ band elongates around the
pole of the egg and anteriorly on the dorsal side.   At first the cells
of the membranes are not greatly different from their sister blasto-
dermal cells, but they rapidly become flattened and attenuated.   Like-
wise the nuclei are greatly flattened.   More rapid growth occurs at
the lateral edges of the invagination and the membranes begin to spread
over the dorsal surface.   It is some time after the opening of the in-
vagination has attained its most anterior position that the membranes
reach their maximum extension.   Fig. 12 is a section of an egg showing
the maximum antero-posterior length.   Shortly after this stage the
amnion is pulled somewhat into the interior of the egg as the hind-gut
anlagen extends posteriorly.

There seems to be a considerable amount of variability in
the extent of the membranes.   In sections of eggs of the same age
they may be present to more or less extent.   The series of transverse
sections shown in Figs. 13, 14 and 15 give a clear representation of

the amnion and serosa.   Here also the nature of the lateral portions
of the dorsal wall are to be noted, especially the absence of mesoderm.

The amnion and serosa are purely transitory structures and
upon the contraction of the germ band they flatten out to form a part
of the dorsal integument.

The Contraction of the Germ Band

The first half of embryonic development involves the
establishment of the germ band and its elongation, during which time
the germ layers and most of the principal organs are laid down.   This
corresponds to Weismann's first two stages of development.   Between
the eleventh and twelfth hours of development the germ band rather
suddenly contracts so that it again lies wholly along the ventral side
of the egg.   This contraction brings the hind-gut to lie in its final
position with the anus at the pole of the egg.   The process of shorten-
ing is best understood by reference to the schematic drawings of Fig. 23.
In Fig. 16 the contraction is just beginning, as evidenced by the appear-
ance of many of the larval segments, while in Fig. 17 contraction has
been completed.   The egg, sections of which are shown in the latter
figure, had been pricked at the point indicated by the arrow, with the
result that the segments do not show in that region.

The relation of this process of contraction to the various
parts of the embryo is discussed in the sections on those parts.

The Hind-Gut and the Malpighian Tubes

The proctodaeal invagination, through which the germ cells
entered the interior, undergoes notable growth and form changes.
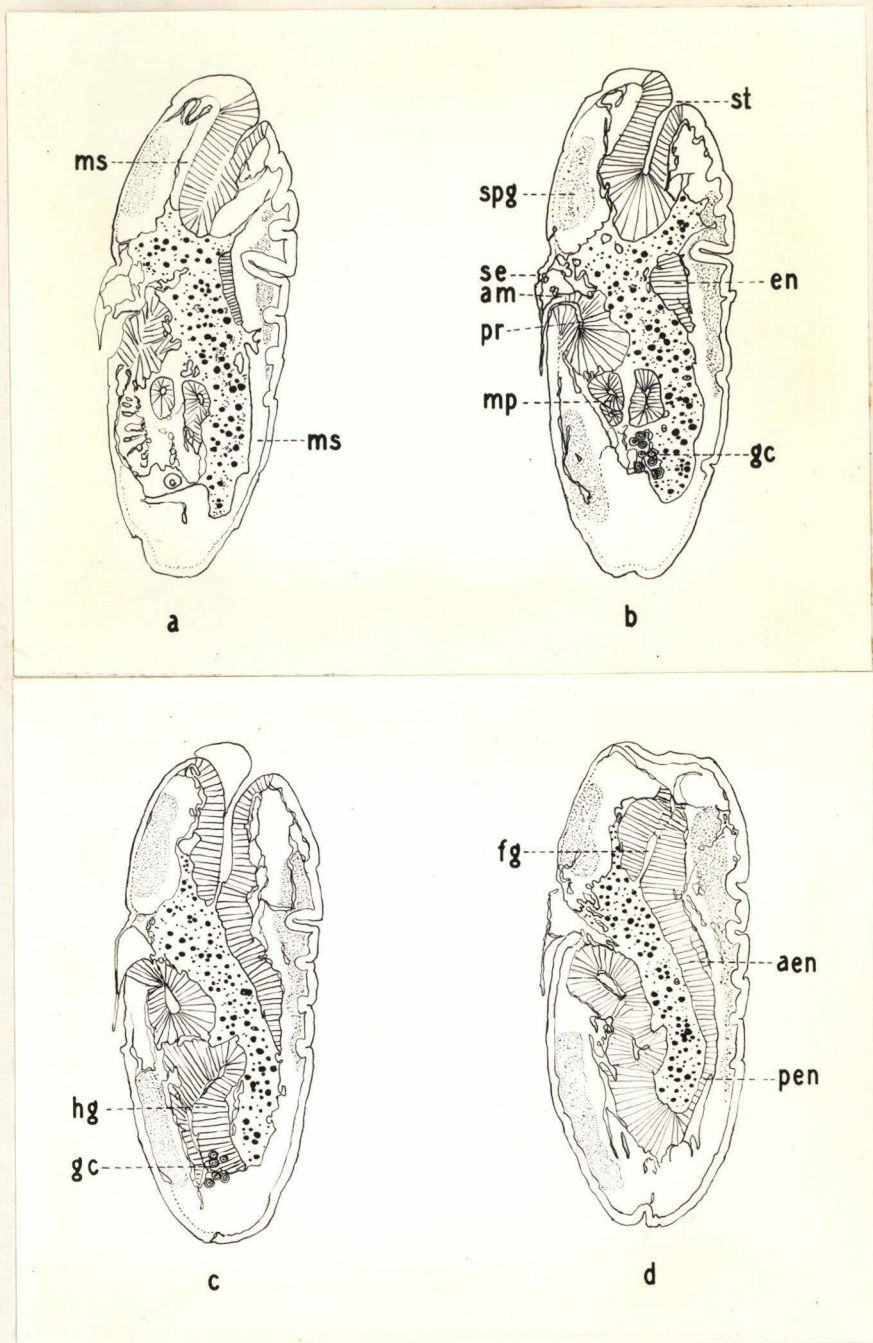
Figure 16. Longitudinal sections just before the shortening of
the germ band (10-11 hrs.). The mesoderm (ms) is still
undifferentiated.   The nervous system (stippled) can
be followed.   The two rudiments of endoderm have united
to form the ventral wall of the mid-gut. The germ cells
(gc) have just escaped into the body cavity. The embry-
onic membranes are still visible.   The hind-gut has its

Figure 16 - Cont'd.

    maximum extension.   The Malpighian tubes are seen in
cross section in "a" and "b".   The regions of the
sub- and supra-oesophageal ganglia are indicated by
sbg and spg.   The nervous system is very schematically
represented.

Figs. 13, 14 and 15, which show the extension of the fore-gut and the
endodermal strands in cross section, also show the hind-gut and its
derivatives, the Malpighian tubes.   The anterior portion of this re-
mains much as in the earlier stages - a simple tube - but posteriorly
four diverticula may be noted at one point (Fig. 13-p, q, r; Fig. 15-f,
g) two of which are more prominent than the others.   These are the
Malpighian tubes.   Further posteriorly the tube is again simple and
ends at the junction of the posterior endoderm.   This point is not
sharply defined, as can be seen by following the two parts in Fig. 15-a,
d.   The germ cells remain at this end for some time before attaining
their final position in the body cavity between the mid-gut and the
dorsal mesoderm.   In many sections they are hard to distinguish at
this stage, but slightly later there is no difficulty (Fig. 16-b, c).
The first signs of the Malpighian tubes are noted between the sixth
and the eighth hours.   By the tenth hour they are well defined and
in the twelfth hour they assume what is nearly their final position.
After this time they become considerably elongate and coil about the
mid- and hind-guts, two of them extending anteriorly and two posteriorly.
This further development of the tubes can be followed in the longitudinal
sections of Figs. 16, 18, 19 and 20.   The cells of the Malpighian tubes
appear little different than those of the hind-gut.

The growth of the proctodaeum caudally brings the posterior
endoderm rudiment nearer to the ventral side along which it extends.
There it fuses with the two anterior strands and forms the mid-gut.
The hind-gut now extends nearly two-thirds the length of the egg.
With the shortening of the germ band between the eleventh and twelfth
hours the proctodaeal opening begins to move posteriorly.   The result

of this is the turning of the hind-gut back on itself to form a U-shaped tube. This represents the first coiling of the hind-gut. The shortening and its attendant changes are best understood from the schematic representation of Fig. 23-d, e and f. The opening of the hind-gut is brought to the posterior tip of the egg and remains there throughout further development. The walls remain thick until the eighteenth to the twentieth hour when the hind-gut begins to coil and elongate considerably. Then, as differentiation proceeds, the thickness of the walls is reduced to about that of the mid-gut walls (Fig. 24).

During this late period of differentiation, through which all portions of the gut elongate considerably, the point of union of the mid-gut and hind-gut comes to lie about mid-way between mouth and anus. Very close to this point the Malpighian tubes leave the hind-gut, one pair coursing anteriorly along the dorsal side of the gut and sometimes coiled in it, the other pair coursing posteriorly in the same fashion. Observations on living eggs show that the pigment of the tubes appears by the twentieth hour, thus indicating the near completion of differentiation by this time. Likewise, in sections, the cells of the tubes can be seen to have the form and disposition of those of the larva as described by Marie Strasburger (1932). The cells are somewhat broad and elongate on the basal side and quite rounded on the side of the lumen of the tube. As a result, in a portion of the tube seen in longitudinal section, the lumen appears "zig-zag" in form.

The nuclei of Malpighian tubes of mature larvae have been shown to have essentially the same structure as those of the salivary glands. In larvae ready to hatch the nuclei of the two are much alike, but are no larger than those of other cells of the gut tract.
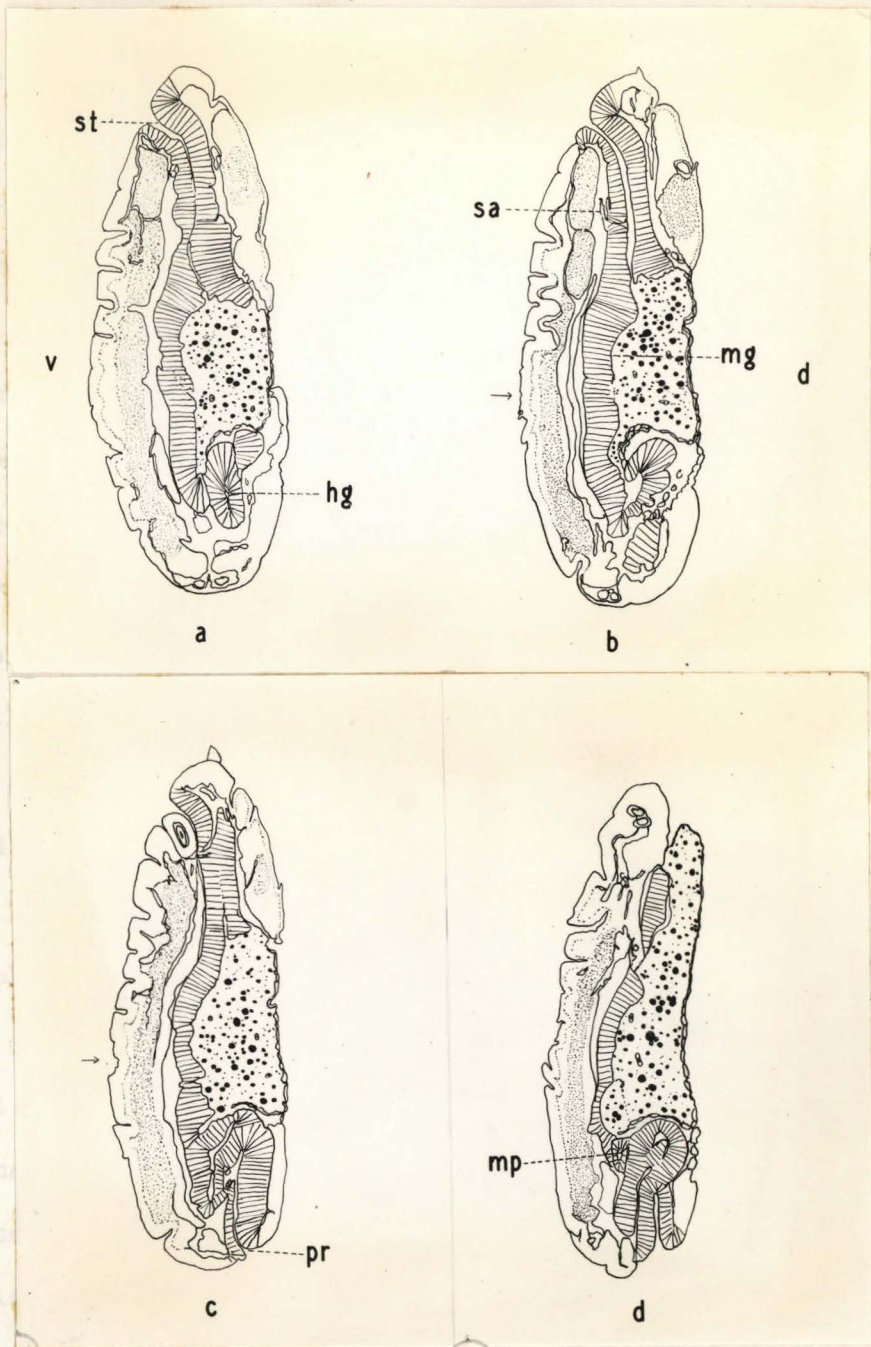
Figure 17. Sections of an egg just after the contraction of the
germ band. Nervous system shown schematically by
stippling. Arrow indicates point of puncture during
fixation. Age = 12-13 hrs.

The Mid-Gut

The mid-gut is formed by the fusion of two rudiments of endoderm, the anterior, which contributes much of the ventral wall, and the posterior, which forms the remainder of the ventral wall and which appears to contribute the dorsal wall. The first evidence of the mid-gut is to be seen in Fig. 13-e to p, which represent the two strands of endoderm extending posteriorly from the point of fusion with the stomodaeum. These fuse along the mid-ventral line to form the larger portion of the ventral wall and begin to extend laterally around the yolk mass. The posterior rudiment of endoderm appears earlier than the anterior, but is not very clearly distinguishable from the cells which will form the hind-gut until the eighth to the tenth hours (Fig. 15-a to e). In eggs nine to eleven hours old it can be found fusing with the anterior endoderm on the ventral side as shown in Fig. 16-c to f. By the time that shortening of the germ band takes place (11 to 12 hours), the cells of the ventral wall have become more elongate, losing little of their breadth, so that the ventral wall is thickened (Fig. 17).

The dorsal portion of the mid-gut wall is seldom noted before the eighth hour, after which it appears in cross sections as a thin strand of elongate cells quite similar in appearance to the more tenuous portions of the embryonic membranes. This appears to arise from the posterior endoderm and extends very quickly around the dorsal side of the yolk, which is completely enclosed when these cells unite with the lateral portions of the ventral wall. Because of its delicate nature it is easily torn in sectioning and is seldom seen complete. Portions
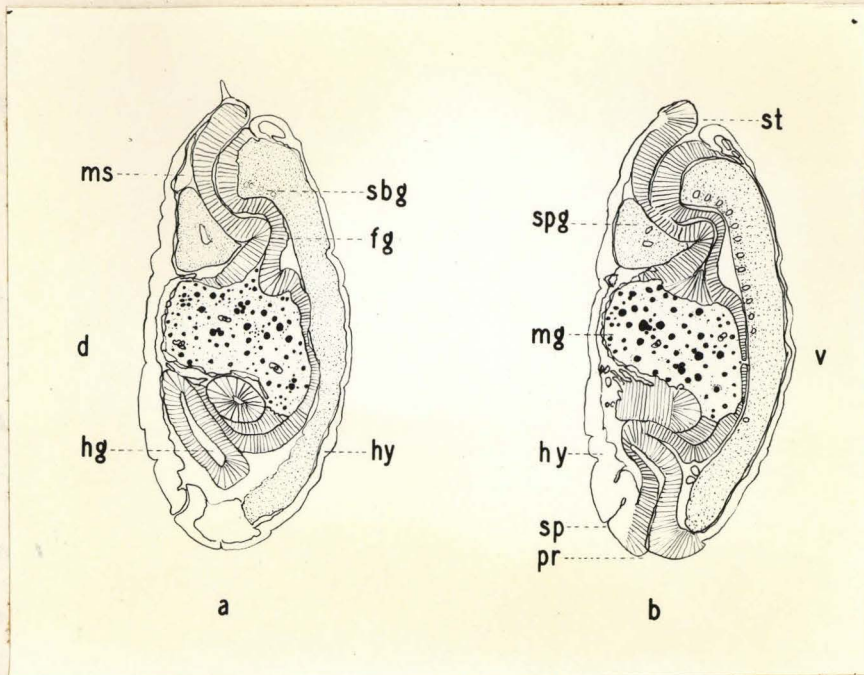
Figure 18.   Adjacent sagittal sections of a 14-hour embryo.   The
extent of the nervous system is seen on the ventral
side.   The spiracle (sp) is visible but there is no
trachea.   Gonads and salivary glands which lie
laterally do not show, but are illustrated in the fol-
lowing figure.   The mid-gut is not yet extended and
coiled.   In "b" the cells indicated in the nervous
system lie medially and extend the length of the rod.
Tracts of nerve fibers differentiate laterally to
them.

of it can be seen in Fig. 15-h to l, also in Fig. 16-c and f. After the shortening of the germ band the cells have become larger and the whole of the mid-gut wall can be made out from sections.

Most, but **not all**, of the yolk is enclosed in the mid-gut. The rest stays in the body cavity. Later, when contraction of the muscles begins, the yolk spheres can be observed moving about in the body fluid. They are particularly noticeable when one observes the lateral portion of a segment, and give a very good notion of how the body fluid, or "blood" is circulated in an "open system".

The photomicrographs in Fig. 22 are of sections of a twelve-hour embryo in which contraction of the germ band is complete. These illustrate very clearly the difference between the dorsal and ventral walls of the mid-gut, as well as the relation of the mid-gut to the fore- and hind-guts and especially to the Malpighian tubes, which are prominent in "b". In the more dorsal section (c) the lobes of the supra-oesophageal ganglion (brain) can be seen laterally to the fore-gut. At the posterior end are the convolutions of the hind-gut.

The subsequent history of the mid-gut involves changes from a simple sac full of yolk to a long and rather tortuous tube filled with smaller aggregations of yolk. By the eighteenth hour the walls of the mid-gut are of uniform thickness, much thinner than the ventral wall, although thicker than the dorsal wall of a twelve-hour embryo. Instead of remaining columnar the cells are of a somewhat intermediate nature.

The anterior end of the mid-gut terminates in the proventriculus, one part of which is made up of endoderm, the remainder of ectoderm. The outer body of this is made up by mid-gut and into it projects the
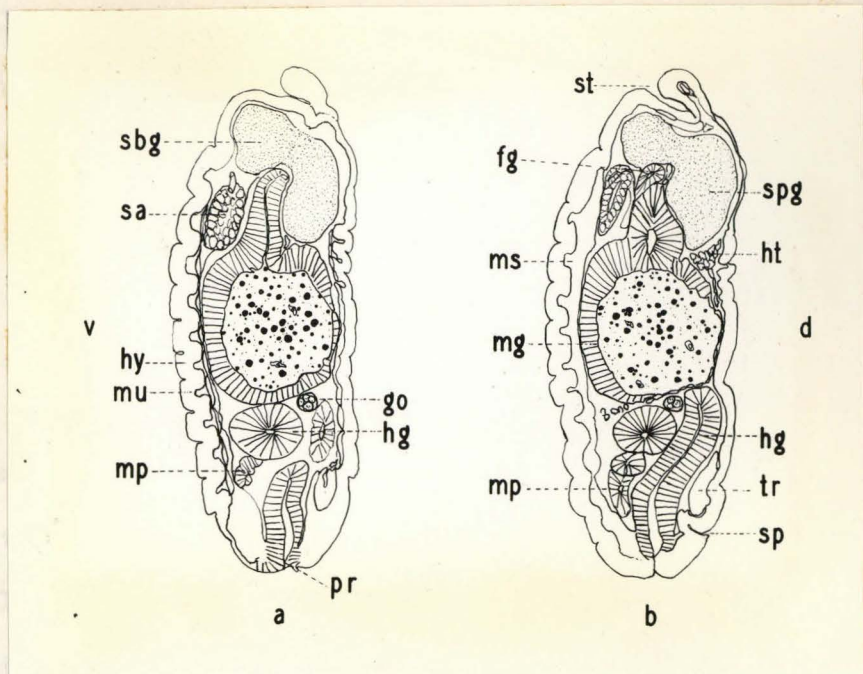
Figure 19. Adjacent longitudinal sections, somewhat oblique,
            (15-16 hrs.) showing the completed mid-gut (mg), the
            coiled hind-gut (hg) and Malpighian tubes (mp), a
            gonad (go), a salivary gland (sa), a spiracle (sp)
            and its extending trachea (tr). The sub- and supra-
            oesophageal ganglia (sbg, spg) are seen to be connected
            laterally enclosing the oesophagous. The mesoderm
            (ms) has begun to differentiate into muscle (mu) and
            heart (ht) rudiment. The hypoderm is indicated by hy.

extended oesophagous.   The proventriculus can be found in sections of embryos more than twenty hours old.

The blind intestinal caeca also appear in this late stage as diverticula from the region of the mid-gut just posterior to the proventriculus.   With respect to cell shape and the form of the lumen, they are similar to the Malpighian tubes.

No observations were made to determine when the gut obtains its muscular layers.. Certainly it is later than eighteen hours.

The Fore-Gut and the Mouth Parts

Consecutive stages in the development of the fore-gut can be followed in the longitudinal sections of Figs. 16, 17, 18, 19 and 20. The originally short, thick-walled tube becomes extended and gradually more convoluted, especially in the later stages.   The formation of the proventriculus at the point of union with the mid-gut has already been described.   The salivary glands which arise from the pharynx are described in the section which follows.

The first sign of the cephalo pharyngeal apparatus appears as a thickening of the dorsal wall of the pharynx between the fourteenth and sixteenth hours of development.   The first figure in which it is indicated is Fig. 20.   Here there has been considerable differentiation and the musculature has begun to attach itself.   The long upper parts of the skeletal bars have become pronounced and chitinization has begun. In later stages these become heavily pigmented.   The development of the cephalo pharyngeal apparatus is indicated rather poorly in the schematic summary of Fig. 23.   The mouth hooks form the most anterior portion of this apparatus.
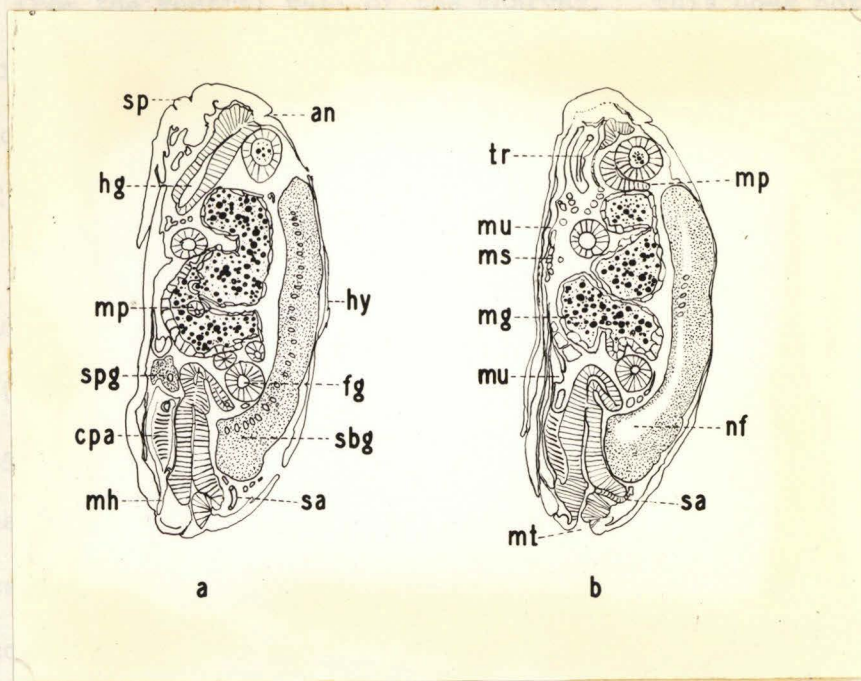
Figure 20.   Adjacent nearly sagittal sections of 16-18-hr. embryo.
              Anterior end is below, ventral side to right. Increas-
              ing convolution of fore-, hind-, and mid-guts. Mal-
              pighian tubes considerably lengthened. The tracheae
              beginning to chitinize. The cephalo-pharyngeal
              apparatus (cpa) and its musculature are beginning to
              differentiate. The mouth hooks are at mh. The duct
              of the salivaries is seen at sa. Differentiation of
              nerve fibers (nf) is marked.

The Salivary Glands

In Drosophila, as in Calliphora, the salivary glands arise by invagination from the ventral wall of the pharynx. This does not occur before the eighth hour of development, for no sign of them can be found in sections of the anterior end (Figs. 13 and 14). They are certainly present at the time that the germ band begins to contract and become prominent in longitudinal sections of embryos of twelve hours or older (Figs. 17 and 19). In these stages they consist of a pair of elongate cell masses (on either side of the anterior end of the ventral nerve cord) uniting anteriorly in a common duct leading to the pharynx.

The difference in structure between the glands proper and the ducts becomes apparent by the fourteenth hour. The cells of the anterior portions become very much smaller and more flattened. In the embryo of Fig. 20 the ducts are clearly differentiated as such.

The time when cell division ceases has not been determined, though it is probably before the eighteenth hour when the glands appear complete in form. Certainly the nuclei of the salivary glands do not assume their remarkable character until the larval stages. Sections of very late embryos were kindly examined by Dr. Hans Bauer, who could find no essential differences between the salivary gland nuclei and other nuclei in those stages.

The Nervous System

The nervous system originates in paired swellings of ectoderm, the neural ridges, which arise along the mid-line of the germ band on either side of what had been the "gastrular groove". These become rather prominent by the fifth hour and are readily seen in living eggs.

Cells of the lateral portions of the nerve cord are proliferated beneath the two ridges while the median cord arises from the base of the groove between, now called the neural groove. The sub-oesophageal ganglion arises directly posterior to the stomodaeal invagination, while the supra-oesophageal ganglion is derived from swellings on the anterior portion of the dorsal side.

The nervous system can easily be made out from transverse sections by the eighth hour (Fig. 13). The neural ridges have become very close together, and the neural groove is little more than a slit between them in the figures shown. In Fig. 14, which consists of photomicrographs of a number of the sections from the previous figure, more details of the early nervous system may be made out. The three parts of the nervous system (two lateral and one median) are rather clearly shown in the upper portion of Fig. 14-e. These lie directly beneath the ectoderm and above the two lateral masses of mesoderm which are connected by a thin bridge. In the center of this figure the fusion of the stomodaeum with the anterior part of the mid-gut rudiment can be seen. In the same series of sections it can be seen that the nervous tissue is more prominent and larger in amount at the points which will become the segmental ganglia.

In longitudinal section the nervous tissue is very difficult to identify until after the contraction of the germ band. In Fig. 16 the representation of the nervous system is rather schematic. The tissue can be made out in the sections, but is not well defined. However, in Fig. 17 and in the succeeding figures, at stages where the nervous tissue has become concentrated, the limits are well defined. The concentration of the nervous system does not stop when contraction

of the germ band ceases, but continues until the time of hatching of the larva when the fused ganglia lie within the limits of the third thoracic and the first abdominal segments. The progress of this concentration is evident in Figs. 18 and 20 and is shown diagrammatically in Fig. 23. In Figs. 18 and 20 the small circles along the median portion of the nervous system indicate the remnant of the median cord referred to above. The ganglion cells lie laterally to this.

Nerve fibers themselves begin to appear between the fourteenth and the sixteenth hours. These extend the length of the cord in two "bundles" on either side of the median cells. They are indicated in Fig. 20 by the unstippled area.

Along with the concentration of the ventral cord the two lobes of the supra-oesophageal ganglion become more closely united with the sub-oesophageal ganglion. Thus the oesophagus becomes completely enclosed in one region by nervous tissue. This can be seen in Fig. 18 and especially in Fig. 19 in which the section is slightly oblique and lateral to the mid-line.

The outgrowth of the nerves was not followed. In embryos of eighteen hours or older they can be found extending to the several segments. Although the fused segmental ganglia lie in the anterior portion of the larva, paired nerves connect the ganglia with the segments from which they were derived.

Fig. 25 is a photomicrograph of a frontal section of the sub-oesophageal ganglion of an embryo twenty to twenty-two hours old. The double structure is evident and the fibers connecting the two ganglia show up rather well. The cells surrounding the central region are very
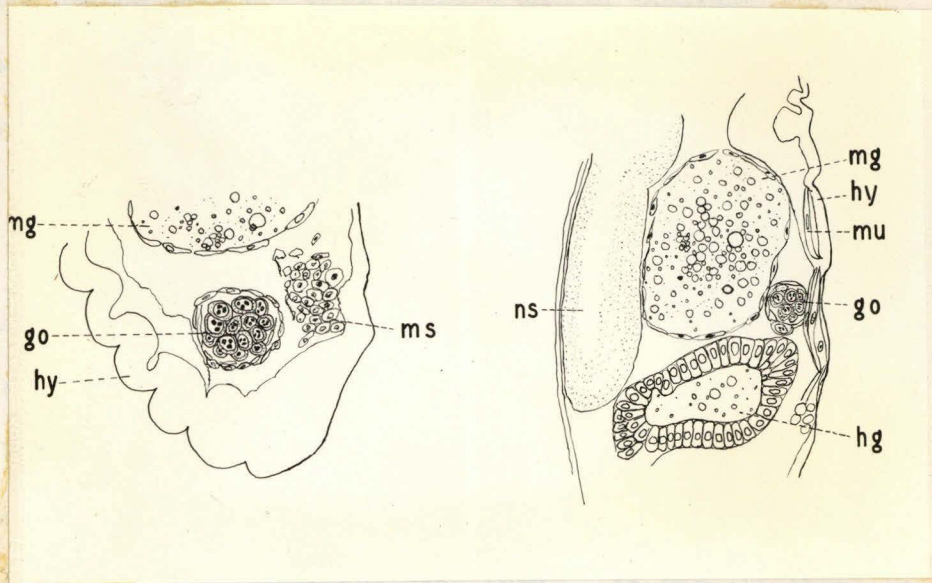
a   b

Figure 21.   a. Drawing of region of 14-16-hr. embryo showing the
                   gonad (go).   The section is somewhat oblique and
                   lateral to the mid-line.   Hypoderm (hy), mesoderm (ms),
                   and a portion of the mid-gut (mg) are shown.   The size
                   of the gonad, i.e., the number of cells, indicates that
                   the embryo is a male.

             b. Drawing of the region showing the gonad of an embryo
                   slightly older than that shown in "a", 16-18 hrs.   The
                   gonad is much smaller than that above, hence the embryo
                   is a female.   The difference is quite marked at this
                   time.   Half of the embryos are found to have large
                   gonads, half small.   The nervous system (ns) is shown,
                   as well as the mid- and hind-guts (mg, hg), muscles (mu),
                   and hypoderm (hy).   Both drawings 440x.

densely packed together and are characteristic of the nerve cord of the later stages. From the time of the contraction of the germ band the cells have become more and more densely packed together.

The larval nervous system and the changes on metamorphosis have been very thoroughly investigated by Hertweck (1931).

The Gonads

The position taken by the germ cells (pole cells) at the point of union of the posterior mid-gut rudiment with the proctodaeum has already been described. While in this position the germ cells are free, though they stay rather closely together, and there is no sign of the envelope which later encloses them. In Fig. 16 they can be seen escaping into the body cavity. By careful study of the sections of this egg it was possible to make out a number of cells which look as though they might soon unite to enclose the germ cells. These appear to be mesodermal cells, as they lie at the inner boundary of that layer. No sections were found which show the germ cells clearly between this time and the later stages (twelfth-hour and on), when they appear as separate gonads lying between the mid-gut and the dorsal wall. At that time they are enclosed in an envelope as shown in Fig. 21. In embryos of these later stages it is possible to determine the sex by the size of the gonads. In half of the embryos observed the gonads were found to be about twice the size of those in the others. This observation extends those of Kerkis (1931) who found a marked sexual difference in the size of the gonads of freshly hatched larvae. The germ cells themselves are of the same size, the difference arising from the number of cells present (see Fig. 21).

a   b   c
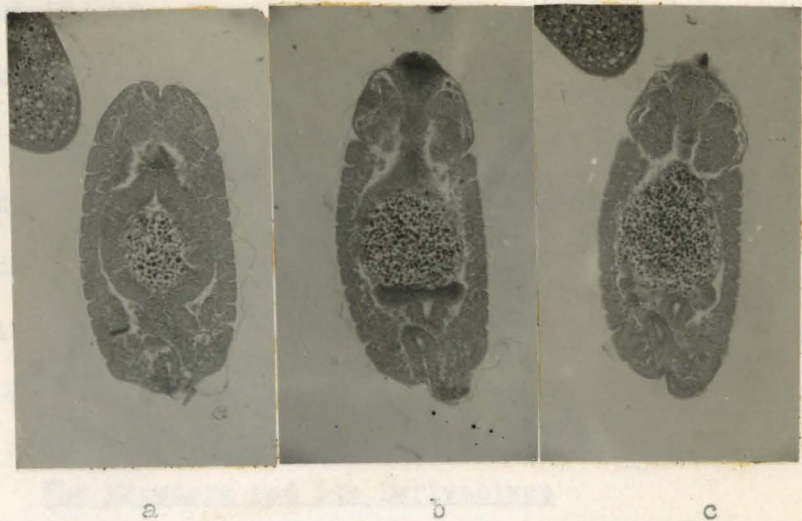
Figure 22.   Photomicrographs of three successive, longitudinal

(horizontal) sections of a 12-hour embryo (150x).

a.   The thickness of the ventral portion of the mid-gut wall

contrasts with the dorsal wall in "c".   Segmentation is

marked - all larval segments are present.   Part of the sub-

oesophageal ganglia is visible anterior to the mid-gut.

b.   Section dorsal to "a" - oesophagus joining the mid-gut;

the rectum and Malpighian tubules just posterior to mid-gut

are clearly shown.   Mesoderm is solid, no sign of coelomic

sacs.

c.   Section dorsal to "b" - mid-gut wall is very thin, con-

volutions of hind-gut visible.   Lateral to the oesophagus

are seen the lower portions of the supra-oesophageal ganglia.

These communicate with the sub-oesophageal ganglia beneath

the fore-gut.

There is no evidence as to just when the separation into two gonads occurs, but it seems reasonable to suppose that it takes place at the time of acquisition of the envelope.

In the later embryonic stages the gonads become embedded in the fat bodies which have formed from mesodermal cells on the dorsal side. The gonads, as Kerkis found, are highly refractive bodies readily distinguishable, at the time of hatching, from the grayish, ir- regular, fat body cells among which they lie.

The Mesoderm and its Derivatives

The origin of the mesoderm has already been described. The mesoderm extends beneath the ectoderm from proctodaeum to stomodaeum, but does not grow laterally until after the contraction of the germ band. Following contraction the mesoderm completes its growth around to the dorsal side. Segmentation of the mesoderm follows closely that of the ectoderm. Upon the dorsal fusion of the mesoderm the larval segments become completed. This occurs by the sixteenth hour.

Mesoderm gives rise to muscles, heart, and fat body. Those cells which are to form muscles become attached to the inner projections of ectoderm which separate the segments. The first indications of this are seen in Fig. 19. Later stages can be seen in some of the following figures, especially in Fig. 21 where muscle fibers are shown in detail. The circular muscles form in between the ectodermal projections to which the longitudinal and oblique muscles attach. Rather large muscles which develop in the anterior region become attached to the cephalo- pharyngeal apparatus.

The heart is formed as an elongate tube at the time of the

fusion of the mesoderm on the dorsal side. The details of the origin were not followed. A portion of the heart can be seen in Fig. 19. Another portion appears in Fig. 20, although it is unlabeled. The heart can be observed beating in larvae ready to hatch. The movement of the "blood" is from anterior to posterior within the tube.

The larval fat body which consists of two parts is formed from mesodermal cells which have reached the dorsal side. In Fig. 20 this is indicated as "ms". Likewise in Fig. 21-a the cells which will form the fat body are simply designated as "ms". In the last few hours before hatching, the gonads are found to be embedded in the fat body.

The Tracheae

The tracheal system is first noted in living eggs shortly after the contraction of the germ band. Two prominent protruberances, or buds, can be seen on the dorsal surface of the last segment just anterior to the anal opening. These are the posterior spiracles. Shortly afterward (within the next hour) the tracheae can be noted extending anteriorly. After the eighteenth hour the tracheae are filled with air and are the most prominent features of the dorsal side.

Likewise, in sections, the tracheae are not to be noted until the contraction of the germ band is nearly complete. They appear to arise by invagination from the surface of the last segment at the points where the spiracles appear. The tracheal tubes extend rather quickly toward the anterior end. In twelve- to fourteen-hour embryos the cuticular lining has not appeared, but by the eighteenth hour, when air begins to fill them, they are completely chitinized, and most

of the branches and cross connections have been formed.   No observations were made on the anterior spiracles.

The Hypoderm

The changes which the hypoderm undergoes through the stages from just after the establishment of the germ band until after its contraction can be followed in Figs. 13, 14, 15, and 17.   At first it is an outer layer of uniform thickness consisting of columnar cells. This becomes modified with the extension of the germ band so that the lateral walls are much thinner.   On contraction of the germ band the ventral wall is thickened and the cells packed more densely.   The lateral walls remain thin until some time after the extension of mesoderm and the completion of the segments on the dorsal side.   The dorsal wall is contributed to chiefly by the embryonic membranes which flatten out with the contraction.   For a time dorsal and lateral walls are exceedingly thin and the underlying mid-gut is clearly visible.

By the eighteenth hour the whole outer integument is of uniform thickness.   The cells become smaller and the thickness of the wall is reduced.   By the twentieth hour the cells have the characteristic appearance of larval hypodermal cells.   After the twentieth hour the rows of setae become visible on each segment.   The outer cuticular layer may be clearly seen in late embryos by the use of high magnification.

Segmentation

The freshly hatched larva of Drosophila melanogaster has eight abdominal segments, three thoracic, and one pre-thoracic or cephalic

secnons. The first foldings of the blastoderm to form what appear to

be anterior segments arising [...] drawn to fol-

lidsme. There is also [...] segments could only

(Fig ?). However, the [...] furrow marks an

important region of the [...] beneath the

intersection of this furrow [...] cular furrow the

anterior mesoderm is form [...] tion of the groove

with the dorsal midline [...] at of the germ

band on the dorsal side d [...] extension. The

groove is most pronounced [...] formation. Dy

[...] ment indistin [...] the stomodaeum.

The ectoderm between all [...]

[...] Section of a later embryo [...] the

im --- fs
cpa ---
sbg --- spg
--- pv
v   d
mp --- tr
--- mg
--- mu
hg ---
--- hy
--- sp

Note on Figure 24. - Invaginations to
form the imaginal discs can be seen in
this figure. fs.- frontal sac from which
the cephalic complex arises; im - two of
the thoracic invaginations which will give
rise to the legs. The latter can be follow-
ed back into the abdominal region.
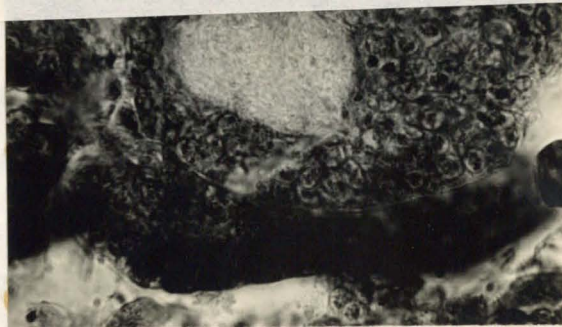
tinctly, leaving [...] tion that

the germ band has begun [...] more marked,

and when contraction of [...] in narrow

views of living eggs. [...] any commoner

then seen clearly as [...] ly led to

the germ band side is nearly complete. In living eggs this extension of

segmentation [...] side can be readily observed. Before the

extension, [...] tion of [...] mesoderm,

the lateral and [...] be seen easily

to made out. After [...] [...] tion of the

segments this is [...]

Figure 25.   Horizontal section through the sub-oesophageal

ganglion of embryo of the same age as above.

Photomicrograph (904x).   Below can be seen one

of the salivary glands.   Anterior is to the

right.
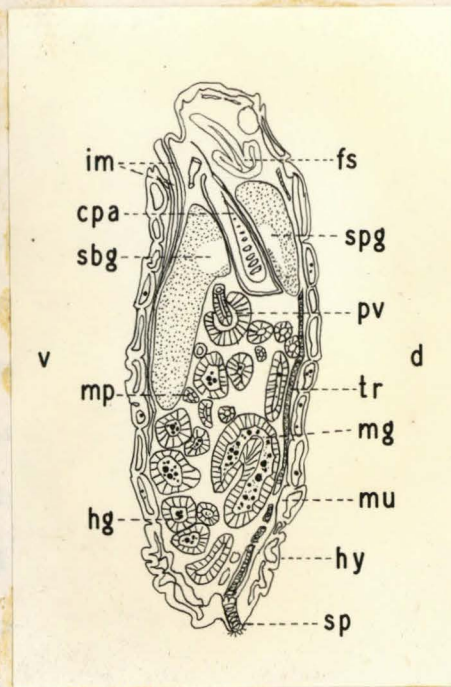
Figure 24.   Section of a late embryo twenty to twenty-two

hours old and ready to hatch.
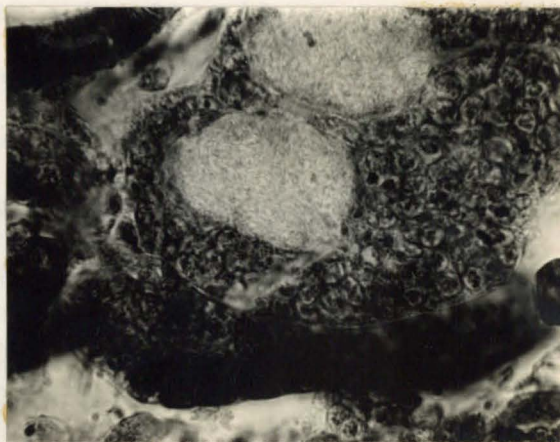
Figure 25.   Horizontal section through the sub-oesophageal

ganglion of embryo of the same age as above.

Photomicrograph (904x).   Below can be seen one

of the salivary glands.   Anterior is to the

right.

segment. The first foldings of the blastoderm to form what appear to be anterior segments are transitory, as Weismann (1863) found in Calliphora. There is also some dorsal folding which disappears completely (Fig. 7). However, the cephalic or anterior cross furrow marks an important region of the embryo. Just anterior to and beneath the intersection of this furrow with the ventral or "gastrular furrow" the anterior mesoderm is formed. The point of intersection of the groove with the dorsal mid-line marks the most anterior extent of the germ band on the dorsal side during the period of maximum extension. The groove is most pronounced at the time of germ layer formation, but becomes somewhat indistinct after the invagination of the stomodaeum. The ectoderm between this furrow and the stomodaeum gives rise to the sub-oesophageal ganglion.

The larval segments appear after the formation of the nervous system. Originally one pair of ganglia are present per segment, but in the later stages the nervous system undergoes a considerable concentration, leaving most segments devoid of ganglia. By the time that the germ band has begun to contract, the segments become more marked, and when contraction is complete, they are much accentuated in surface views of living eggs. Fig. 19 includes sections which show segmentation very clearly on the ventral side. Extension laterally and to the dorsal side is nearly complete. In living eggs this extension of segmentation to the dorsal side may be readily observed. Before the extension, which is accompanied by growth of the underlying mesoderm, the lateral and dorsal portions of the thinly covered yolk mass can easily be made out. With the growth of the mesoderm and the completion of the segments this is obscured.

The mouth parts of the Drosophila larva are degenerate. The rudiments of some of these parts may be seen at different stages (eight to twelve hours), but all are transitory. Because of this fact no careful study was made of them.

All of the larval segments are clearly recognizable both in living eggs and in sections after the shortening of the germ band is completed, and the positions of the internal organs are constant with respect to them. The most anterior segment is at first the longest, but undergoes diminution so that by the time of hatching it is shorter than the others.

Imaginal Discs

The evidence of Geigy (1931) from irradiation of Drosophila eggs indicates that imaginal determination occurs at about the seventh hour of development (beginning of the Zweite Entwicklungsperiode). This is during the period of maximum extension of the germ band. There are, however, no morphological indications of this until very much later.

The first visible evidence of the anlagen of adult organs is the invagination of ectoderm from the pharynx to form the frontal sac which later gives rise to the cephalic complex. This can be noted in the eighteen-hour embryo in Fig. 20 and is indicated in the late embryo of Fig. 24. Thoracic discs have not been noted in the longitudinal sections, and good transverse sections will probably be necessary in order to obtain a clear picture of their formation. See note on Fig. 24.

Late Embryonic Development - Hatching

With the exception of the imaginal discs, the larva is essen-

tially complete by the twentieth hour and moves vigorously within the egg membranes until it escapes, between the twenty-second and twenty-third hours. Most of the development after the fourteenth hour is concerned with differentiation, the later stages of which will require thorough and detailed histological investigations, such as are outside the scope of this work. Now that the course of development and the general topography have been elucidated, such studies should proceed with a minimum of difficulties.
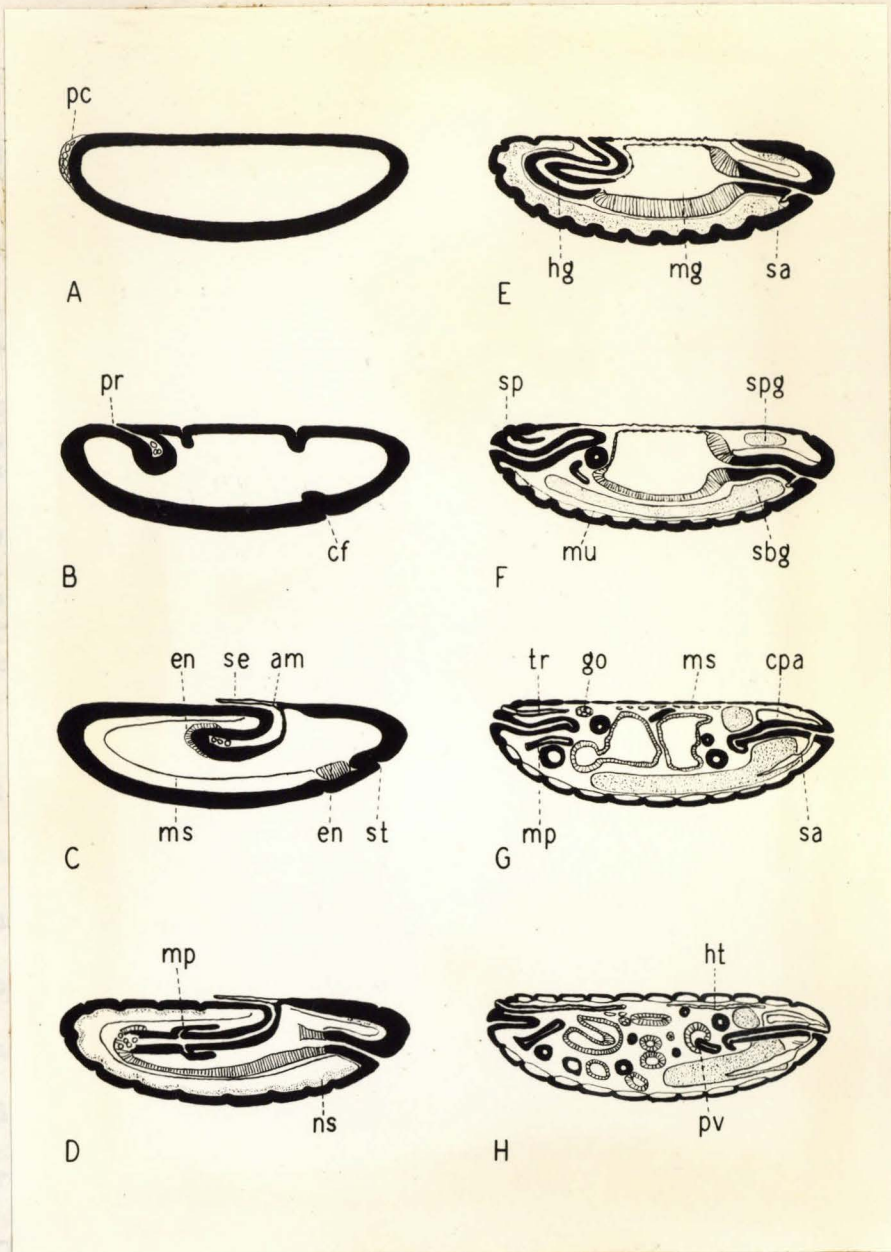
Figure 23.   Schematic summary of the embryology of Drosophila.

(Explanation on following page)

Black indicates blastoderm and organs of ectodermal

origin; the endoderm is cross-lined, the nervous system

stippled, and mesoderm is left unshaded.

A. Second hour = blastoderm and pole cells.

B. Third-fourth hours - proctodaeal invagination.

C. Fifth-sixth hours - extended germ band and germ layers.

D. Eighth-tenth hours - completion of mid-gut, origin of the nervous system.

E. Tenth-twelfth hours - shortening of the germ band.

F. Twelfth-fourteenth hours - embryo on the final position, tracheal invaginations, and beginning of differentiation.

G. Sixteenth-eighteenth hours - mesoderm extended dorsally, increasing convolution of the gut, first movements of gut and muscles.

H. Twentieth-twenty-second hours - larva virtually complete, pulsating gut and heart, tracheae and mouth hooks chitinized, proventriculus and mid-gut caeca present, setae appearing on surface of the hypoderm. Hatching will occur within the next hour.

am	=	amnion	ns	=	nervous system
cf	=	cephalic (anterior cross) fold	pc	=	pole cells
cpa	=	cephalopharyngeal apparatus	pr	=	proctodaeum
en	=	endoderm	pv	=	proventriculus
go	=	gonad	sa	=	salivary gland
hg	=	hind-gut	sbg	=	sub-oesophageal ganglia
ht	=	heart	se	=	serosa
mg	=	mid-gut	sp	=	spiracle
mp	=	Malpighian tubes	spg	=	supra-oesophageal ganglia
ms	=	mesoderm	st	=	stomodaeum
mu	=	muscle	tr	=	trachea

SUMMARY

Time Relations of the Development of Normal Eggs at 22-23° C.

Hours after
fertilization:

1st: Early cleavage, synchronous divisions; migration begins.

2nd: Migration continues, germ cells appear; incipient blastoderm;
completion of blastoderm.

3rd: Growth of blastodermal cells at expense of inner blastema;
formation of cephalic fold.

4th: Invagination of proctodaeum and inclusion of pole cells;
"gastrulation".

5th: Stomodaeal invagination; extension of germ band.

6th: Growth of proctodaeum and appearance of Malpighian tubes;
amnion fold noticeable; neural ridges.

7-8th: Extensive growth of endoderm and the hind-gut; greatest extent
of amnion.

9-10th: Completion of the gut by union of two parts of the mid-gut;
first signs of segments; proliferation of nervous tissue.

11-12th: Contraction of the germ band; rudiments of the tracheal system;
segmentation complete on ventral side.

13-14th: Differentiation begins; concentration of the nervous system
continues; coiling of the gut; extension of mesoderm to dorsal
side; muscles attach; salivary glands present; cephalopharyngeal
apparatus; hypoderm sharply demarcated; gonads lie dorsally.

15-16th: Further concentration and differentiation of nervous system;
increasing convolution of gut; further differentiation of
muscles and mouth parts; heart and fat body appear; beginning
of movements.

17-18th: Continued differentiation in all parts; frontal sac appears;
tracheae chitinized.

18-22nd: Completion of differentiation; active movement up to the time
of hatching.

THE EFFECTS OF CHROMOSOMAL DEFICIENCY UPON EMBRYONIC DEVELOPMENT

Introduction

The role of the chromosomes in developmental processes has become increasingly evident since the first demonstration by Van Beneden (1883) that the diploid chromosome set of the zygote is derived equally from the sperm and from the egg. That the character of embryonic development is determined by the chromosomes has been established, especially by the work on reciprocal crosses among Echinoderms (Baltzer 1909, 1910). From this work and the many later studies upon artificial parthenogenesis it became clear that the diploid chromosome number is essential for wholly normal development. Normally diploid organisms will develop with the haploid number, but usually with great difficulty (Delage 1901; Baltzer 1922). In his ingenious experiments on the production of multipolar mitoses, Boveri (1902, 1907) demonstrated that the chromosomes are qualitatively different and that the developmental process is dependent upon the completeness of the set.

The studies on development and those on heredity, in the years that followed, took somewhat separate lines. Material most favorable for developmental studies (Amphibia, Echinoderms) proved to be rather difficult material for the geneticist, who found insects, in particular Drosophila melanogaster, most suitable. The work of modern genetics has made it ever more apparent that the genes which lie in the chromosomes have intimate control over the developmental processes, and that these genes, which for practical purposes can usually be considered as unit factors, are certainly not restricted in their effect to particular

organs or characters.   Instead they exert manifold effects (Dobzhansky 1927, 1930).

In order to find out what genes actually do in development it is necessary to study more than the differences in end results between a given gene and its allelomorphs.   The information so gotten is valuable and interesting of itself and certainly aids in elucidating some steps between the gene and its manifestation, but it does not tell us what genes really do.   What is the role of the gene in development?   Are there certain genes which are essential for the developmental process, or are genes only determiners of superficial characters?   There are biologists to this day who believe that the latter is true, although there are few geneticists in their company.   Much has been written on the role of genes in development and more probably will be.   In spite of this there are very few facts known.   One of the facts is profoundly significant.

This is the existence of so-called lethal factors.   The first case was found and studied very early in the revival of Mendelism by Cuenot (1905, 1908).   Lethal factors may or may not be genes.   In a great many cases where cytological studies, as well as genetical, have been made, these have been proved to be deficiencies for a greater or lesser number of genes and for greater or lesser portions of chromosomes. The correlation between the genetical and cytological evidence for deficiency and its lethal effect is of considerable import for it provides an approach to the problem of genes and development.   It is possible to follow the development of a lethal embryo and find out what fails to happen in the absence of definite chromosomal regions.   There is nothing new in this method which is the cardinal principle of classical physiology;

the determination of function through extirpation of an organ.

The first case of genetical deficiency was found by Bridges (1916, 1917). This was the Bar deficiency which survived in heterozygous females, but was lethal in the male. A second case, vermilion deficiency, was found also by Bridges (1919). Both were small deficiencies and consequently impossible of demonstration by the then available cytological techniques. In addition it was known from Bridges' work that complete deficiency for the X-chromosome is lethal (1916), as well as complete deficiency for the fourth chromosome (1921). Some of the best known cases of deficiency are the Notches which behave as dominants and are lethal in the male. Notch-8 was first described by Mohr (1919) who later made more exhaustive studies paying special attention to the exaggeration effects of the genes lying opposite the deficiency (1923). Li (1927) determined the stages of development at which the lethal effects appear in these and in other chromosome aberrations. He found that the homozygous deficiencies always die in the egg stage and that heterozygous large deficiencies are lethal in later development, e.g., larval and pupal stages. Any analysis of the latter is difficult because of the complexity of the situation. However, he did not attempt to analyze the lethal effects on the basis of developmental processes.

Since the introduction of the X-ray method for the production of chromosome aberrations (Muller 1927) the number of deficiencies available in Drosophila melanogaster has become almost unlimited. It was decided, therefore, to investigate in terms of developmental processes cases in which the lethal effects appear in the egg stage, especially in very early development. The X-chromosome was chosen as

the logical chromosome for such an analysis, for there it is possible
to obtain deficiencies ranging in size from the whole length of the
chromosome to a few bands of its "salivary" length, all of which are
lethal in the egg stage.   The deficiencies used were nullo-X (no X-
chromosome), half-X (both left and right halves), Notch-8, and De-
ficiency scute-8.

Nullo-X: Deficiency for the X-Chromosome

Irregularities of meiosis may give rise to unusual distributions
of chromosomes.   In the first case of non-disjunction (primary) of the
X-chromosome Bridges (1916) obtained four classes of offspring: normal
females (XX), females which give secondary non-disjunction (XXY), normal
males (XY), and sterile males (XO).   On the basis of Bridges' analysis
of the case two other classes should appear: individuals with three
X-chromosomes (XXX) and individuals with no X- and one Y-chromosome (YO).
Later the XXX individuals were found to survive occasionally and were
called super-females.   The other class never appeared.   In case of
secondary non-disjunction two classes were found to be absent: super-
females and those with no X- and with two Y-chromosomes (YY).   In the
case of complete non-disjunction, attached-X females, found by L. V.
Morgan (1922) the female offspring always obtain their X-chromosomes
from the mother and the males from the father.   When the attached-X
female also carries a Y-chromosome (X$\widehat{X}$Y) the YY class always fails to
appear.   Li (1927) found that eggs with two Y-chromosomes (nullo-X)
fail to hatch and that no recognizable embryo is formed.

In the study of such eggs two different stocks of attached-X
females were used, one homozygous for forked (f$\widehat{f}$ Y), the other homozygous

for yellow-2, vermilion, forked, and carnation (y^2v f cr Y) both
carrying Y-chromosomes. No differences between the two strains were
noted either in the normal or in the "deficient" eggs. Eggs of known
age were obtained and prepared for study as described in the section
on materials and methods.

In both cases one-fourth of the eggs failed to hatch and pre-
liminary examination of the living eggs showed that no blastoderm had
formed in these eggs. Freshly laid eggs were collected, dechorionated,
and observed at intervals for four hours under the microscope. During
the first hour all of the eggs appeared alike externally and no distinc-
tion between normal and deficient eggs could be made. In the second
hour, when the nuclei began to arrive at the surface and the pole cells
began to cut off, the difference became clear. During this time few
or no nuclei could be seen at the surface of the nullo-X eggs and when
they could be made out they were usually somewhat irregularly distributed
in the mid and anterior regions of the egg. Throughout the next hour
(when germ cells become included in the proctodaeum of a normal embryo)
the number of nuclei increased a good deal, although there was no more
regularity in their position. Only a few were scattered posteriorly
and many lay in the anterior part of the egg, but most of them remained
in the region in which the first cleavage occurred. The appearance of
the nuclei was very different from that of the normal, especially with
respect to size. In the normal egg the nuclei are of uniform size
and as the blastoderm begins to form these produce a very regular pattern
over the surface of the egg. No such thing was ever observed in the
deficient eggs. Pole cells were never observed to form in living
nullo-X eggs, although in sections made of an egg six hours old such

Figure 26.   Sagittal section of a nullo-X egg four to six

hours old.   Photomicrograph (150x).   Anterior

end is to the right and dorsal side is up.   The

mass of nuclei is seen to form an anterior cap

and extend posteriorly along the dorsal side.

Complete separation between yolk and protoplasm

has not occurred.   The yolk spheres are very

darkly stained, the nuclei very little stained.

Below is a transverse section of a normal egg

in which the two parts of the germ band, the

proctodaeum, and yolk spheres can be seen.

were once found.   This case will be described at another point.

All development that occurs after the early cleavages is abnormal.   Separation of yolk spheres and protoplasm occurs after the second hour.   In older eggs the clear yolk spheres usually fill the posterior end of the egg while the central region contains most of the protoplasm, now somewhat vacuolated.   By means of the relatively clear posterior region, it is possible to pick out nullo-X eggs four hours or older under the low power binocular after dechorionating.

Sections of these eggs demonstrate clearly the positions of the nuclei relative to the other egg contents, and provide much more information about the nuclei than can be gained from the living eggs. First of all it is evident that division of the nuclei may continue for some hours.   It is also clear that not all of the nuclei behave in the same fashion and that they may be considered in two groups: those which remain in the anterior portion of the egg and those which are included in the coalesced mass of protoplasm which occupies the center of the egg.

In the earliest stage at which sections of the nullo-X eggs can be distinguished from the normal, the time of migration to form the blastoderm, the nuclei are found to lie anterior to the center of the egg.   At this time the nuclei appear little different than in normal eggs, except that a few are larger than the others and apparently have failed to divide.   Very few sections of these early stages were obtained and none was really good.   As a result, much of the evidence for the behavior of nullo-X eggs comes from eggs four hours or more in age.   From that time on it is easier to pick out the eggs, and many more were obtained for sectioning.   In general the chief difficulty

encountered in this work was in getting good sections of the early eggs. The early stages of normal eggs and all stages of the deficient eggs gave considerable trouble in sectioning because of shattering. The deficient eggs in which the nuclei and yolk spheres were no longer held together by the protoplasm were most troublesome in this respect. In order to get a clear picture of what was going on it was necessary to section at least twenty at a time. This proved laborious, to say the least.

In eggs four to six hours old the nuclei are largely packed in the anterior end with some protoplasm lying between them, but no such things as separate cells can be said to exist in any case. The nuclei are mostly of the same size, but scattered here and there are larger nuclei containing much more chromatin. There may be ten or twenty such polyploid nuclei in a section which contains two hundred smaller nuclei. Although no counts of chromosomes could be made, the number of chromosome ends which appear in the large nuclei may be two or more times the number in other nuclei. With advancing age the nuclei become smaller and smaller and many appear to disintegrate.

The section shown in Fig. 26 is of an egg five to six hours old. The nuclei are seen to lie chiefly in the anterior part of the egg. On the dorsal side the nuclei extend from anterior along to the posterior end. The nuclei are embedded in a certain amount of protoplasm, but have definitely not formed cells. The few nuclei which lie in the region of the polar granules have formed cell bodies about themselves and have all the appearances of pole cells, except that they are much smaller than normal pole cells. This is the only case in which pole cells have been found in nullo-X eggs. In this case they have been

formed at a much later time than usual, and then probably only by chance. Although in the older eggs nuclei are sometimes found to extend some distance along either dorsal or ventral sides (forming a large cap over the yolk and protoplasm) this is the only case out of nearly a hundred eggs observed in which they reached the region of the polar granules.

In the same figure the separation between the yolk and the protoplasm has not become so marked. Nuclei can be found in the protoplasm. These nuclei are very much larger than those found in the anterior region, but appear to have only the normal amount of chromatin in most cases. About the same proportion of them are polyploid as among the anterior nuclei. Some of the nuclei have begun to disintegrate. On counting the nuclei in this section there were found to be approximately 250 in the anterior mass and its extension along the dorsal side, 17 in the protoplasm, and at least a dozen more which had disintegrated. In the whole egg there were more than 2000 nuclei present. This is about half as many as normally present in eggs of this age. In eggs eight hours old the anterior end is densely packed with small nuclei which, however, are by no means uniform in size. Scattered more or less at random among the small nuclei are other larger nuclei, some of which appear to be tetraploid, while others may even be octoploid. The chromatin in all of the nuclei is always very vesicular and usually clumped so that it is impossible to make any chromosome counts. The size and the relative amounts of chromatin are the only factors on which a judgment may be based. It is evident from this that division of the nuclei into two frequently ceases before the division of chromatin ceases. Some protoplasm remains around these nuclei and the whole mass remains as a syncytium. The mid-region of such eggs contains most of the proto-

plasm fills the polar plasmre along the posterior portion of the egg.

The anterior end of ... ... dorsal or

ventral side; usually ... ... ly to the

center of the egg. ... ... mass of

protoplasm. These ... ... the nuclei of

the anterior region ... ... of normal

eggs, but after one ... ... the blasto-

derm and must have und... ... ... be seen

nuclei of the anterior ... ... concts

not-like as in resting ... ... nuclear

membrane has frequently ... ... more or less

scattered in the proto... ... ... nuclei are

found to have disinte... ... ... ct is shown

in Fig. 27.

Figure 27. Longitudinal (horizontal) section of a nullo-X
            egg six to eight hours old. Photomicrograph
            (352x). The anterior mass of nuclei is above.
            Nuclei of unequal sizes may be found. The
            largest are the polyploids described in the text.
            In the central region is the vacuolated mass of
            protoplasm in which only a few nuclei are to be
            found. At the posterior end are the yolk
            spheres.

plasm while the yolk spheres occupy the posterior portion of the egg. The anterior mass of nuclei may extend slightly down the dorsal or ventral side; rarely do any of these nuclei lie posteriorly to the center of the egg. Nuclei are always found in the central mass of protoplasm. These are very different in appearance from the nuclei of the anterior region. They resemble very closely the nuclei of normal eggs just after the final division before the formation of the blastoderm and must have undergone very few divisions as compared to the nuclei of the anterior region. In most cases the chromatin appears net-like as in resting nuclei. In these older stages the nuclear membrane has frequently broken down and the contents lie more or less scattered in the protoplasm. The older the eggs the more nuclei are found to have disintegrated. Such an egg as that described is shown in Fig. 27.

Nullo-X Eggs: Summary

In eggs wholly deficient for the X-chromosome division of the nuclei proceeds and may continue for as long as eight to ten hours. After the early divisions, development becomes abnormal, for the nuclei fail to migrate to the surface. As a result no blastoderm is formed. The protoplasm begins to coalesce and withdraws from the periphery of the egg, where in normal eggs it forms a thick layer as the blastoderm is completed. A separation between yolk spheres and protoplasm ensues and becomes marked between the fourth and sixth hours of development. The nuclei remain in the region of the first cleavage spindle and continue division. By the fourth hour division begins to be disrupted as evidenced by the presence of polyploid nuclei. The anterior nuclei

form a cap over the separate masses of yolk and protoplasm which lie posteriorly to them. A number of nuclei are found in the coalesced mass of protoplasm. These always appear to have undergone many fewer divisions than the anterior nuclei, but are incapable of forming cell bodies. Many of them begin to disintegrate by the fourth hour.

The formation of cells, their growth, and all subsequent morphogenesis fail in nullo-X eggs.

Deficiency for Half of the X-Chromosome

For the study of the effects of the removal of half of the X-chromosome a translocation in which that chromosome had been broken near its genetic middle was used. In order to get eggs of the desired types males bearing the translocation T 1,4-CRB were crossed to attached-X females of the stocks described in the section on materials and methods. The two stocks gave identical results. The CRB translocation was first described by Muller and Stone (1930) and subsequently in more detail by Stone (1934) and by Painter (1934) who determined its nature by the "salivary method". In this translocation one-half of the X-chromosome (to the right of lozenge and including Bar) has been intercalated between the fourth chromosome and its spindle fiber, while the other half of the X including lozenge carries the X-spindle fiber. This translocation was used by Dobzhansky and Schultz (1934) to obtain duplications for half of the X-chromosome in their studies on the distribution of the sex factors.

The expected types from such a cross are shown in Fig. 28. For simplicity only the X- and Y-chromosomes have been indicated. Of the twelve types five (41.7%) die in the egg stage. One egg count

	X^r Y	X^l Y	X^r	X^l	X^r X^l	Y
$\widehat{XX}$	X^r Y $\widehat{XX}$ 1	X^l Y $\widehat{XX}$ 2	X^r $\widehat{XX}$ 3	X^l $\widehat{XX}$ 4	X^r X^l $\widehat{XX}$ 5	Y $\widehat{XX}$ 6
Y	X^r Y Y 7	X^l Y Y 8	X^r Y 9	X^l Y 10	X^r X^l Y 11	Y Y 12

Figure 28. Zygotes from the mating of $\widehat{XX}$Y female by T 1,4-CRB male. Eggs to the left, sperm above. All of the individuals in the upper row survive the egg stage. All but one of the individuals in the lower row die in the egg stage.

1.- 4.	Duplication females
5.	Super female
6.	Normal $\widehat{XX}$Y female
7 and 9.	Deficiency for X^l
8 and 10.	Deficiency for X^r
11.	Translocation male
12.	Nullo-X

showed 67 unhatched eggs out of 155 (or 43.2%), a value very close to the expected. A few unfertilized eggs found among the sectioned material probably account for any differences.

Of the five types one is nullo-X (YY). Such eggs can readily be identified as described above. There remain (if the extra Y be neglected) two types of eggs: those deficient for the left half of the X- and those deficient for the right half. At the time that the blastoderm is normally formed, these are readily distinguishable (both in sections and in living eggs) from the normal and the nullo-X eggs. No distinction can be made between the two deficiencies themselves.

The nuclei do migrate to the surface so that for a time a single layer of nuclei can be found on the surface of the egg. Pole cells are usually formed, but do not become sharply separate from the other nuclei. On reaching the surface the nuclei continue dividing and do not undergo the period of growth characteristic of normal blastoderm nuclei. Nor do they become elongate. Together with a portion of the blastema they form a syncytial mass. In living eggs the regular pattern which begins to appear on the surface as the nuclei reach it becomes very irregular, and forms a striking contrast to that of the normal blastoderm. The difference in surface appearance between these eggs and the nullo-X is also marked. Thus the most notable feature of the half-X eggs is that no blastoderm becomes established. In consequence of this all further development is abnormal. The nuclei continue to divide and lie four or five deep over the central contents of the egg, the yolk and protoplasm. The marked separation between yolk and protoplasm found in nullo-X eggs does not occur in these. Fig. 29 shows in detail the nature of this layer of nuclei and its relation to the yolk
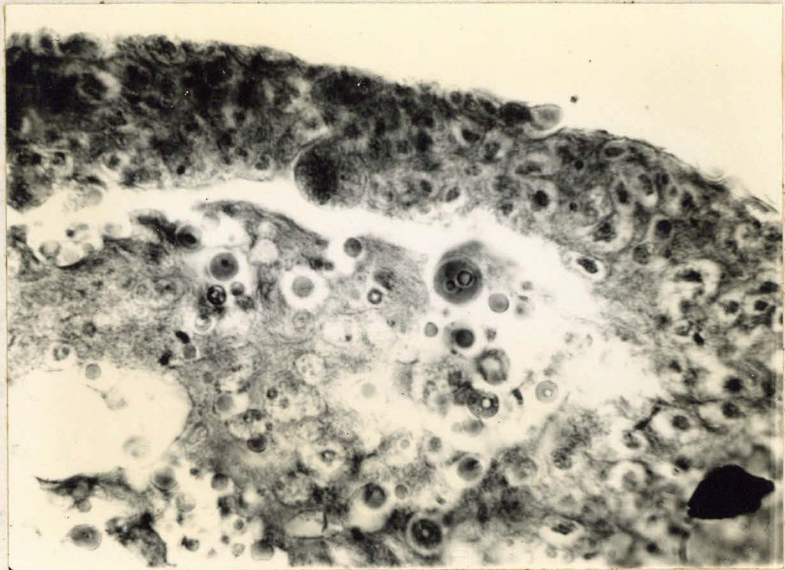
Figure 29. Detail of half-X egg (four to five hours old),
from sagittal section showing nuclei on dorsal
side. Photomicrograph (904x).

The nuclei and blastema form a syncytium; nuclei
are of nearly uniform size. Yolk and protoplasm
in central region.

and protoplasm. The nuclei are seen to be embedded in the peripheral blastema which, however, is not cut up by cell limits of any kind. (Compare with Fig. 4.)

Fig. 30 is a longitudinal section, slightly oblique, of an egg in the sixth hour of development. The layer of nuclei is seen to be thicker on the ventral than on the dorsal side, one portion of which is only a few nuclei thick. At the ends of the egg many more nuclei are concentrated, especially at the anterior end. The nuclei in this egg, as in all of the half-X eggs, are remarkably uniform in size and appearance. Occasionally at the anterior end a few larger nuclei are to be noted, but they are exceptional. The distribution of the nuclei in such eggs is remarkably constant. The great mass of the nuclei lie in those regions which the germ band normally fills at the time of its maximum extent (six to ten hours). This is admirably illustrated in Fig. 30.

Division of the nuclei may go on until after the sixteenth hour of development, but it is rather slow. Eggs of this age to not contain many more nuclei than the eleven-hour egg shown in Fig. 30. No signs of nuclear disintegration are to be noted up to the sixteenth hour. Later stages were not studied.

In the sections of older eggs (twelve to sixteen hours) a few of the deficient eggs show signs of what might be called a furrow or constriction around the anterior portion, very nearly in the region where in normal eggs the cephalic furrow appears. Beyond this there is nothing to indicate that two types of deficient eggs are present. So far it has been impossible to demonstrate which is which, or whether this difference is significant. A scheme for doing so was devised, but proved to be
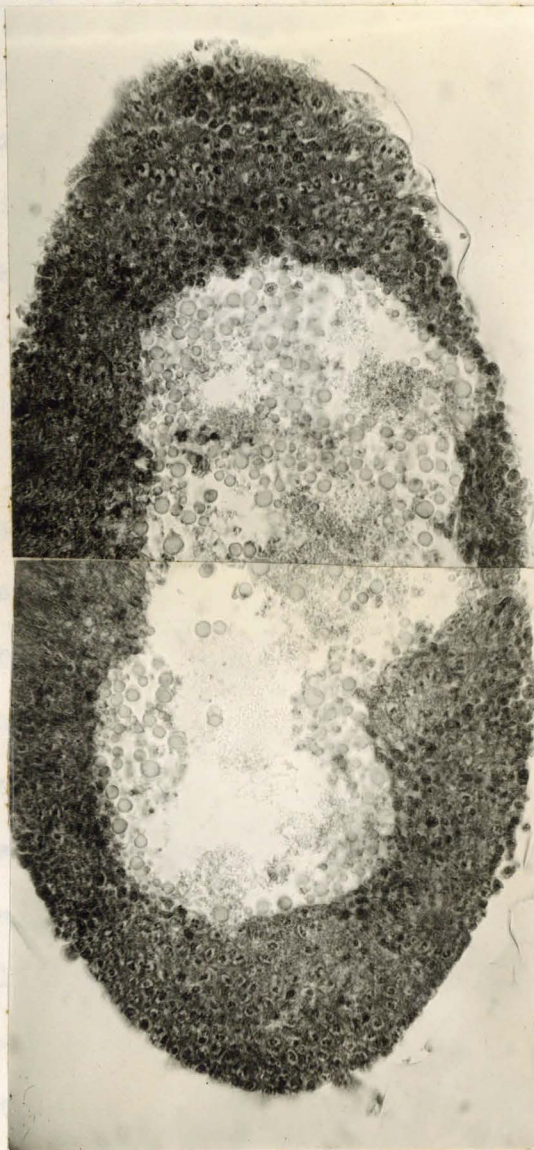
Figure 30.   Nearly sagittal section of half-X egg eleven

hours old.   Photomicrograph (600x).   Anterior

end above, ventral side to left, dorsal to right.

The nuclei are uniform in size and are distributed

throughout the region normally occupied by the

germ band.   Yolk and protoplasm in center.

impractical. Attached-X females bearing duplications for each of the two halves of the X-chromosome respectively were crossed to wild type males. Among the eggs of each there would exist only one kind of deficiency (aside from nullo-X). Unfortunately such duplication bearing females are extremely unfertile and it proved impossible to obtain enough eggs for sectioning, to say nothing of the numbers required for observation of living eggs.

Half-X Eggs: Summary

Deficiencies for one-half or the other of the X-chromosome produce nearly identical effects upon the development of the egg. Nuclei migrate to the surface, germ cells are sometimes formed, but the blastoderm is never completed. All subsequent development is abnormal. Instead, the nuclei continue to divide and form a layer around the central yolk. The protoplasm does not coalesce and the nuclei remain embedded in the peripheral blastema, forming a syncytial mass. No cells are formed and only infrequently are pole cells found. The nuclei continue dividing until the eleventh hour and are distributed chiefly throughout the region which the germ band normally occupies during the time of its maximum extent. The nuclei are uniform in size and in appearance. In the latest stages studied (sixteen hours) no sign of nuclear disintegration can be found.

Notch-8 Deficiency

Notch-8 is a deficiency extending from just to the left of the white locus (1.5) to, but not including, the locus of echinus (5.5) in the X-chromosome. Mohr (1923) was unable to demonstrate the de-

ficiency cytologically in the metaphase X-chromosome. Recently it has been demonstrated "salivarily" by Mackensen (1935) who found it to include the white bands and those lying to the right of white, but not including the echinus bands, i.e., the bands between 1C and 2C on Bridges' (1935) map are missing.

One-fourth of the offspring of Notch-8 females fail to appear. These should be Notch-8 males. Li (1927) found that the lethal effect occurred in the egg stage after some development had taken place (just how much was not investigated).

Observations made on living eggs show that the pole cells and the blastoderm form normally and that no apparent irregularities occur in the early stages. Hence the normal and deficient eggs are indistinguishable until somewhat later. The most marked irregularity which is noted in living eggs occurs in the middle stage (11-12 hours) when the germ band normally contracts and segmentation becomes pronounced. Only the faintest signs of segmentation appear in the Notch eggs and contraction never occurs. Beyond this the observations on living eggs give little information. Accordingly, sections were prepared of eggs of this age and older. From them the story of what happens is clear.

The most striking feature of such eggs is that they contain no mesoderm or endoderm. Separation of the germ layers has failed to occur with the consequence that only ectodermal parts are formed at all. The proctodaeal and stomodaeal invaginations occur, the ventral blastoderm elongates without the formation of underlying layers, and the embryo looks superficially normal. The yolk is never enclosed, for the mid-gut fails to form. The hind-gut and its diverticula, the Malpighian tubes, never attain their normal position. The ectoderm

proliferates in the regions where the nervous system normally forms, but there is no differentiation of ectoderm into hypoderm. A number of such embryos have been examined.

Fig. 31 is a sagittal section of a Notch-8 embryo fourteen to sixteen hours old. The extension of the ventral blastoderm is clearly visible and is comparable to that of a normal embryo before contraction of the germ band. For comparison with the normal, reference should be made to Fig. 16. The fore-gut is in the normal position at the anterior end and has become thickened in the region where the cephalopharyngeal apparatus normally arises. It is, however, highly abnormal and the lumen cannot readily be followed. Dorsal and ventral to it are the supra- and sub-oesophageal ganglia which are in a more or less extended state. Continuous with the sub-oesophageal ganglion is the tissue of the ventral portion of the nervous system, which extends just around to the dorsal side at the posterior end of the egg. In this mass of cells there are more or less clear and fibrous looking areas which have much the appearance of the nerve fiber areas of the normal nervous system. In extent the nervous tissue corresponds to that of the normal egg in which contraction is just being completed. There is no demarcation between the nervous tissue and the outer ecto-derm. Thus there is no hypoderm.

The hind-gut and the Malpighian tubes form a rather complex and tangled mass in the mid-region of the section. To follow the parts through was found extremely difficult. There are tubes of two sizes, the smaller of which are Malpighian tubes and look very much like those in normal embryos of twelve to fourteen hours. The hind-gut lies against the posterior portion of the central yolk mass. The

yolk, however, is completely free and no sign of any mid-gut wall can be made out in this or other sections. Some of the yolk is scattered between the hind-gut and the posterior portion of the nervous tissue. The hind-gut forms a very much larger proportion of the embryo than in normal eggs, filling nearly half of the interior. It is not inconceivable that some endoderm is present, especially that of the posterior portion, for even in normal embryos this portion is difficult to identify until the time of fusion of the two parts to form the mid-gut. On the other hand it is impossible to find any cells or tissue in the Notch embryos which can be interpreted as mesoderm. If any is formed at all it must be very small in amount.

In only one case have the germ cells been tentatively identified in the confused mass of the hind-gut. They appear to have acquired no sheath. This is not surprising, for the identification of the germ cells in normal embryos, up to the time of the contraction of the germ band, is very difficult.

There is no sign of formation of the tracheal system which is ordinarily laid down at the close of contraction. No trace of the embryonic membranes can be found.

Development, abnormal though it is, continues for some time after this and death and disintegration of cells does not occur before twenty hours, the age of the oldest embryos examined.

To obtain all the details of the Notch case it would be necessary to make an extended and thorough histological investigation of each of the stages from "gastrulation" to the time of contraction of the germ band.

Although attachment to the egg layers may fail that much is normal. Later development of the embryo layers is hindered by the absence of mesoderm ... embryos at the time when the gut is normally ... and tissues which are formed (although the ... ll) are all of ectodermal origin. The ... of ectoderm into hypoderm and the ... organs which ... next differentiation and ... the ... hind-gut.
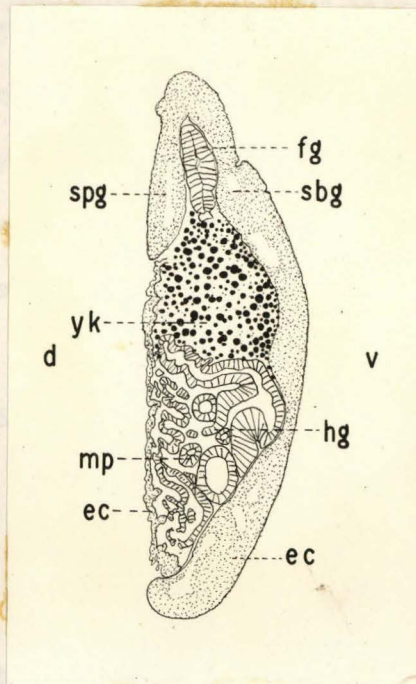
Figure 31. Notch-8 deficiency embryo fourteen to sixteen
            hours old.   Mesoderm is absent.   Fore- and
            hind-guts are present, but abnormal.   The
            nervous system extends along the ventral side.
            There is no hypoderm.   Yolk is not enclosed.

A number of other ... closed, but not studied in detail.   One in particular, deficiency-notch-8, which is a very ... and ... of the integument ... versions.   It ... the ... described by ... Sturtevant (19...) ... There ...

Eggs bearing this deficiency (which should be ... a deficiency males) die in the late embryonic stages after the dams head has penetrated but ... ... is complete.   The trachea begin to form but never ... filled with air.   Apparently they are never ... ... does not proceed much beyond this stage. ... ... is complete and the hypoderm takes its ... the ... ...   Further details are not available.   The eye ...

Notch-8 Eggs: Summary

Although development in the early stages up to four hours is normal, N$_o$tch-8 embryos fail to form the germ layers as evidenced by the absence of mesoderm and endoderm from the embryos at the time when the gut is normally completed. The organs and tissues which are formed (although they may become highly abnormal) are all of ectodermal origin. There is no differentiation of ectoderm into hypoderm and the embryo is without skin. Those organs which undergo most differentiation and development are the nervous system and the hind-gut.

Other Deficiencies

A number of other deficiencies were examined, but not studied in detail. One in particular, deficiency scute-8, which is a very small deficiency for the loci of yellow and achaete near the left end of the X-chromosome is associated with one of the longest known inversions. It was found by Sturtevant (unpublished) and has been described by Noujdin (1935). It was used extensively by Beadle and Sturtevant (1935) in their inversion crossover studies.

Eggs bearing this deficiency (which should be scute-8 deficiency males) die in the late embryonic stages after the germ band has contracted and segmentation is complete. The tracheae begin to form, but never become filled with air. Apparently they are never chitinized. Development does not proceed much beyond this stage. The gut appears to be complete and the Malpighian tubes lie in the normal position. Further details are not available. The few

sections made of such eggs proved useless for study.

However, these eggs develop normally through the early stages of morphogenesis. Failure of development occurs during the subsequent period of differentiation.

The stock of this deficiency is kept balanced with a lethal gene in the opposite chromosome, scute-3D. This lethal is probably not a deficiency for males carrying scute-3D are perfectly viable, but the homozygous females die. The stage of the lethal action was not known. When egg counts were being made and eggs obtained for the examination of the scute-8 deficiency, it was found that not one-quarter of the eggs, but one-half of them failed to hatch. Out of 724 eggs 332, or 46%, failed to hatch. Examination of the unhatched eggs showed two types: apparently complete and active larvae attempting to escape the egg membranes and embryos in which the tracheae were incompletely developed. Most of these larvae never hatch and die within the membranes. The few which do hatch do not survive. These probably die in early larval life. A number were released from the membranes and supplied with adequate food. None developed into mature larvae.

It was originally proposed to investigate the case of nullo-IV, in addition to the X-chromosome deficiencies. So much difficulty was encountered in getting sufficient eggs from haplo-IV females, and the percentage of early abnormalities in the eggs obtained was so high that this project had to be abandoned for the time. There was considerable difficulty in keeping the stock, which later was found to be contaminated by a lethal.

Discussion

Homozygous deficiencies differ from other types of chromosome aberrations, e.g., heterozygous deficiencies of duplications, in that they represent qualitative as well as quantitative changes in the gene complex. Whereas in the case of quantitative changes many of the effects can be accounted for on the basis of slowing up of processes, this is not necessarily so in the case of homozygous deficiencies. Genes are wholly removed and when the time comes at which their action or coöperation (or those of their products) is essential, development becomes abnormal. Practically all homozygous deficiencies die. All those studied by Li (1927) were found to die in the egg stage. The survival of small patches of deficient tissue appears to be dependent upon which genes are missing. The behavior of small deficiencies has been studied by means of somatic chromosome elimination by Demerec (1934) and Ephrussi (1934). The former found that of twenty-four small deficiencies affecting eleven regions of the X-chromosome, only four affecting one region produced visible patches of deficient tissue. From this Demerec concludes that most deficiencies are "cell lethal". By this rather unfortunate and misleading term, in which there is no implication of the time or stage at which the cells die, he means presumably that the cells carrying the deficiency fail to survive long enough to become differentiated into visibly distinguishable imaginal tissue. No investigations have been made into the number of cells required to form a visible patch of tissue or into the histological structure of the hypoderm in the regions of such mosaic spots. The deficiencies studied by Ephrussi were found not to be lethal and to form

clearly recognizable patches of tissue. One of them was the scute-8 deficiency. Notch-8 was found by Demerec to be "cell lethal". The present work shows that embryonic cells of Notch-8 are certainly not inviable, nor are the nuclei of half-X eggs.

One of the exceptions to the rule that homozygous individuals die is the viable deficiency found by Muller and Prokofyeva (1935). This showed violent phenotypic effects "produced by absence of just two specific genes (those for "yellow" and "achaete") lying in the region in question". Other deficiencies in and about this region proved lethal. A very probable explanation for such a viable deficiency is to be found in the "repeat" regions which Bridges (1935) has demonstrated in the salivary chromosomes. Loss of a repeat would involve only a quantitative change in the genes and in the case of small regions would probably not prove lethal.

In plants deficiencies are uncommon, as they seldom survive in the gametophyte generation. Stadler (1933) who studied many X ray induced variations in maize found most of them to be eliminated in this way. One deficiency was found to be transmitted through the female gametophyte, but not through the male. In this case viability and fertility were markedly reduced, although sufficient genes were present to allow for the survival of the haploid generation.

From the results obtained with the nullo-X eggs it is evident that the presence of at least some of the genes in the X-chromosome are essential for the normal distribution of the nuclei following the early divisions, for the formation of cells, and for the establishment of the blastoderm. That the genes which are required for cell formation and the production of the blastoderm lie in more than one region of the X-

chromosome is clear from the behavior of the half-X eggs. The genes of either half alone are insufficient for the proper completion of this stage of development. On the other hand the regions included in Notch-8 and in scute-8 deficiency are probably not essential for these particular processes, but become indispenable in the later stages.

In speaking of the action of genes it need not be supposed that the individual gene is concerned with only one process or that it is active (in the production of substances) at only one time. It is entirely possible that genes are active all the time. In this event one would expect that in an egg like that of Drosophila there would remain in the deficient eggs some of the substances produced by the genes before the genotype of the future embryo was determined by the reduction division and fertilization. Substances might even continue to be produced for some time by the polar body nuclei. However, when these substances reached too low a concentration or were used up entirely, development would become abnormal.

Division is not interfered with; even the nullo-X nuclei go on dividing for some time before degenerative changes set in. Such interference would scarcely be expected for it has been rather clearly demonstrated that cleavage of eggs will proceed to some extent in the absence of chromosomes. Long ago McClendon (1907) described division of this kind in the egg of a parasitic copepod, Laemargus. More recently Dalcq (1927, 1929, 1931) has carried out investigations on the relations between chromosomes and cleavage in the frog's egg and has found cleavage in the absence of the chromosomes. Cleavage of enucleated fragments of Arbacia eggs treated with parthenogenetic agents has been obtained by E. B. Harvey (1935).

The presence of polyploid nuclei in the nullo-X eggs indicates

that the chromosomes may retain their power of reproduction even after the nucleus as a whole has lost the ability to divide. Similar polyploid cysts have been found in the testes of A-B hybrids of Drosophila pseudoobscura by Dobzhansky (1934).

The case of Notch-8 is probably the most interesting of those studied for it represents the loss of a considerably smaller number of genes than the nullo-X and half-X. In addition the nature of the failure is clear cut. Genes whose action is essential for the normal separation of the germ layers are absent; as a result of this, further normal development is impossible. The other abnormalities, e.g., the failure of differentiation of the ectoderm into hypoderm and the extensive proliferation of the hind-gut, indicate that genes whose participation is required for numerous other processes are missing as well, or that the substances normally produced by the missing genes are necessary for numerous other reactions.

In the mouse there are several cases of lethals which look superficially similar to the Notch-8 case in that the embryos die in an incomplete stage of development. Homozygous brachyurie embryos die on the tenth day of development (Chesley 1932). In these there is no sign of somites and the posterior limb buds fail to appear. Numerous other abnormalities are present including deformation of the neural tube. Ephrussi (1934) found that cells and even tissues of such embryos could be cultivated successfully in vitro. Whether or not this lethal factor is a deficiency has not been demonstrated. The one case proved both genetically (Gates 1930) and cytologically (Painter 1930) to be a deficiency has not been studied developmentally. In another case which is probably a deficiency (Snell 1933) the abnormal

development of the heterozygotes has been followed (Snell, Bodemann, and Hollander 1934). The neural tube usually fails to close and becomes distended.

The present work is the only one thus far in which the effects of homozygous deficiencies upon embryonic development have been followed in detail. Here it has not been possible to determine what the individual gene does. For that it will be necessary to carry out the same kind of investigations using single gene deficiencies or small overlapping ones.

Conclusions

1. The presence of genes lying in more than one region of the X-chromosome is essential for normal distribution of nuclei to the surface of the Drosophila egg and for the subsequent formation of cells and blastoderm.

2. In the absence of the genes lying in either half of the X-chromosome nuclei are capable of normal division and continue to live for more than twenty hours. Such nuclei are incapable of forming cells.

3. The failure to form a normal embryo in the above cases results primarily from the failure to form cells and blastoderm.

4. Genes lying within the region included in the Notch-8 deficiency are essential for the separation of the germ layers and for the proper differentiation of the ectodermal structures which appear.

5. Genes included in the scute-8 deficiency are essential for differentiation during the late embryonic stages, especially of the tracheae.

6.   The genes included in the latter two deficiencies are not essential for the establishment of the blastoderm.

SUMMARY

1.   An account has been given of the normal embryology of the fly, Drosophila melanogaster, from the time of the fertilization of the egg to the time of hatching of the larva.   This account has been correlated with previous knowledge of the embryology of the Diptera.

2.   The effects upon embryonic development of complete deficiency for the X-chromosome and smaller portions thereof have been presented in detail.

3.   In the absence of the X-chromosome normal distribution of the nuclei to the surface, the formation of cells, and the establishment of the blastoderm fail.   Nuclear division continues for some hours in the absence of the X-chromosome.

4.   Deficiency for either half of the X-chromosome does not interfere with the distribution of nuclei to the surface, but does prevent the formation of cells and consequently a normal blastoderm.   Nuclei of these eggs remain alive and continue dividing at a time when morphogenesis is complete in the normal embryo.

5.   In the absence of the genes removed by the deficiency known as Notch-8, the pole cells and blastoderm form normally, but later development becomes highly abnormal because of the failure of the separation of the germ layers.   Organs of ectodermal origin appear and begin to differentiate. Much of the differentiation is abnormal.   The hypoderm fails to appear.

Abbreviations

am	amnion		mt	mouth
an	anus		mu	muscle
cf	cephalic furrow (anterior cross furrow)		nf	nerve fibers
			ns	nervous system
cpa	cephalopharyngeal apparatus		pc	pole cells
d	dorsal		pm	pharyngeal musculature
ec	ectoderm		pr	proctodaeum
en	endoderm		pv	proventriculus
fg	fore-gut		sa	salivary gland
gc	germ cells		sbg	sub-oesophageal ganglion
go	gonad		se	serosa
hg	hind-gut		sp	spiracle
ht	heart		spg	supra-oesophageal ganglion
hy	hypoderm		st	stomodaeum
mg	mid-gut		tr	trachea
mh	mouth hooks		v	ventral
mp	Malpighian tubes		yk	yolk
ms	mesoderm			

In all of the figures except Fig. 23 the whole gut tract is indicated conventionally with cross lines. Nervous tissue is stippled.

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