SOME PROBLEMS ON PHYSIOLOGICAL GENETICS IN DROSOPHILA:

EFFECTS OF GENETIC LETHAL FACTORS ON EMBRYONIC DEVELOPMENT

by

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Introduction

The special studies of animal embryology and animal genetics grew up independently, using different organisms and with little reference to each other. Embryology was concerned with elucidating the developmental mechanisms by which an apparently simple egg became transformed into a complex organism, and omitted the equally important problem of how they came to be repeated uniformly in each generation. Genetics, on the other hand, following the lead of Mendel, was establishing the basic rules of inheritance by concentrating upon characters which were clear-cut, and which showed much inherited diversity, such as the form and colour of the skin and its derivatives.

As a result, it was commonly charged against Mendelism that it applied only to "superficial characteristics", and it was suggested that the genes played no part in fundamental developmental processes. More-over, there was much confusion between "genes" and "characters", so that it was frequently assumed that the relation

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between the two was both necessary and simple. The difference between this outlook and the one which has replaced it has been well put by Sewall Wright: "Most people seem to be born preformationists. They tend to take for granted a separate heredity for each part of the adult organism. Even physiologists sometimes attribute a character partly to physiological factors, partly to heredity, as if heredity could operate by some sort of sympathetic magic, independently of physiological channels. The attitude of physiological genetics is that characters are determined 100 per cent by physiological processes, but that genes are the ultimate internal physiological agents." (Sewall Wright, 1945).

The term "physiological genetics" was used by Goldschmidt (1938) as the title of a book in which he stated, for the first time, some of the fundamental concepts of the subject. The most important of these were: 1) that genes act by the production or regulation of enzymes, 2) that their effects are frequently on the velocities of reactions, which bring about qualitative differences by their relations to various threshold situations. Further stages in the development of the subject were marked by the publications of Waddington (1940a), and Sewall Wright (1945), and it is

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now established as one of the fields in which biological research is most active, uniting embryological and genetical work.

Genetical studies are made by comparing organisms which differ with respect to certain chromosomal factors, which may be point mutations of particular genes, chromosomal rearrangements of various kinds, or deletions of parts of chromosomes. Usually, one organism is a "normal" with respect to a particular factor, and the other is a "mutant". If the mutant factor affects a fundamental developmental process, its effect is usually so drastic that the organism dies before reaching the adult stage; consequently, most genetical work has been done with the "superficial" mutants mentioned earlier. For physiological genetics on the other hand, the "lethals" provide excellent material, and developmental studies on such mutants have been made in all groups of animals in which genetical techniques exist for maintaining lethal stocks. These include the fowl (reviewed Waddington, 1952), the mouse (reviewed Gluecksohn-Waelsch, 1951), and Drosophila (reviewed Hadorn, 1951); a general review has been done by Gluecksohn-Waelsch (1953).

Drosophila has particular advantages for such work:

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1) Much research is done into the induction of mutations in Drosophila by various mutagenic agents; almost all of this work is on lethal mutants, which are therefore available in large numbers for developmental studies.
2) There is an enormous background of genetical knowledge relating to Drosophila, into which the findings of physiological genetics must be integrated.
3) There is a correspondingly large array of genetical techniques, which may be drawn upon for the testing and extension of these findings.

Against these advantages on the genetical side must be placed serious disadvantages on the physiological. All physiological studies in insects lag behind those on vertebrates, and this is particularly the case with their embryology. At the descriptive level it is extremely complex, and no unifying principle has been found underlying the great diversity of developmental patterns in the different orders (reviewed Johannsen and Butt, 1941). And experimental investigations have been hampered by the recalcitrance of insect eggs to the standard techniques of causal embryology (reviewed Richards and Miller, 1937), so that the basic developmental mechanisms remain obscure. In both these respects, the Diptera is the most difficult of all the insect orders.

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For this reason, comparatively little work has been done on the Drosophila lethal mutants. A number of workers have surveyed series of them, noting the stages at which death occurred, but not doing developmental studies, in order to find the relative numbers of embryonic, larval and pupal lethals (Medvedev, 1939; Hadorn and Chen, 1952; Oster, 1952 and 1954; Rizki, 1952). Several studies have been made upon larval and pupal lethals (reviewed Hadorn, 1951). Li (1927) found that deficiencies of the whole of the X-chromosome or IVth chromosome caused death in the egg stage, but did no developmental studies; he also found that Minute-II and Plexate deficiencies allowed complete embryonic development, but that the larvae did not hatch. Similar non-hatching larvae were formed in the case of Star (Sivertzev-Dobzhansky, 1927), the X-chromosome deficiency 260-2 (Kaliss, 1939), the IIIrd chromosome deficiency Lyra (Counce, 1950), and scute deficiency (Poulson, 1940). Other cases were found in which the larvae occasionally emerged, but died in the 1st instar; the ClB homozygote was of this type (Brehme, 1937).

Most dominant lethals induced by X-irradiation of sperm were found to act in the embryonic stage (Demerec and Fano, 1941), and a cytological study by Sonnenblick (1940)

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showed that this was due to early cytological abnormalities which affected the cleavage nuclei and prevented further development. Early cessation of development was found also in the case of deficiencies of the whole, or either of the halves, of the X-chromosome (Poulson, 1940), and the female sterile mutant deep-orange (Counce, 1953).

The most interesting mutants from the developmental point of view are those which permit extensive organization and cellular differentiation, but disturb it in a particular way. The best accounts of such lethals published so far have come from Poulson (1940 and 1945), on the "Notch"(facet) and white series of deficiencies. Gloor (1950) did a study of the Krüppel mutant. Farnsworth (1951) and Bull (1952) studied a Minute-IV deficiency and three vestigial deficiencies respectively, but have not published detailed accounts of their observations. Counce (1953) has done intensive studies on the female sterile mutants fused and rudimentary.

In the case of such lethals, where there is a concatenation of abnormalities in the arrangement and development of many structures, it is probable that in each case they might be traced to a single primary

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abnormality. Such a programme has been largely successfully carried out in some organisms, particularly in certain studies on rats and mice by Grüneberg (reviewed Grüneberg, 1948). But in the case of the embryonic lethals of Drosophila it must be admitted that attempts to furnish similar explanations have had a rather hollow sound. This is undoubtedly due to the general absence of information concerning its normal developmental physiology. However, the study of the abnormalities in itself contributes to the normal embryology in two ways: 1) by requiring and making a basis of comparison for a more careful enquiry into normal processes; 2) by furnishing a means of interfering with development analogous to the classical techniques of causal embryology.

The work of Seidel on Platycnemis (reviewed Seidel, 1936), which has thrown much light on insect embryogenesis, still gives little help when dipteran embryos are in question. Experimental work on these has been retarded by technical difficulties caused by the small size of the egg and its unusual development. Rather crude techniques have had to be resorted to, including cauterization (Reith, 1925; Strasburger, 1934; Howland and Robertson, 1934), centrifugation (Pauli, 1927; Howland, 1941) and ligaturing (Pauli, 1927; Rostand, 1927), using variously Drosophila,

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and Calliphora. Comparatively little has emerged from these experiments except that embryos are completely mosaic from a very early stage, with almost no capacity for regulation. Rather more interesting results have recently been obtained by using ultra-sonics (Selman and Counce, 1953; Counce, 1953), which are referred to later (page 190).

The disturbances induced by lethal factors may be regarded as experimental tools of much greater refinement than any of the above, and much is to be hoped from their use as such; but their usefulness is impaired by ignorance as to their nature. Consequently this sort of investigation has to work from both ends, seeking to discover the original gene action by observing its effects on development, and elucidating its developmental mechanisms by observing the effectsmof the factors upon them. Such work is bound to consist largely of painstaking observations, relieved by a number of guesses at interpretations. When a sufficient number of lethals has been surveyed, general principles regarding dipteran embryology may emerge, and refined experimental techniques may be available for testing them. It is as a contribution to such a survey that the following study is intended.

Materials and Methods

Mutant stocks:

The mutants are all sex-linked recessive lethal factors which have arisen in the mutation research of Dr C. Auerbach and co-workers. They are maintained by Dr H. Slizynska for cytological study, but only preliminary investigations have been made on the mutants used in the present work, so that it is not yet possible to say whether they are point mutations, chromosomal rearrangements, or deficiencies.

They are maintained in Müller-5 stocks, in which the X-chromosome carrying the lethal factor is balanced against another X-chromosome containing a double inversion, so that crossing over is almost completely suppressed. This chromosome usually carries a recessive marker, apricot, and a dominant one, Bar, but in all the stocks used here except Lff 11 and Lff 16, Bar was replaced by the recessive cut. The stocks are made up in each generation by selecting heterozygous females, and mating them to the males, which are always hemizygous for the Müller-5 chromosome ($\frac{\text{M-5}}{\text{lethal}}$ female X $\frac{\text{M-5}}{---}$ male). The progeny consists of ¼ homozygous Müller-5 females, ¼ hetero-

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heterozygous females, ¼ hemizygous Müller-5 males, and ¼ hemizygous lethal males (¼ $\frac{M-5}{M-5}$ females; ¼ $\frac{M-5}{lethal}$ females; ¼ $\frac{M-5}{---}$ males; ¼ $\frac{lethal}{------}$ males). The hemizygous lethal male class is the only one in which the lethal factor is not masked by its normal allele; consequently, only one quarter of the eggs produced are expected to show the lethal abnormality in their development. The normal embryos provide material for control studies on normal development. The stocks are kept at 18°C, and the embryos for study are reared at 25°C.

Egg collection:

Females from which eggs were to be collected were aged for three days after emergence, and given a good supply of yeast, in order to obtain the maximum number of eggs. They were mated to an excess of males, in order to reduce the number of unfertilized eggs. The collections were made by putting the females, usually about 50, in a cream jar which was inverted over an "agar lid". The latter consists of a watch glass containing a gelled agar solution (100ccs. water, 3ccs. alcohol-acetic acid, 3grms. agar); more eggs were laid when the smooth surface of the agar was scraped off. For developmental studies the lids were changed every hour; the first hour's collection was always discarded, on account

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of it's consisting largely of eggs which had been held within the female, and whose developmental age would therefore be unknown. The succeeding collections would consist of newly fertilized eggs, whose developmental age could be reckoned from the time of laying.

Hatchability tests:

Preliminary tests for embryonic lethality were made by counting the numbers of hatched eggs (the empty chorion is easily seen) and unhatched eggs, either 30 or 48 hours after collection. After 30 hours, unfertilized eggs and some unhatched larvae are included among the unhatched eggs; after 48 hours all the normal larvae are hatched, and abnormal eggs in which any cellular material has formed become brown, making them distinguishable from unfertilized eggs, which remain white.

Observations on living embryos:

The opaque chorion may be removed by scratching it gently with a tungsten wire needle, and the embryo observed through the transparent vitelline membrane. The egg is placed in tap-water in a depression slide, and in most cases development goes on to completion. The main external features of late embryos may be seen fairly clearly, but the early events are difficult to

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follow by direct observation. On the other hand, time-lapse cinéphotography gives a clear picture of developmental events at all stages. A film of normal development, "The Embryonic Development of Drosophila melanogaster", has been made in connection with the present work in collaboration with SJJCounce, with photography by E.C.A. Lucey. The majority of the sequences in this film were of embryos from the lethal stocks Lff 11 and X2, since sequences of these mutants were also required, and it was impossible to tell whether any particular egg set up for photographing was of a normal or a mutant embryo until development was under way.

Sectioned material:

When observations on the late mutant embryos showed marked structural abnormalities, their developmental history was studied by means of large series of serial sections, made at all necessary stages.

Eggs were fixed, dehydrated and embedded according to Smith's method for eggs which require softening (Darlington and La Cour, 1947). The fixative consists of formaldehyde (6), alcohol (16), acetic acid (1), plus water (30). Penetration of the vitelline membrane is slow, so that each egg has to be pricked with a tungsten

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needle when it is put into the fixative. In dehydrating, the eggs are taken through mixtures of ethyl and butyl alcohols up to pure butyl alcohol with 4% phenol. The eggs were embedded about six to a block, orientating them by reference to the chorionic filaments. Sections were cut at 5 microns, and stained with Heidenhain's iron haematoxylin, counterstained with eosin, following the method given by Pantin (Pantin, 1946).

Preliminary Survey of Lethal Stocks

The stocks investigated were of three sorts: 1) mutants induced by feeding larvae formaldehyde in the food; 2) mutants which arose spontaneously; 3) mutants induced by X-irradiation of male parents. These gave the following proportions of embryonic lethals: formaldehyde - 2 out of 25 examined, plus one egg/larval boundary lethal; spontaneous - 2 out of 16; X-ray - 8 out of 29, plus 3 egg/larval boundary lethals; total - 12 out of 70, plus 4 egg/larval boundar lethals.

This ratio of embryonic to larval and pupal lethals is similar to that found by other workers in Drosophila melanogaster (Medvedev, 1939; Hadorn and Chen, 1952; Oster, 1952 and 1954), and in Drosophila willistoni (Rizki, 1952).

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Six of the embryonic lethals thus discovered (1 formaldehyde; 1 spontaneous; 4 X-ray) were found to complete their embryonic development without any apparent structural abnormality, but remained unhatched, presumably because of some physiological defect; these were not investigated further. The other six showed marked developmental abnormalities, and are described in detail below.

An Outline of normal Development

In the accounts of the mutants which follow the development of each is described by comparing it stage by stage with that of the normal embryo. Details of the latter, including original observations made from serial sections or from the film "The ebbryonic development of Drosophila melanogaster" are inserted as they are required in the elucidation of the mutant types. In the present section an outline of the complete normal embryogenesis is given, following the schematic division of the process which it has been found convenient to adopt in describing the mutants. In general the account follows that of Sonnenblick and Poulson in their two chapters of "The Biology of Drosophila" (Sonnenblick, 1950; Poulson, 1950).

The description is illustrated by a series of photographs and diagrams (pages 32 - 51) including:

1. Diagrams of five developmental stages constructed from serial sections (figs. 7-19).
2. Series of camera lucida drawings made from the film (figs. 9-14).
3. Corresponding series of still photographs from the film (figs. 1-8).

In the case of each drawing and photograph made

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from the film a time given in hours and minutes indicates the stage of development. These times are accurate with respect to the intervals between successive frames of the film, but not to the beginning of development. This is due to the editing and cutting having been done before the analysis reported here was undertaken; consequently, an approximate estimate of the time which had elapsed between the beginning of each sequence and the start of development was made, and the intervals were added to that.

In addition, the times of events in the film do not always correspond with the times given in the description, usually lagging behind them, especially in the later stages. There are two reasons for this. Firstly, it was not possible to control the temperature during filming; usually it fluctuated between 21 and 23 degrees centigrade. The times given in the description are for embryos at 25 degrees, at which development is more rapid. Secondly, the conditions under which the embryos were filmed were abnormal, and probably deleterious; several did not complete their development. So it is most likely that the particular conditions decreased the speed of development in some cases, especially towards its conclusion. The times given in Poulson's

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account have been accepted here as standard, and deviations from them in the illustrations may be taken as accounted for by these considerations.

The unfertilized egg and fertilization:

The structure of the egg and the earliest events in development have been long established (Huettner, 1923, 1924, 1927). The egg is 400 to 500 microns in length, and is about three times as long as wide. It is boat-shaped, being more convex ventrally than dorsally and more pointed anteriorly than posteriorly. There is a thin vitelline membrane, and a loose covering membrane secreted by the follicle cells of the ovary, the chorion. Internally the cytoplasm is only clear at the periphery, where it is called the Keimhaut-blastem. The inner part of the egg is occupied chiefly by the yolk, in the form of distinct granules which stain heavily with haematoxylin. There are many cytoplasmic vacuoles, one surrounding each yolk granule, and others which are empty. At the posterior end is an area of cytoplasm containing fine darkly staining granules, called the polar plasm. There is a space at either end between the egg material and the vitelline membrane, which disappears when the blastoderm is formed. At the anterior tip is

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an extension of the vitelline membrane bearing the opening, the micropyle, through which the sperm enter at fertilization. The egg is in the uterus when this occurs, and it is usually deposited immediately afterwards (Nonidez, 1920). Between five and eight sperm enter, one of which forms the male pronucleus. The female pronucleus lies in a clear area of cytoplasm about one third of the way back from the micropyle. The details of the meiotic divisions are irrelevant for the present purpose; the pronuclei fuse, and cleavage begins at this point.

Cleavage, the pole cells, and blastoderm formation:

(Figs. 1,2,3,4,9).

An early study of cleavage was mde by Huettner (Huettner, 1933), but problems concerned with it have still not been settled. The chief of these is that of the fate of the cleavage nuclei, which may become variously blastoderm nuclei, pole cell nuclei, or yolk nuclei. Parks looked for a regular pattern of cleavage, but concluded that the fate of any individual nucleus was a matter of chance (Parks, 1936).

The main events of this period are as follows.

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The dividing cleavage nuclei migrate towards the surface of the egg. At 1½ hours between three and seven nuclei are extruded at the posterior end, each one surrounded by a portion of the polar plasm. These are the pole cells, which by division give a cap of cells which remains outside of the blastoderm at the posterior end of the embryo (fig. 2,1.41, 2.18). Most of the other nuclei reach the surface at 2 hours, at the ninth cleavage, where they are evenly distributed to form a syncytial blastoderm. The Keimhautblastem deepens and the yolk is concentrated in the middle of the egg. The mitotic divisions are extraordinarily rapid, succeeding each other at ten minute intervals. The early cleavages, including the first three at the surface, occur synchronously, but when cell boundaries appear in the blastoderm they become irregular. This happens at 2½ hours, when furrows extend inwards from the surface between the nuclei, which are completely isolated by the formation of a basal boundary slightly later.

Some nuclei remain behind in small cytoplasmic islands in the yolk, forming the yolk nuclei (Rabinowitz, 1941b). Rabinowitz noted that some of the yolk nuclei are formed by the migration of some of the pole cells back into the yolk. He believes tht they degenerate later, but this is disputed by Poulson, who believes them to

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form the primitive mid-gut (Poulson, 1950).

Further details of these events will be given when the mutant S9 is discussed (page 225). The essential feature of the embryo at 3 hours is the presence of a blastoderm of deep columnar cells, surrounding a central yolk reserve, with a cap of pole cells external to it at the posterior end.

Gastrulation:

(Figs. 5,9,10, 11, 15).

Gastrulation occurs in three stages which follow each other rapidly.

1. At 3.30 hours the ventral furrow is formed. This is an invagination along the mid-ventral line of the cells which give rise to the mesoderm. It remains open only for a short time, and ~~forms~~ the mesoderm cells thus cut off, with their underlying ectoderm, form the germ band.
2. At 3.45 hours the cephalic furrow appears as an oblique groove on each side anteriorly. It is discussed in detail under mutant Lff 11 (page 93).
3. Almost immediately afterwards the germ band begins its extension dorsally and anteriorly, carrying with it the pole cells. These are carried in a pocket, the posterior mid-gut rudiment, which

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represents the posterior endoderm material. When it has reached a point just behind the cephalic furrow it becomes invaginated into the interior of the embryo. As it deepens, ectodermal material is also invaginated, forming a proctodaeal invagination which is continuous with that of the posterior mid-gut rudiment. This is now a backwardly directed sac, containing the pole cells.

At about the same time, the anterior endoderm is formed by the invagination of a group of cells antero-ventrally. The ectoderm soon closes over them, and they form a mass of cells, the anterior mid-gut rudiment, which is carried into the interior of the embryo at the tip of another invagination which arises immediately in front of them, the ectodermal stomodaeum.

The extension of the germ band, marking the end of this period, is completed at 5 hours. The three germ layers have now been separated as follows:

1. Ectoderm: This consists of the external layer of cells, plus the proctodaeal and stomodaeal invaginations.
2. Mesoderm: This forms a layer of loose rounded cells underlying the ectoderm of the germ band.
3. Endoderm: This exists as the two rudiments of the

/mid-gut

mid-gut, the anterior one at the tip of the stomodaeal invagination, the posterior one continuous with the proctodaeal invagination.

Differentiation between 5 and 9 hours:

During this period, in which there are no mass movements ofthe embryo, the organ systems are "roughed out" by the following developments in the germ layers.

1. Ectoderm: Between 4 and 7 hours certain of the ectodermal cells of the germ band round off and move internally to become neuroblasts, which produce the nervous tissue. Details of this process are given when mutant X20 is considered (page 178). The cells which remain externally give rise to the hypoderm. At about 7 hours a series of small invaginations appears along the germ band, the tracheal pits. The openings are transitory, and the material which is invaginated forms groups of cells which later unite along each side to produce the tracheal system. At about the same time a group of cells on each side in the ventro-lateral region thickens and invaginates to give the rudiments of the salivary glands. At eight hours the hypoderm is distinctly segmented. The proctodaeal and stomodaeal invaginations continue growing by cell division.

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2. Mesoderm: The segmental arrangement of the mesoderm becomes apparent at about 6 hours; in the next two hours a separation occurs between the somatic mesoderm, which gives rise chiefly to the somatic musculature, and the splanchnic mesoderm, which becomes attached to the rudiments of the gut which have already formed, where it gives rise to the visceral musculature of the gut wall.

3. Endoderm: At 5 hours the yolk is enclosed in a thin layer of cells, the primitive gut, which has probably been formed by the migration to its surface of the yolk cells, which there unite with the thin layer of cytoplasm surrounding it (Poulson, 1950).

The two rudiments of the mid-gut press against the primitive gut and, by rapid proliferation of their cells, each becomes Y-shaped, with two arms growing out around and under the yolk. The arms of the anterior and posterior rudiments meet on each side, temporarily enclosing an aperture through which the primitive gut extends backwards and forwards. These extensions can be seen in the film to be drawn into the main body of the yolk, possibly by contraction of the walls of the primitive gut. As they are drawn in the mid-gut wall becomes completed ventrally.

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During this period the pole cells emerge from the posterior mid-gut rudiment, but opinion is divided as to how this happens and as to what becomes of them. This matter will be taken up when mutant X27 is discussed (page 153).

Shortening, involution of the head, and dorsal closure:

(Figs. 6,7,8,12,14,16,17,18).

At 9 hours a period of mass movements of the embryonic material begins. The movements may be classified, for the purpose of description, as those of shortening of the germ band, those of the involution of the head, and those of dorsal closure. But they occur to some extent simultaneously, and are integrated aspects of a general rearrangement ofthe embryonicmaterial. Shortening is completed in an hour, at the end of which the proctodaeal opening is at the posterior end of the embryo, and the yolk is covered dorsally only by the thin embryonic membrane. This is replaced by the extension of the lateral body walls over the dorsal side, where the segments of each side meet and unite medially. This movement is dorsal closure. At the same time, the ectoderm of the head region begins to move into the embryo, following up the previous invagination of the stomodaeum. This is the beginning of head involution,

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which continues up to 15 hours. Towards the end of the process the thoracic segments move forwards and completely enclose the head.

Organ formation:

(Figs. 6, 8, 13, 19).

From 10 hours the development of the various organ systems may be considered separately.

The gut:

During dorsal closure, the extension of the lateral body walls over the dorsal side is accompanied by a corresponding enclosure of the yolk by the completion of the wall of the mid-gut dorsally. This is complete at 12 hours as a simple sac, rather conical in shape, with its broad end anteriorly. The gut is now present as a tube running through the whole body, and its regions may be considered separately.

1. The fore-gut: The first part of the fore-gut is the pharynx, which is formed from the ectodermal material invaginated during the involution of the head. The salivary gland material on each side becomes differentiated into duct and gland cells at 11 hours, and at 12 hours the glands

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become filled with a substance which stains intensely with haematoxylin; the ducts fuse distally, and the common opening is carried into the pharynx in involution. Above the pharynx a double series of large muscles forms the pharyngeal pump, which is roofed by the floor of frontal sac. This is a deep invagination, also formed in the process of head involution. Further details of this process are given in the section on Lff 11 (page 74). The stomodaeum gives rise to the oesophagus, a narrow tube connecting the pharynx to the mid-gut. Cuticle is laid down throughout the fore-gut from 13 hours. At the sides of the pharynx a heavily cuticularized cephalopharyngeal apparatus is formed, and just inside the mouth opening there are two mouth hooks and a median tooth of cuticle.

2. <u>The mid-gut</u>: The oesophagus protrudes into the first part of the mid-gut, the proventriculus, of which it forms the valve. Immediately behind the proventriculus four mid-gut caecae are formed, two extending forwards on each side. The proventriculus appears first at 12 hours as a bulge of the mid-gut sac, at its junction with the stomodaeum. At 13 hours a constriction appears in the middle region dividing it into two sections,

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and shortly afterwards another constriction is formed around each of these, so producing a series of four compressed chambers. No cell division occurs after 12 hours, but changes in cell shape bring about an increase in length of the mid-gut. The chambers buckle against each other, and eventually lose their compressed shape, becoming first globular, then tubular, so that eventually a tortuous arrangement of coiled gut fills the abdomen. The posterior part is the first to become tubular; the anterior part is more capacious. While the lumen of the gut decreases, the amount of yolk becomes steadily diminished, until at the end of embryonic development it has nearly all gone. Malpigian tubules appear early, b before shortening, at the junction of the mid-gut and the proctodaeum, as four rudiments. These give rise to four elongate tubules which meander through the posterior part of the abdominal cavity.

3. The hind-gut: After the change brought about in its position by the shortening of the germ band relatively little change is undergone by the proctodaeum in becoming the hind-gut. It remains as a nearly straight tube, arched over the coils of the mid-gut, to which it is united at a point

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about mid-way along the body. From 13 hours it becomes lined by cuticle.

All parts of the gut, with the exception of the pharynx which has its own musculature, become invested with a thin layer of visceral muscle.

The body wall:

This consists of the walls of the thoracic and abdominal segments, which are essentially similar in structure. The thoracic segments are more sharply defined than the abdominal ones, each overlapping the one in front. There is a single layer of hypoderm cells, which begins to secrete a cuticle at 13 hours. The intersegmental hypoderm and cuticle forms the apodemes, to which the muscles become anchored. The muscles are spindle shaped, each one formed by the fusion of several myoblast cells derived from the somatic mesoderm. Myofibrils are visible in them between 10 and 11 hours. The ventral anterior muscles which effect the burrowing and feeding movements of the larva are particularly large. Between the muscles and the hypoderm in each segment are a number of small unattached cells. Muscular activity begins between 13 and 14 hours.

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The nervous system:

From 9 hours the neuroblast cells and their derivatives become increasingly compacted together, to form a distinct nervous system, consisting of a brain and a ventral nerve cord, connected by circum-oesophageal commissures. From 10 hours it is completely differentiated into ganglion cells and fibres. In the ventral cord the fibres are arranged in two longitudinal strips, with a cross connection in each segment. At the end of shortening the ventral nervous system is a distinct mass of tissue extending the whole length of the embryo. At 14 hours it begins to condense; its anterior end and the brain move towards the middle of the body, coming to lie in the last thoracic and the first abdominal segments, while at the same time the posterior end moves forwards. At the end of development the nervous system is extremely compact.

The tracheae:

The cell groups invaginated as the tracheal rudiments in each segment unite along each side of the embryo at about the time of shortening. The tracheae become visible at about 14 hours, when the cuticular lining is laid down. There are two main 1 longitudinal trunks, with an anterior and a posterior

/segment.

segment. At about 18 hours they become filled with gas, secreted internally; this is well shown in the film.

The gonads:

It is generally thought that the germ cells are formed from some of the pole cells which emerge from the posterior mid-gut rudiment (Poulson, 1947). A radically different opinion has been expressed recently by poulson, who suggests that they may arise from those pole cells which migrate into the yolk before the onset of gastrulation (Poulson, 1950). Whatever their origin, 4 to 12 germ cells become aggregated in a gonad on each side in the 4th from last abdominal segment, with roughly equal numbers on each side. Between 16 hours and the time of hatching one or two mitoses occur, so that finally there are between 8 and 38 germ cells in each gonad. At 14 hours a delicate gonad sheath is visible around the germ cells and the gonad is embedded in the tissue of the fat body which extends for a few segments in both directions. Both gonad sheath and fat body arose from the somatic mesoderm.

Other mesodermal organs:

These consist of the dorsal vessel and its alary

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muscles, the pericardial and paracardial cells, the nephrocytes and the dorsal diaphragm. They are developed from those parts of the somatic mesoderm of each side which are untied medially at dorsal closure at 11 hours. Their development is difficult to follow in normal embryos, and virtually impossible in the most of the mutants. However, the dorsal vessel often shows well, especially posteriorly, where its lumen is broad. It is contractile at 16 hours.

Fig.1. Arrival of the cleavage nuclei at the surface, and blastoderm formation; anterior region of the egg.

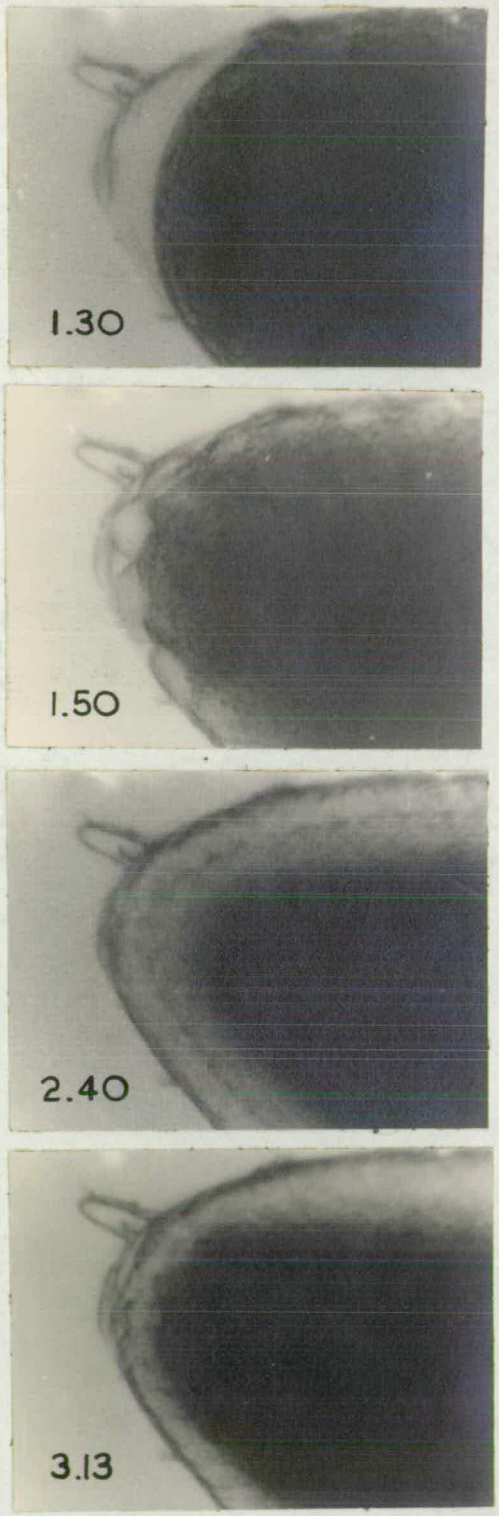
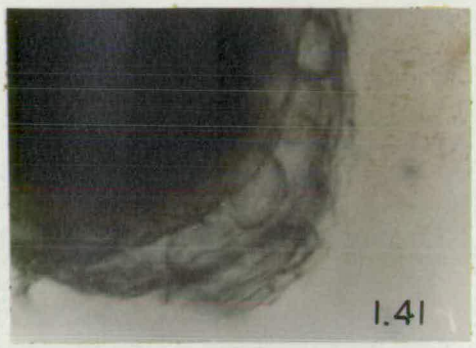

1.30
1.50
2.40
3.13

Fig. 2. Extrusion of the pole cells, and formation of the posterior mid-gut rudiment; posterior region of the egg.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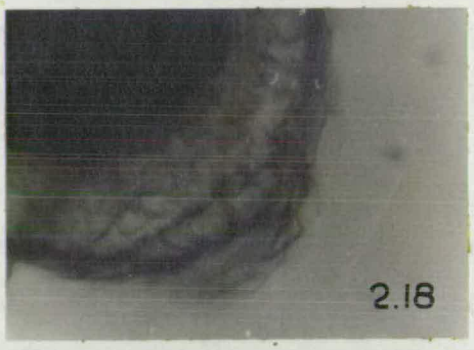
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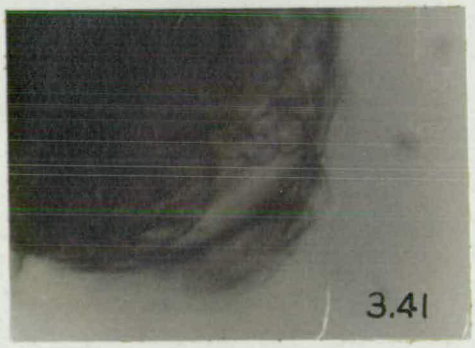
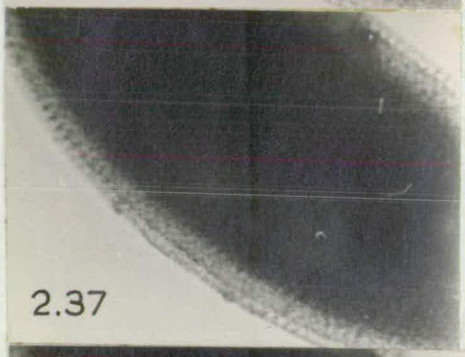

3.41

Fig.3. Blastoderm formation and invagination of the ventral furrow; middle region of the egg, anterior at the left.


2.30


3.09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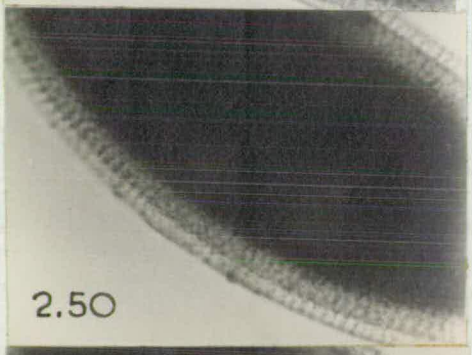
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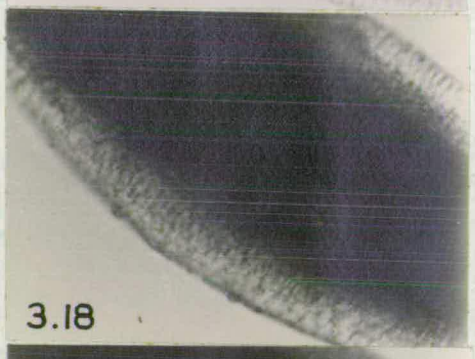
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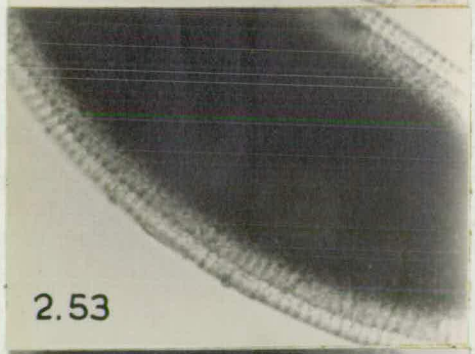
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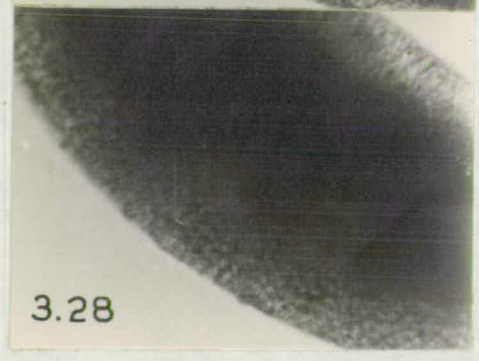
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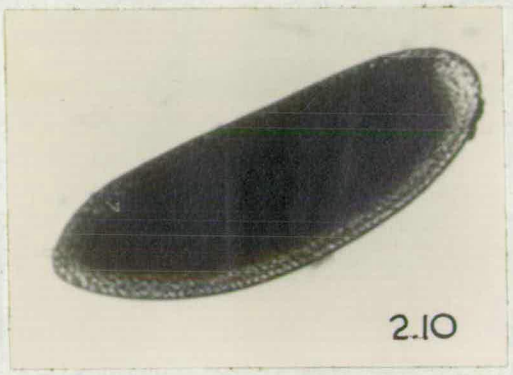
Fig. 4. From blastoderm formation to the beginning of gastrulation.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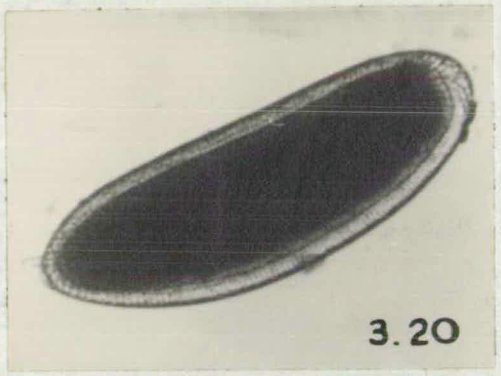
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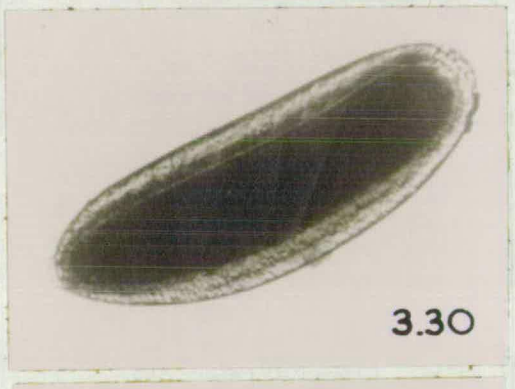
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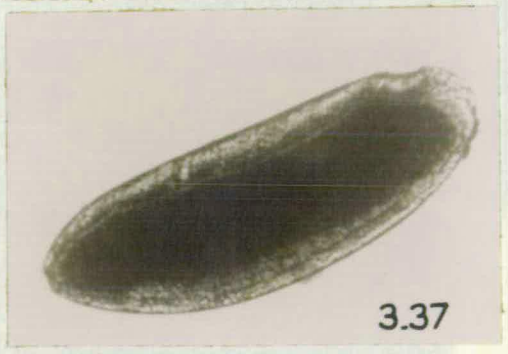
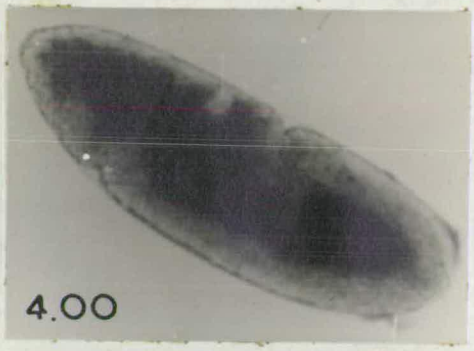

3.37

Fig. 5. Gastrulation: invagination of the posterior mid-gut rudiment, and the extension of the germ band.


3.45


3.47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3.48


5.22


3.53

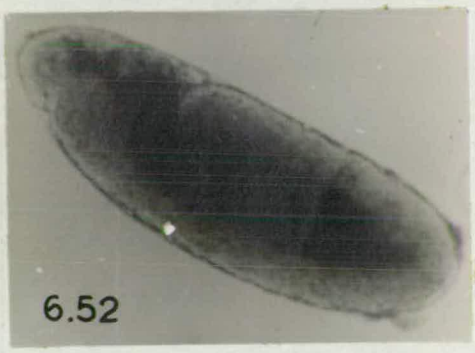
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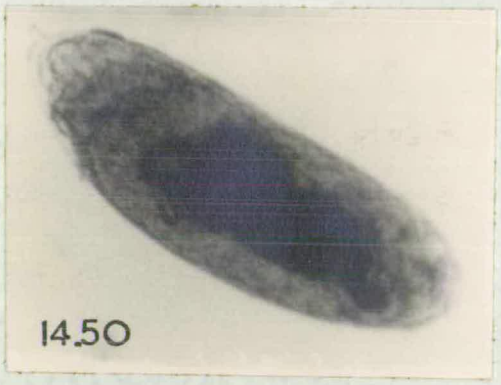
Fig.6. Organogenesis: shortening of the germ band; dorsal closure; involution of the head; gut formation; concentration of the nervous system.


11.10


12.30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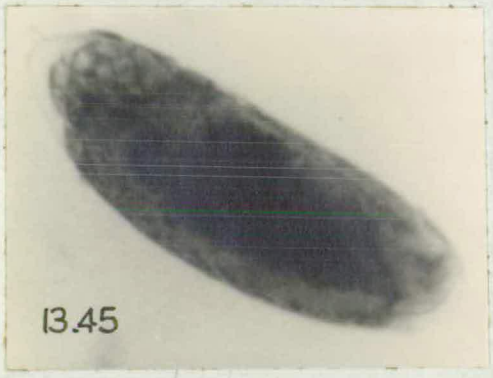
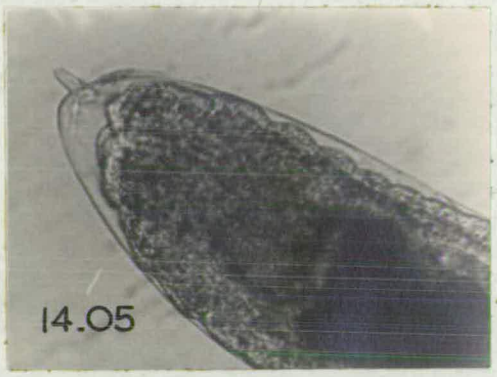

13.45


16.10

Fig.7. Involution of the head.


9.30


11.27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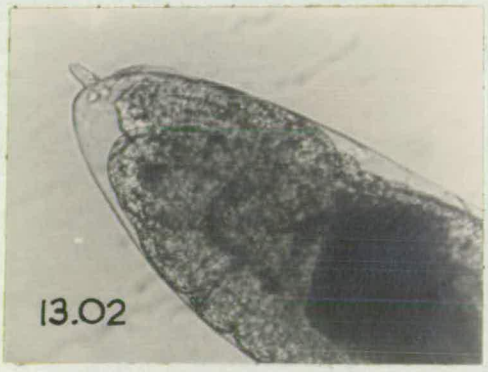
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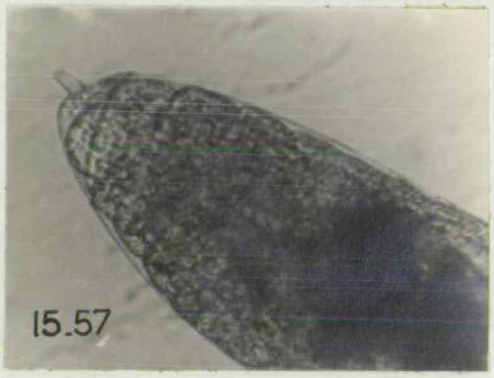
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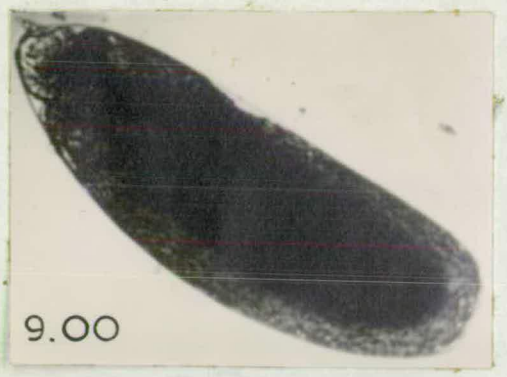
Fig.8. Organogenesis: shortening of the germ band; dorsal closure; involution of the head; gut formation; segmentation; concentration of the nervous system.


9.00


9.30


13.13


10.22

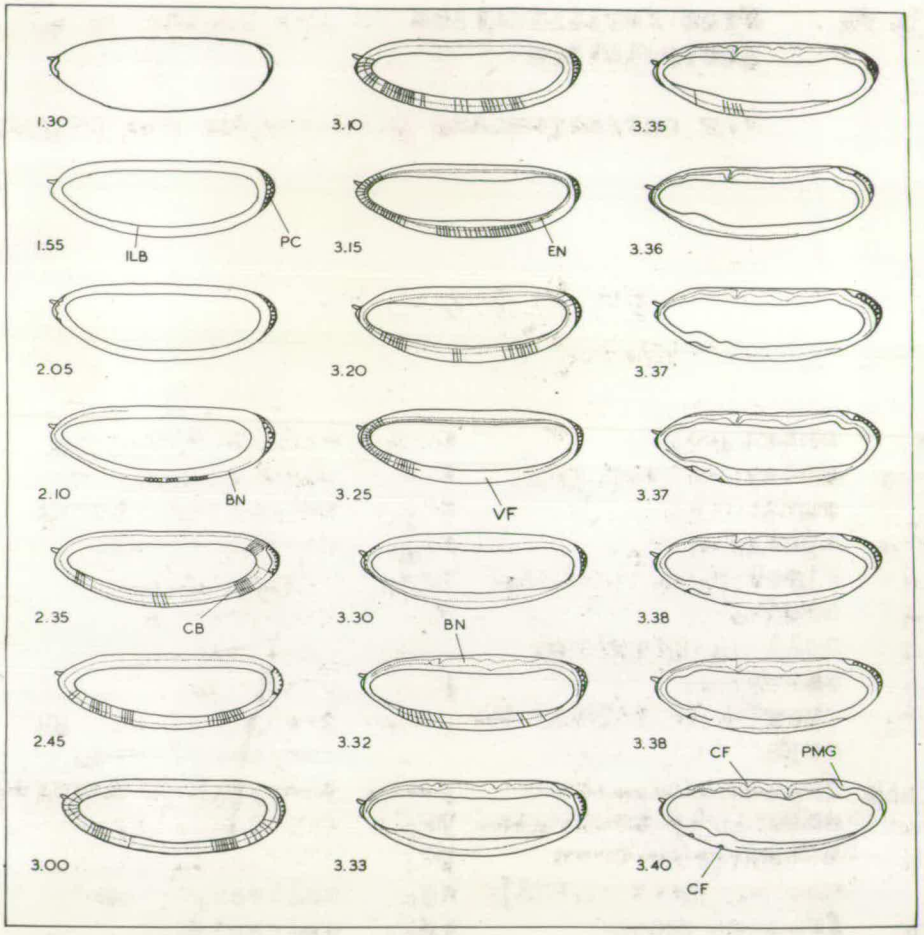

14.10

Fig.9. From fertilization to the beginning of gastrulation.

For corresponding photographs see fig.4.

Key to figs.9-21.

A, anterior.
amg, anterior mid-gut rudiment.
apd, apodeme.
bn, blastoderm nuclei.
br, brain.
cb, cell boundaries.
ch, chorion.
chf, chorionic filaments
cf, cephalic furrow.
dmus, dorsal muscles.
em, embryonic membrane.
en, extensions from nuclei (see p.226).
fs, frontal sac.
gb, germ band.
gnso, sub-oesophageal ganglion.
hg, hind-gut.
hy, hypoderm.
ilb, inner limit of blastoderm.
ll, lateral lobe of head.
mg, mid-gut.
mgc, mid-gut caecae.
mmg, middle mid-gut.
mus, muscles.
mt, malpighian tubules.
nf, nerve fibres.
oes, oesophagus.
P, posterior.
pc, pole cells.
ph, pharynx.
pmg, posterior mid-gut rudiment.
pmus, pharyngeal muscles.
pr, proctodaeum.
pv, proventriculus.
sg, salivary gland.
sp, spiracle.
st, stomodaeum.
T, boundary between head and thorax regions.
vf, ventral furrow.
vll, ventro-lateral lobe of head.
vns, ventral nervous system.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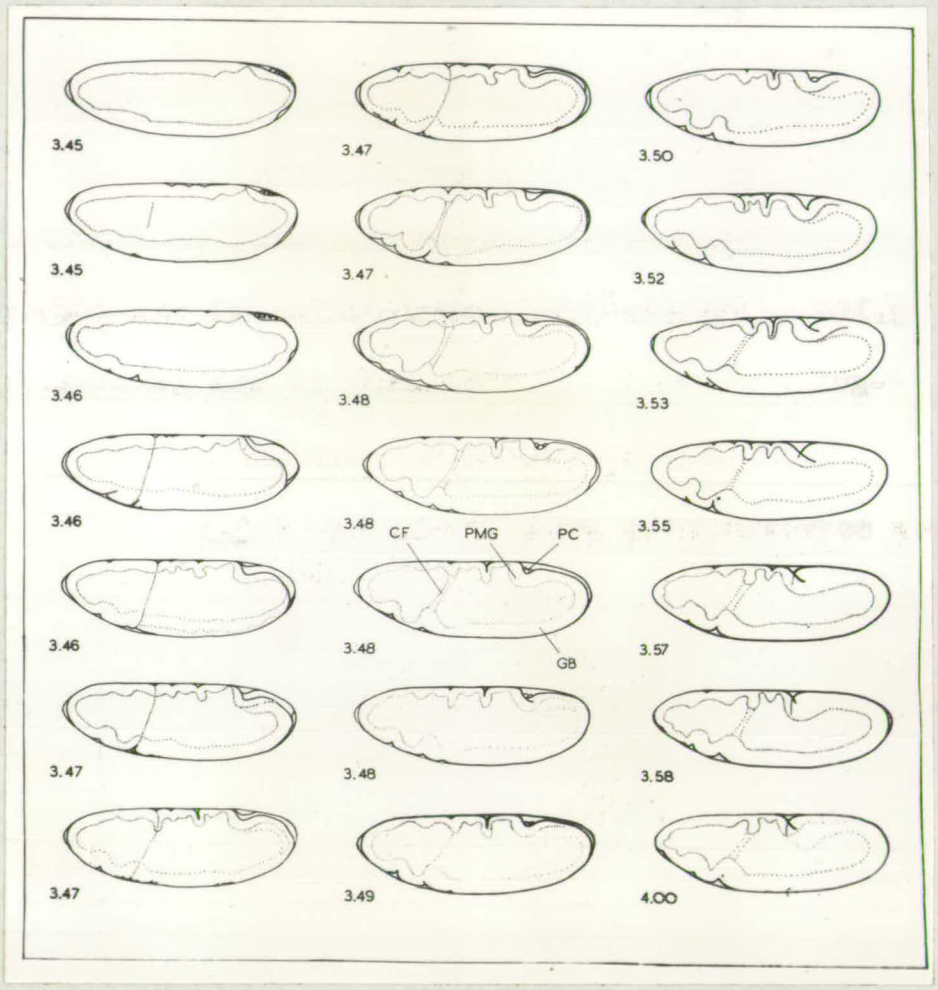
3.25
VF
3.30
BN
3.32
3.33
3.35
3.36
3.37
3.37
3.38
3.38
CF
PMG
3.40
CF

facing page 41

Fig.10. Gastrulation: invagination of the posterior mid-gut rudiment, and extension of the germ band.

For corresponding photographs see fig.5.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CF
PMG
PC
3.46
3.48
3.57
GB
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3.48
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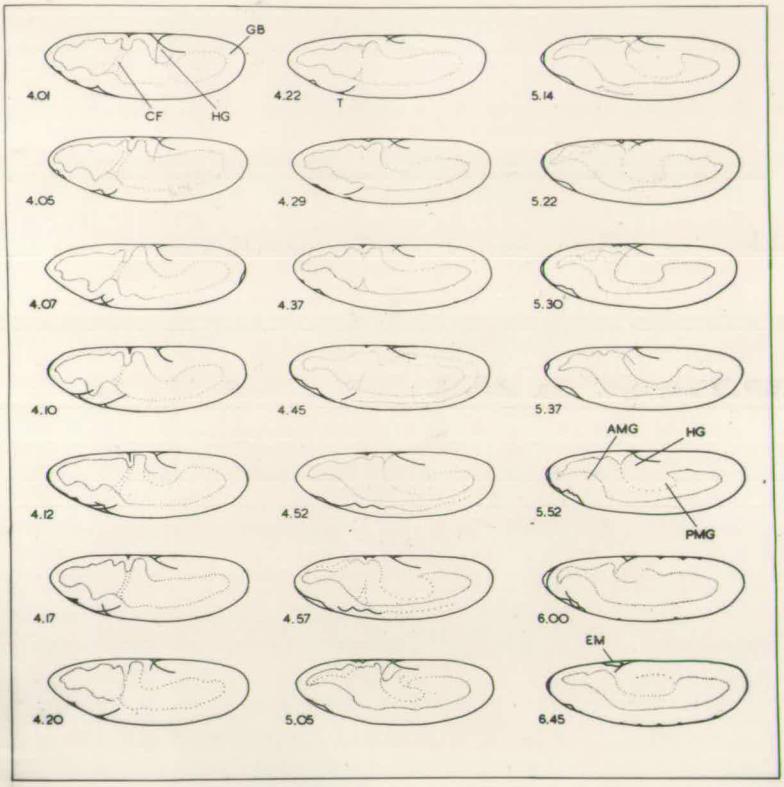
3.47
3.49
4.00

Fig.11. Completion of gastrulation.

For corresponding photographs see fig.5.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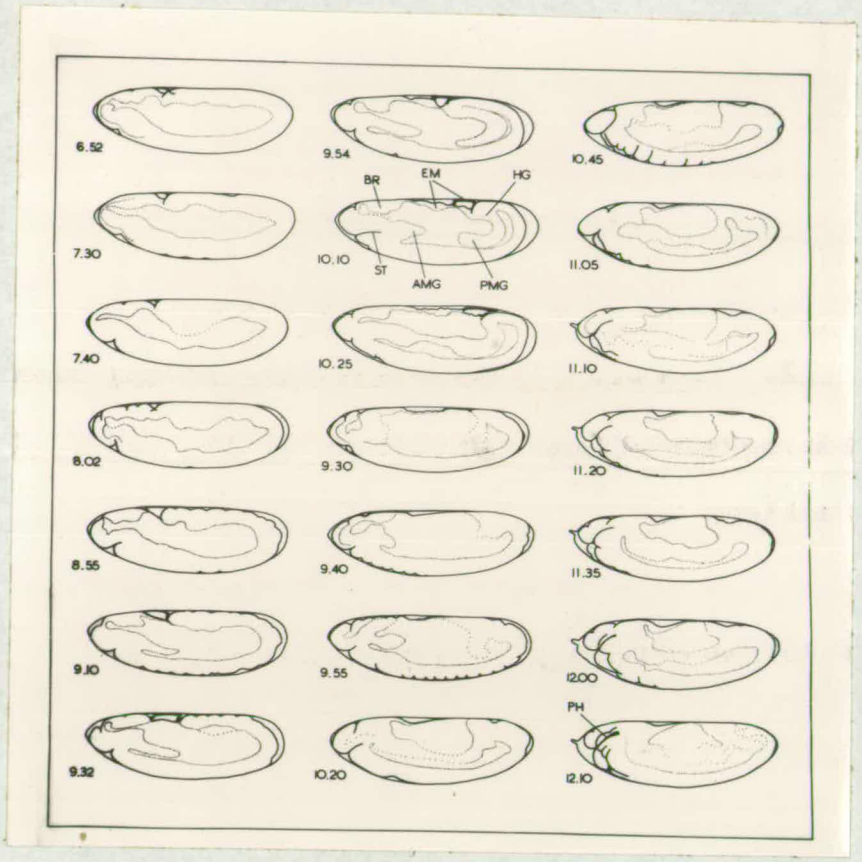
5.30
4.10
4.45
5.37
4.12
4.52
5.52
4.17
4.57
6.00
4.20
5.05
6.45

Fig.12. Organogenesis: shortening of the germ band; segmentation; involution of the head, gut formation.

For corresponding photographs see fig. 6.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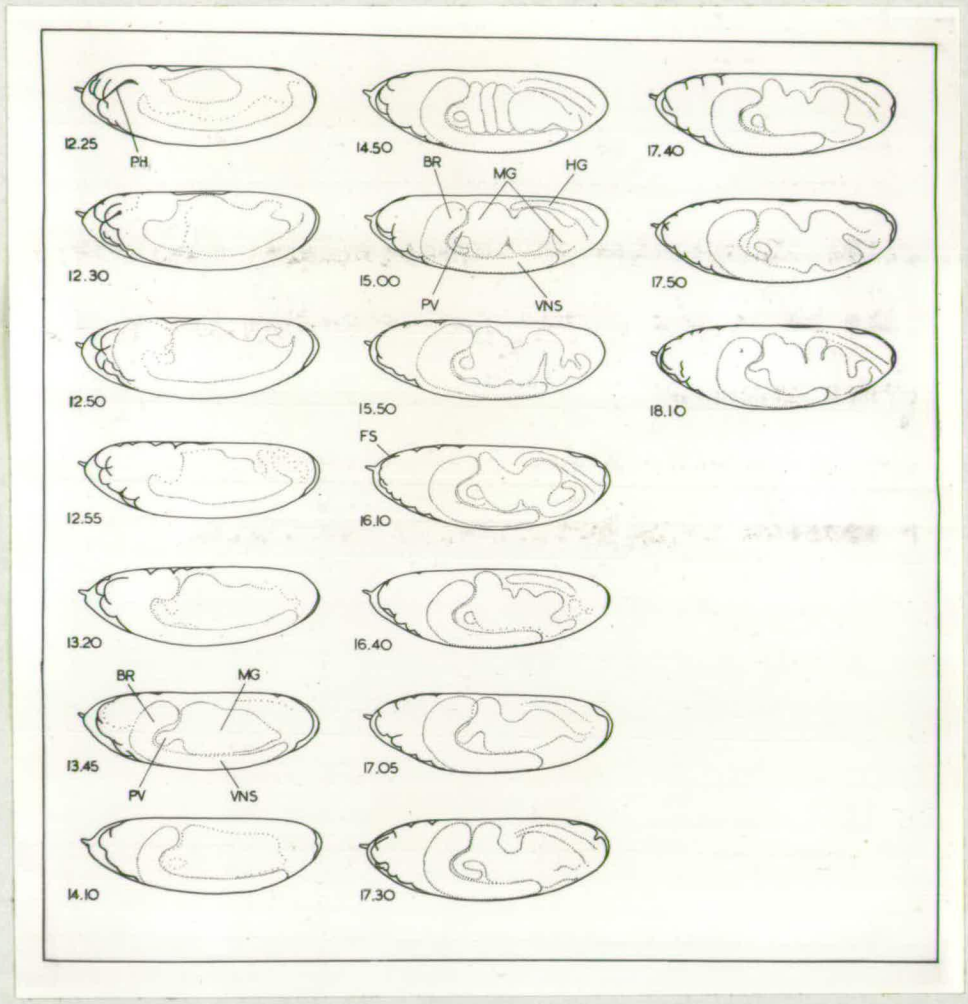
10.45
BR
EM
HG
7.30
10.10
ST
AMG
PMG
11.05
7.40
10.25
11.10
8.02
9.30
11.20
8.55
9.40
11.35
9.10
9.55
12.00
PH
9.32
10.20
12.10

Fig.13. Completion of organogenesis: involution of the head; gut formation; concentration of the nervous system.

For corresponding photographs see fig.6.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BR
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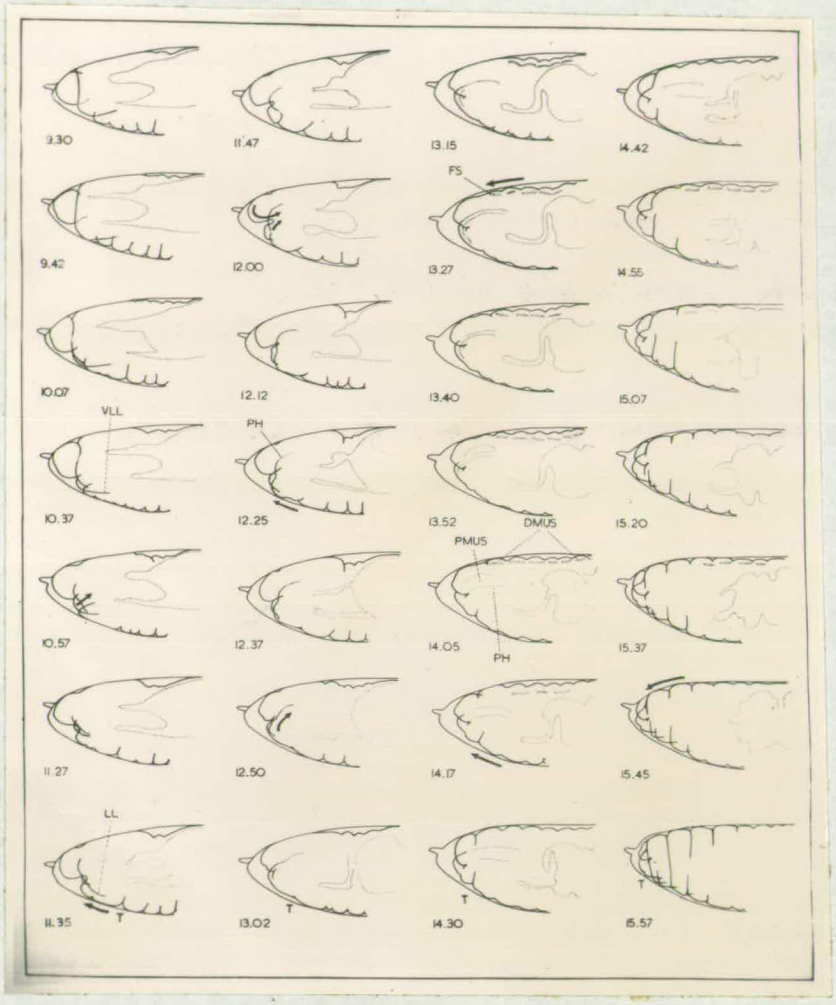
HG
15.00
PV
VNS
15.50
FS
16.10
16.40
17.05
17.30
17.40
17.50
18.10

Fig.14. Involution of the head.

For corresponding photographs see fig.7.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VLL
PH
10.37
12.25
13.52
DMUS
15.20
PMUS
10.57
12.37
14.05
PH
15.37
11.27
12.50
14.17
15.45
LL
T
11.35
13.02
T
14.30
T
15.57

Fig.15. Structure of the embryo at 7 hours.

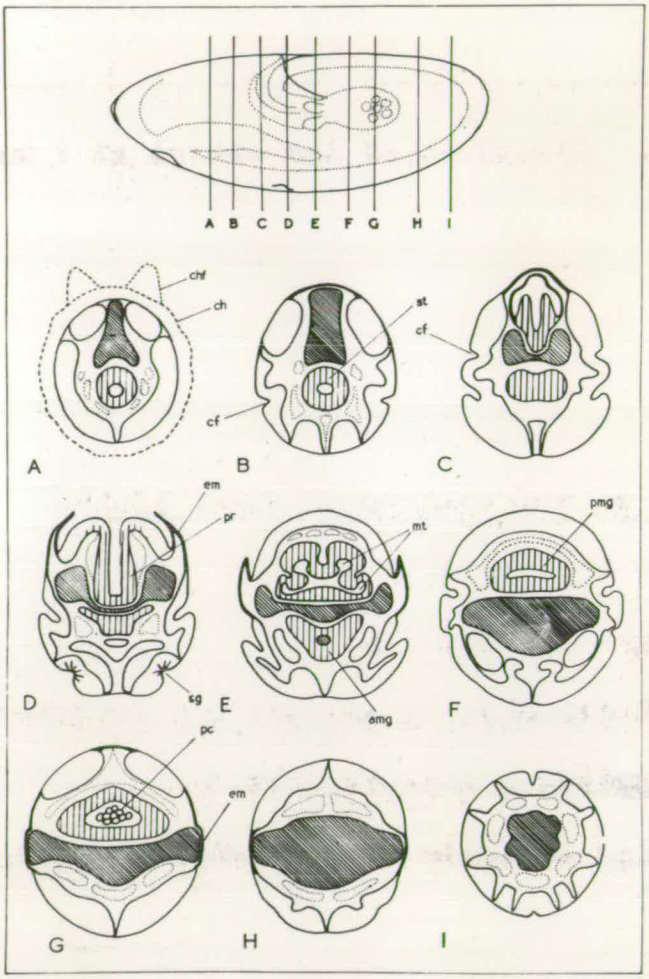
Key to the shading in figs. 15-19.

Diagonal lines - yolk.

Vertical lines - gut and gut diverticula.

Stippling - nervous tissue.

Dotted outlines - mesodermal tissues.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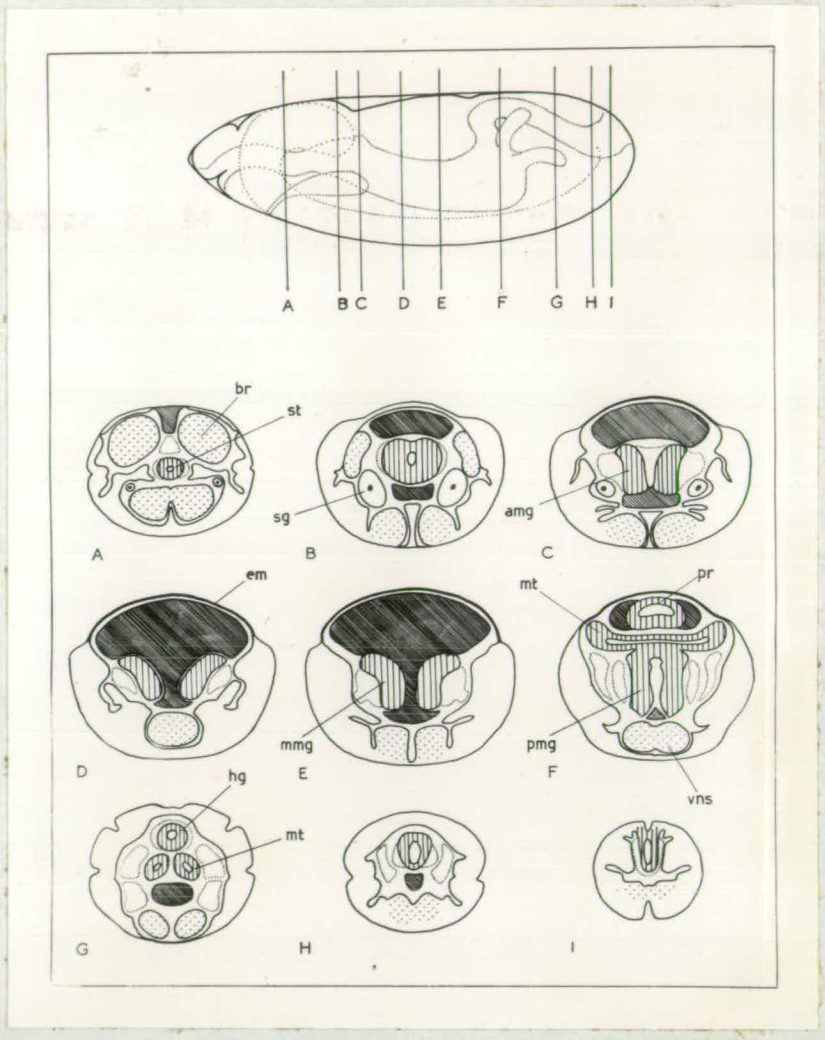
em
G
H
I

Fig.16. Structure of the embryo at 10 hours.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mt
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H
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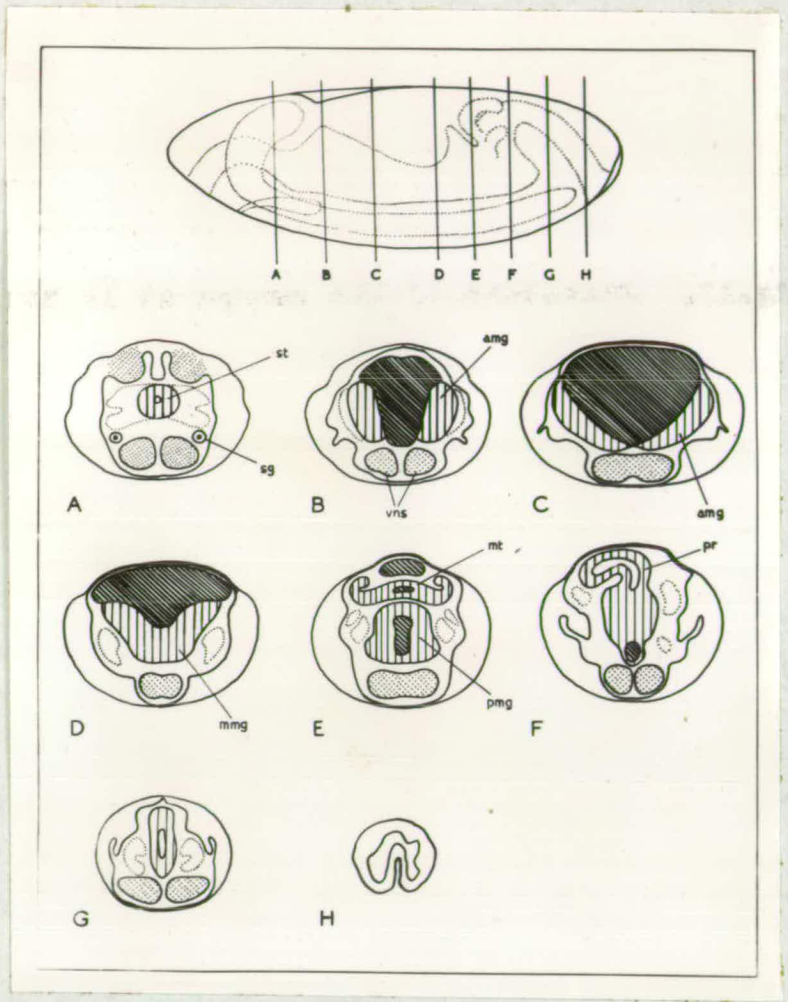
Fig.17. Structure of the embryo at 11 hours.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C
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mmg
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pr
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G
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Fig.18. Structure of the embryo at 12 hours.

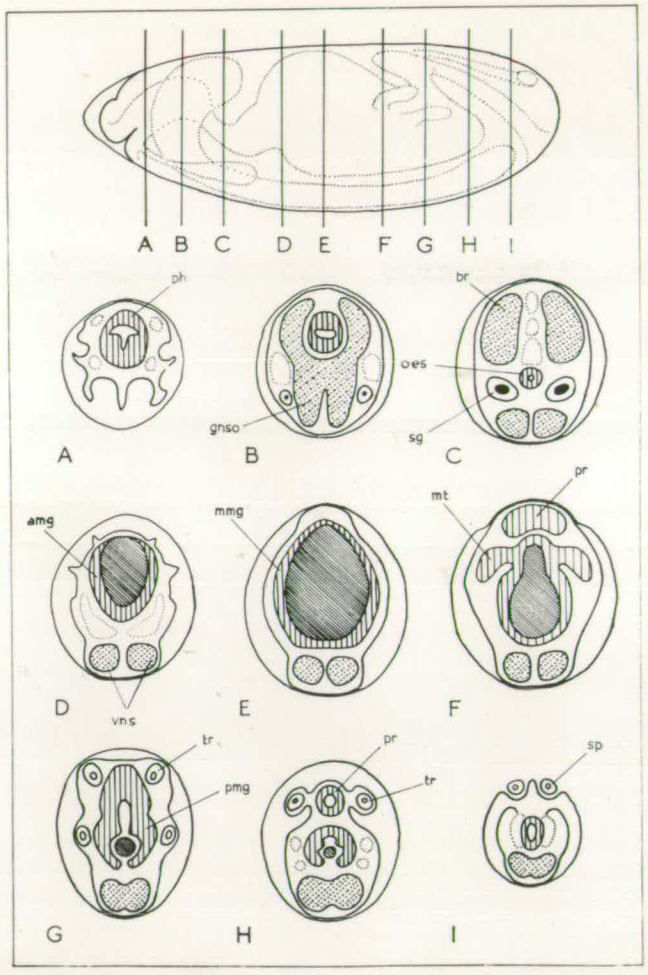
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Fig.19. Fully developed embryo just prior to hatching - at approximately 22 hours.

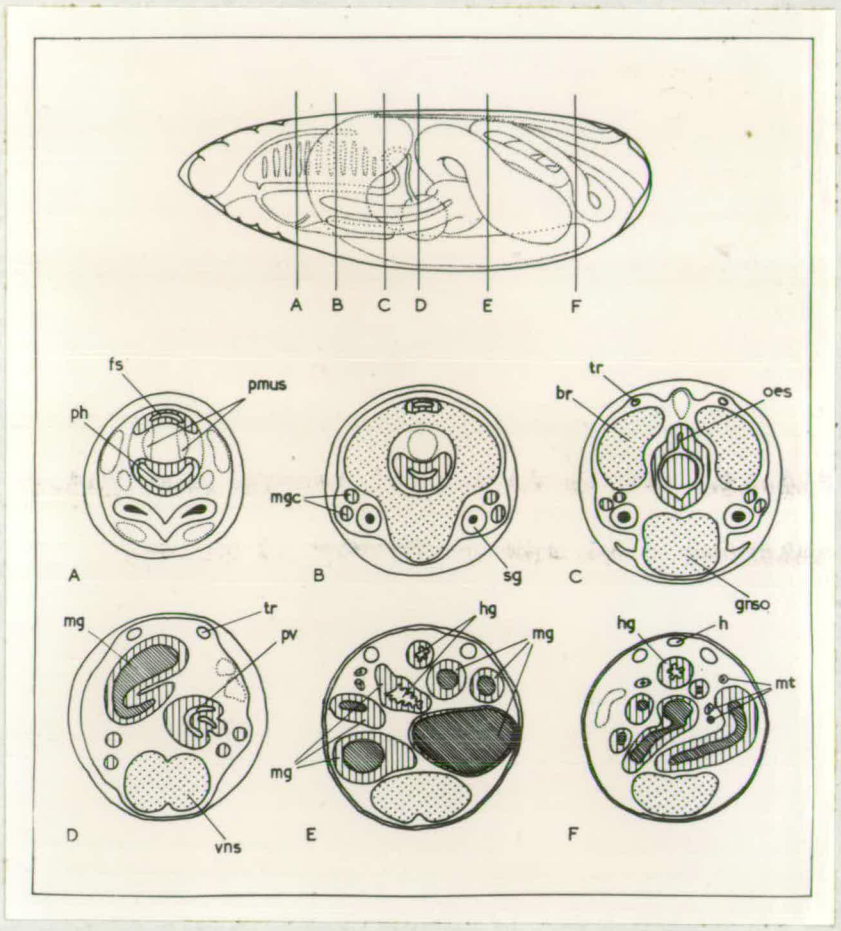
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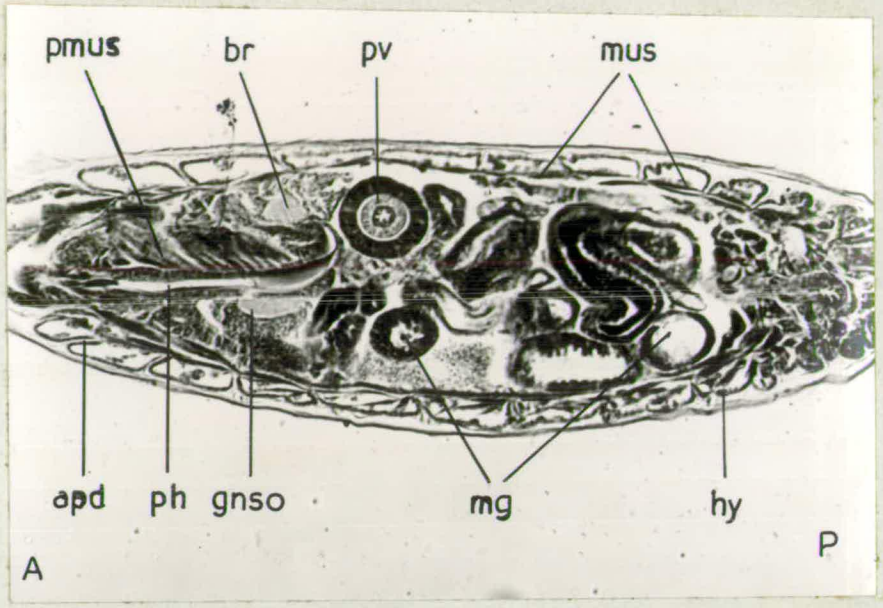
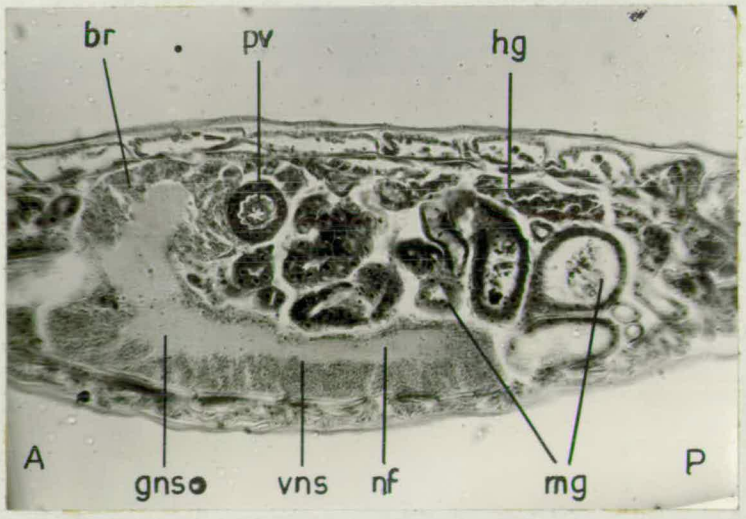
Fig.20. L.S. fully developed embryo just prior to hatching, 22-23 hours. x325.

Fig.21. L.S. the same embryo, through the nervous system. x350.





Mutant Lff 11

Material:

Observations were made upon living material; two embryos were filmed, one of which completed the whole of its development, while the other died soon after gastrulation. Most information was obtained from sectioned embryos, taken at all developmental stages.

Preliminary data:

1. Failure of eggs to hatch in a collection from massed females:

Eggs laid	Unhatched at 30 hrs.	% unhatched
758	220	29.09

2. Analysis of sectioned material:

Period	Unfert-ilized	Unident-ifiable	Normals	Typical Mutants	Other AB-normals
0-5hrs.	4	7	158	18	-
5-18	62	11	304	99	6
23-26	1	3	19	34	3
Totals	67	21	481	151	9

In the sectioned material, embryos in the period 5-18 hours are distinguished as normals or mutants, and none of the former have hatched. Not counting unfertilized eggs, or uncertain cases, almost exactly a quarter - 99 out of 403 - of the embryos are mutants. /Thus

Thus the lethal factor is completely effective in the embryonic stage. This is shown also in the egg count in which the proportion of eggs failing to hatch is rather over 25%; the additional 6% may be explained as consisting of unfertilized eggs, plus the residue of failures in development which occurs in normal stocks.

Final appearance of the Embryo:

Mutant embryo never hatch, though at 22 hours, when the normals are emerging, they may be seen moving vigorously within the vitelline membrane. This activity occurs in the posterior part of the body, and involves the body wall, and also the gut, which often protrudes through it. Segments, bearing chaetae, are present, but are distorted. The anterior region is grossly abnormal, and there is much undigested yolk, not only within the gut, but distributed in masses both inside and outside of the body. Some of these features may be seen in the final photograph from the film (fig. 42 , 17.40). The muscular activity persists for several hours, but in sections of 25-26 hour embryos many cells are seen to be degenerating (fig. 36).

Development of the Embryo.

Events up to Gastrulation:

These first stages are undisturbed, occurring as

/described

described in the outline of normal development. The similarity of the mutant and the normal development during this period is illustrated in the film sequences up to about 3½ hours (Normal: figs. 4 and 9 , 3.37; Mutant: figs. 41 and 43, 3.48). These illustrations show the pole cell cap formed posteriorly, and the cephalic furrow anteriorly, i.e. gastrulation has begun. The differences in their appearance lie only in their photographic qualities, which differ even among normal embryos owing to variation in density. The sequences show that beyond this point the pattern of development of the mutant diverges markedly from that of the normal.

Gastrulation:

Gastrulation in normal embryos:

The normal process of gastrulation has not yet been adequately described, and probably will not be until an adequate marking technique is devised which will make it possible to follow the movements of specific regions of the blastoderm. A successful technique has been used in studying spider embryos (Holm, 1952), which might be adapted to Drosophila work; but the difficulty of penetrating the vitelline membrane without damaging the embryo has so far discouraged attempts along these lines. The film sequences of the movements have been helpful, but only show the changes in the outline profile, not the surface migrations. Con-

/tributions

-tributions from this source, as well as from observations of living embryos and sections, supplement information taken from the existing literature in the following account.

At the time of completion of the cellular blastoderm, the latter is of a uniform thickness, and the yolk which it encloses is similar in shape to the whole embryo. The cell outlines radiate outwards, roughly at right angles to the surface of the yolk. (Fig. 9 , 3.00). During the next half hour changes occur in the form of the blastoderm and of its cells, and of the yolk, which culminate in the invagination of the ventral furrow. These changes will be described in more detail elsewhere (page 225); for the present it may be noted that there is a withdrawal of blastoderm material from the extremeties and a heaping of this material in the middle. The yolk undergoes a corresponding change, becoming slightly contracted medially (fig. 9 , 3.15). The withdrawal of material from the posterior end is obscured by the beginnings of the posterior mid-gut rudiment, but at the anterior end the blastoderm becomes quite thin, especially ventrally. This rearrangement of the blastoderm material involves a change in the orientation of its cells, whose outer boundary remain more or less stationary, while their inner margins follow the general movement. The direction of the latter is consequently

/indicated

indicated by the direction taken upby the blastoderm cells; the ventral cells at the anterior tip point almost horizontally backwards; those behind become increasi gly upright; while the posterior cells point forwards. The junction of the two movements thus indicated is not in the middle of the embryo, but about a third of the distance from the anterior end, i.e. at the position of the cephalic furrow.

During the later stages of these general movements the ventral furrow is formed; they must contribute to its formation, but the most direct movement involved cannot be seen in the profile view. This is the movement of the lateral material towards the ventral midline, and its turning in at this point to form the long ventral furrow. In cross sections there is to be seen a contrast between this movement and that of the rest of the blastoderm movements; in this it is the inner boundaries of the cell which move least, while the outer boundaries follow the general direction of movement. This suggests that this mobement may originate in the blastoderm itself, whereas the other may be caused by movements originating in the yolk. This point is taken up in the discussion. In the film the formation of the ventral furrow shows first as a disturbance of the clear outline of the blastoderm ventrally (fig. 9, 3.25; fig. 3, 3.18), then of a heaping-up of the cells in the middle region as the furrow cells turn in (fig. 3, 3.22), and finally a spreading of

/the

the furrow material anteriorly and posteriorly (fig. 3 , 3.28). The opening of the ventral furrow to the exterior is transient; the cells turned in are separated from those remaining externally, and become the mesoderm material. Together with the external cells of the ventral surface, they form the germ band.

Following quickly upon these events, the cephalic furrow is formed. This seems to appear first, in both the film and in sectioned material, as an inward movement of the nuclei, the inner boundaries of cells being unaffected. But very soon after, the cells themselves press into the yolk, first laterally, and then dorsally and ventrally. From the side the furrow appears oblique, slanting forwards ventrally (fig. 9 , 3.40).

Next follows the most spectacular of the movements of the embryo, that of the extension of the germ band, the formation of the posterior mid-gut rudiment at its hinder end, and the carrying of the pole cells, in the rudiment, forwards over the dorsal side of the embryo, and finally backwards into the interior.

The formation of the mid-gut rudiment involves the aggregation of a number of columnar cells of the blastoderm underneath the pole cell cap, and their

/changing

changing in shape as the migration begins, so as to form a pocket which catches up the pole cells into itself as it moves over the dorsal side in the van of the extending germ band (fig. 2 , 3.41; fig. 5 , page 36).

From the dorsal side the advancing posterior mid-gut rudiment appears pear-shaped, the depression containing the pole cells being deepest and widest anteriorly, with a heaping-up of cells at its edge. It is interesting that in living embryos it usually shows a slight assymetry in its position but that it moves back tothe dorsal mid-line in the course of its forward movement. In front of the advancing rim the dorsal cells buckl e slightly in about three tranverse rows behind the cephalic furrow. Like that of the cephalic furrow, these bucklings at first affect only the nuclei; they involve the complete blastoderm wall as they become deeper, and project into the yolk. The folds are quickly resolved into a single deep transverse buckling fold, which itself disappears as the mid-gut rudiment advances. These changes may be followed in the film sequences (fig. 9 , 3.33-3.40; fig. 10 , 3.45-4.00; fig. 5 , 3.45-4.00). The films also illustrate the formation of another buckling furrow, which remains shallow, anterior to the cephalic furrow, which is not mentioned in the published accounts.

/When

When the posterior mid-gut rudiment reaches a point about half-way along the dorsal side, its cells begin to turn inwards, and anterior to the cephalic furrow the mid-gut rudiment is invaginated, becoming a pouch-like pocket which turns posteriorly and carries the pole cells deep into the interior of the embryo (fig. 11, 4.01-5.05; fig. 5, 4.00 and 5.22).

This stage of gastrulation is the most critical in the development of the mutant, and it is also one which has given much trouble to investigators. Until the appearance of Poulson's latest account (Poulson, 1950), this invagination had not been recognized as having a connection with the mid-gut; instead it had ba been referred to as the proctodaeal, or proctodaeal-amniotic, invagination. Poulson has explained that the proctodaeal rudiment, though continuous with the posterior rudiment, is distinct from it, and is only invaginated after the latter, which it follows into the interior of the embryo. However, the distinction between the two is still not entirely clear; even less so is the place of origin in the blastoderm of the material of which they are composed. These points are important in the present connection.

The proctodaeal invagination includes as its

/anterior

anterior border a part of the embryonic membranes, which must be considered next. In fact there is only one membrane, which extends backwards as a fold, and therefore as a double sheet, a short distance over the opening of the proctodaeal invagination. The dorsal part of the fold is continuous with the thin layer of lateral cells which remain along the sides of the embryo after the bulk of the material has moved into the germ band; the ventral part extends down into the proctodaeal invagination, there it becomes continuous with the proctodaeum itself.

During these gastrulation movements the germ band has been clearly demarcated. Starting with the invagination of the future mesoderm cells, it is consolidated by the compression together, in the midline, of most of the blastoderm cells, those remaining at the sides becoming very thin. As the germ band becomes turned over upon itself, rather in the manner of an omelette, the yolk becomes flattened in cross section by compression between the dorsal and ventral regions. The mesoderm cells are rounded and loosely arranged; they divide rapidly and spread along the extending band. The mechanism of expansion of the external cells is not known. Sonnenblick (Sonnenblick, 1950) believes that rapid cell division immediately behind the posterior mid-gut rudiment may provide the force. But it

/may

may be that these mitoses merely keep pace with the extension of the band; the movement in the film suggests a rather sudden expansion due to physical causes.

As the invagination becomes deeper, the cephalic furrow becomes shallower; at 6 hours it is reduced, and at 7 hours only shallow lateral depressions remain. The ventral portion of it becomes indistinguishable from an intersegmental division, most likely that separating the head from the thorax. The dorsal portion appears at first to merge with the proctodaeal-amniotic invagination but this is not the case: closer investigation of sectioned embryos always reveals a small nick in the embryonic membrane, which is the vestige of the dorsal portion of the cephalic furrow.

This concludes the gastrulation process in the normal embryo. The relation of the parts at this stage are shown in a sectioned embryo (fig. 27, page 103), diagramatically, at a rather later stage (fig. 15, page 46), and the period of development is covered in the film sequence (fig. 11, 5.14-6.45; fig. 5, 5.22 and 6.52).

Gastrulation in the mutant:

Blastoderm formation is quite normal in this mutant, and so also are the first two stages of

gastrulation

gastrulation - the formation of the ventral furrow, and the formation of the cephalic furrow. The third stage that of the extension of the germ band and the invagination of the posterior mid-gut rudiment, is extremely abnormal; its effect on the structure of the embryo at the end of gastrulation may be seen in figs. 25 and 26 (page 103), which exhibit the two strikingly characteristic features of the mutant. These are:

1. The abnormal position of the opening leading to the posterior mid-gut rudiment; instead of being just behind the cephalic furrow it is at the posterior end.
2. The deep constriction at the region of the cephalic furrow, which separates, except for a narrow neck, the anterior third from the posterior two thirds of the body.

The first feature is brought about by the mid-gut rudiment turning into the embryo immediately, or shortly after, the beginning of the extension of the germ band so that instead of being carried dorsally over the embryo it moves anteriorly inside of it. The slight asymmetry which has been commented on in the normal embryo at the beginning of extension is often present in the mutant, and in this is case is not corrected, so that the germ band becomes displaced to one side or the other. The beginning of the movement is shown in the film (fig. 43

3.53-4.05). There is some variation in the time at which the mid-gut rudiment is turned in; in the other film sequence (not illustrated) the germ band travels a short distance along the dorsal side before it is invaginated.

As the invagination extends further anteriorly, it forces much of the yolk in front of it into the head region. The lateral impushings of the cephalic furrow are turned forward by this pressure.

The origin of this feature appears to lie in certain abnormalities in the distribution and shape of the blastoderm cells, which precede the process of extension. In transverse sectionsof normal embryos the blastoderm at this period is seen to consist of a dorsal and ventral region of columnar cells, with thinning lateral cells between the two regions. In the mutant, there is a thinning of the dorsal cells, which become cuboidal, and a gradual increase in depth of the cells towards the ventral side, where an apparently normal germ band is formed.

It seems likely that the "diving" of the germ band beneath the blastoderm cells in its extension may be due

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to this difference in the shape of the dorsal cells. It has been noted above that, in normal development, the buckling which takes place between the advancing mid-gut rudiment and the cephalic furrow at first involves only the nuclei. It is suggested that the thick layer of cytoplasm formed by inner portion of these deep columnar cells serves as a stabilizing factor, which allows a steady compression of this region, so that the buckling furrows are concentrated into a single furrow as the mid-gut rudiment presses up behind it. The cytoplasmic tails of the dorsal cells degenerate as the movement continues, and finally the nuclei of the furrow disappear (figs. 22 and 23, page 102 ~~end~~).

In the mutant the dorsal cells are cuboidal, with much less cytoplasm than in the normal; virtually, then this layer corresponds to the layer of nuclei of the normal, without the stabilizing thickness of cytoplasm (fig. 24, page 102). When the germ band extends, it is as if pressure were to be exerted against a thin layer of paper, which would "give", so that the object applying the pressure would slip underneath it; whereas the normal condition would be represented by a thick layer of cardboard, which the object pressing against it would be ~~un~~able to compress, while continuing its direct

/advance.

advance. The vitelline membrane prevents the germ band slipping outwards over the dorsal blastoderm cells, so that it goes instead into the interior of the embryo.

The second striking feature of the mutant - the deep constriction anteriorly - appears to be a modification of the cephalic furrow, which cannot be seen as a separate structure. As the mid-gut invagination advances through the embryo, the cephalic furrow becomes exaggerated. As gastrulation is completed it does not tend to disappear, but becomes very much deeper. In some embryos it encircles the egg in a short spiral, so that sections cut it in two places on each side. Its anterior wall becomes thin, while the posterior one consists of rather deep columnar cells which resemble those of the proctodaeal invagination. The resemblance to the proctodaeal invagination is increased by the growth over it of a fold of the anterior wall, representing the embryonic membrane of the normal embryo, though it never extends back so far.

The invagination of the anterior endoderm is normal. It is invaginated as the anterior mid-gut rudiment, and is carried into the embryo at the tip of the stomodaeum, which grows in normally.

This concludes the peroid of gastrulation. Its characteristics are not very well shown in the film illustrated here (fig. 43, 4.10-4.45; fig. 41, 4.45); in the other film sequence the deepening of the cephalic furrow shows more clearly, but the embryo died shortly after this stage.

Differentiation between 5 and 9 hours:

Ectoderm:

The ectoderm is now divided into that part which will give rise to the hypoderm and nervous tissue, and that which will form the fore and hind gut i.e. the stomodaeum and proctodaeum. The stomodaeal invagination, and the anterior mid-gut rudiment which it carries in at its tip are quite normal, and the usual intense mitotic activity is seen in both of them as they grow posteriorly. They always grow through the neck of the constriction; in no embryo was this observed to hinder them (fig. 38).

The proctodaeum is abnormal, and it is difficult to determine with certainty how it develops, largely because of the difficulty of clearly distinguishing the proctodaeum from the posterior mid-gut rudiment. The posterior wall of the furrow resembles that of the

/normal

normal proctodaeum; and in spite of the difficulty of distinguishing it here, it may be said that in some embryos the proctodaeum is absent between the posterior mid-gut invagination, or at any rate very short (fig. 24). This suggests that the material of the two invaginations, the ectodermal one and the endodermal one, may be separated in some instances, and invaginated at different points. But in many embryos it is possible to distinguish a well defined proctodaeum (fig. 28), and the posterior wall of the furrow in later embryos is always found to have taken on the character of hypoderm. Probably the proctodaeum is always present to some degree in its normal position between the posterior mid-gut rudiment and the posterior end of the germ band, but is sometimes very much less extensive than normal.

The other part of the ectoderm develops normally in the region anterior to the furrow, with a separation taking place between hypoderm and neuroblast cells. The latter are seen in the usual dorsal position on each side (fig. 25), where normally they would produce the material of the brain.

Posteriorly there are important deviations from the normal course of events. The position of the ectoderm of the germ band is unusual, as has been described, and

it becomes roofed over, as it moves through the embryo, by the cuboidal cells of the dorsal side of the embryo. The invagination of the germ band from the posterior end means that, not only will the position of the ectoderm of the germ band be abnormally placed, being internal instead of external, but it will form the floor of a tube which is roofed over by the cuboidal dorsal cells of the embryo, which have been turned in as the germ band extends. The drawing in of these cells probably accounts for the excessive thinness of the layer which now encloses the embryo dorsally. This region now bears a resemblance to the normal embryo, with its stretched embtyonic membrane, after shortening has been completed (compare figs. 25 and 30).

The other important abnormality is the precocious formation, in the mutant embryos, of the tracheal pit invaginations. In the normal embryo these appear between 6 and 7 hours, showing as a series of pits, in which the cells are undergoing active mitosis, along the sides of the germ band. They are segmentally arranged, and are the first external signs of segmentation. In the mutant they appear between 5 and 6 hours; the most anterior of them occur within the walls of the anterior constriction, the middle ones along the sides

/of

of the body, while the posterior ones occur in the posterior invaginated ectoderm. Two to three pairs occur in this position, showing that this number of segments is included in the invagination (fig. 26). The segments themselves become apparent in the ventral germ band at the end of this period.

The normal separation of the ectoderm cells of the germ band into hypoderm cells and neuroblast cells occurs, the latter coming to lie internally. However, the position of the nervous system which forms from these cells reveals that there has been a general tendency to the rotation of the posterior part of the embryo, so that its ventral surface comes to lie more or less laterally with respect to the orientation of the chorion (fig. 25). This disorientation is possibly related to the assymetry which has been noted in the gastrulation process.

In normals the salivary glands make their first appearance, as a pair of ventro-lateral thickened plates of cells, about a third of the distance from the anterior end of the embryo. This is precisely the region which is most disturbed in the mutant by the development of the anterior constriction; consequently the salivary glands are never well formed, and usually one is absent completely (fig. 33).

/This

This may represent the fusion of material from both sides.

Mesoderm:

The mesoderm cells become quite normally arranged along the inside of the germ band. Its division into segmental masses is not easy to see, owing to the distortions of the embryo, but in view of the later development there is no reason to suppose that it does not occur normally. Separation between somatic and splanchnic regions occurs, the former chiefly accumulating against the body wall, while the latter spreads along the anterior and posterior parts of the gut (fig. 28).

Endoderm:

The anterior endoderm develops normally, as has been mentioned in connection with the stomodaeum. The posterior endoderm i.e. the posterior mid-gut rudiment which invaginates into the embryo ahead of the germ band, has been described above. As it approaches the region of the anterior constriction it turns posteriorly, as it does in the normal embryo (figs. 25 and 27). This turning is sometimes inhibited by the disturbance of the spacial relations of the embryo.

/Mitotic

Mitotic activity is very evident in the rudiment, and it extends back as a sac, containing the pole cells, behind which the buds of the malpighian tubules develop. (fig. 25). The fate of the pole cells will be discussed later.

It has been mentioned that the abnormal gastrulation usually has the effect of forcing much more of the yolk than normally into the anterior region. At about 5 hours this anterior yolk has been cut off as a separate mass, while the yolk remaining posteriorly has become enclod enclosed in a primitive gut formed by the yolk cells.

Shortening, Involution of the Head, and Dorsal Closure:

It is at this period, from about 9 hours onwards, that the two primary disturbances already described begin to produce secondary effects by interfering with other processes. They do this particularly where there are large-scale movements, which are prevented or distorted by the unusual arrangement of the parts.

Shortening:

Shortening is normally a process in which the germ band concentrates into something like half its length, in the space of about an hour, so that it comes to lie wholly along the ventral side of the embryo.

/It

It is a very smooth movement, as it is seen in the speeded motion of the film, in which the germ band draws away from the vitelline membrane posteriorly; particularly noticeable is the way in which the gut pivots round as shortening goes on. The pivot is at about the junction of the proctodaeal invagination with the posterior mid-gut rudiment; this remains more or less in the same place. So the opening of the proctodaeum into the mid-gut remains where it was, while its opening to the exterior is carried over the top, to eventually arrive at the posterior end of the embryo. During this movement, the posterior mid-gut rudiment on the other hand moves forwards, partly because of the shortening movement, but largely by growth, whereas previously it was directed backwards. These events are shown in two film sequences (fig. 12, 9.32-10.25; fig. 8, 9.00-10.22), and the arrangement of the parts of the embryo is shown diagramatically in fig. 16. At this time, the tip of the posterior rudiment is open, and the pole cells move out of it.

In the mutant shortening is interfered with, presumably by the disturbed relationships of the parts, though no clear reason can be given for it. Whatever the reason, the germ band remains invaginated dorsally, though some degree of shortening - an "attempt"at it -

/occurs.

occurs. This is seen in the film, where there is a concentration of the dorsal germ band material towards the posterior end, but it is never carried round to the ventral side (fig. 44, 8.55-9.45). This abnormality has a striking effect upon the development of the gut, for the pivoting movement of the proctodaeum nd the posterior mid-gut rudiment does not occur; instead they proceed with their development anteriorly, and within the growing extensions from the anterior mid-gut rudiment (compare figs. 28, 31 and 30, 32). The effects of this upon the development of the gut will be described later.

Dorsal Closure:

The extent to which this is disturbed varies. In some cases the body wall comes to enclose the embryo completely dorsally (fig. 33), while in others this region remains to a greater or lesser extent closed only by the thin dorsal membrane. In this case the gut will eventually break through it and protrude dorsally, and the development of those medial parts which are formed from material brought up from each side is prevented (fig. 35).

Involution of the Head:

/Of

Of the three processes this is the most drastically affected. It is disturbed in nearly all of the abnormal developments described here, for one reason or another. Occurring late in development, and involving as it does so much of the embryonic material, it is almost inevitable that it should be affected by serious abnormalities in any earlier stages of development. For this reason a more detailed description of the involution process will be given here. It has not been clearly described in the literature, and the exact movements have not been clearly followed, since before some adequate technique is devised this is very difficult. The film has made possible a more careful analysis, but even with its help the absence of any marks by which different parts of the hypoderm can be distinguished makes it impossible to do much more than describe the movements of the surface outlines. The film shows that these sometimes change only slowly when there is very active movement of the material within them.

Involution in the normal embryo:

At the time of shortening, the first head segment is easily distinguishable as a sharply pointed region, clearly demarcated from those behind it (fig. 8 , 9.00; fig. 7 , 9.30; fig. 14 , 9.30). The head segments

/behind

behind it have characteristic lobes in the ventral region (fig. 14 , VLL); it is not easy, at this stage, to pick out the boundary dividing the head from the thorax among the intersegmental divisions, but it becomes obvious later as the segmental structure of the head disappears (fig. 14 , T).

In the first hour of head involution, the outline of the tip of the embryo changes as the material of the first head segment is withdrawn into the embryo; it becomes blunter, and the clear line marking it off from the succeeding regions disappears. Material also moves in laterally and ventrally, causing the head lobes to change their form; the ventrolateral ones are "swallowed", and lateral ones come to form a boundary at each side of the opening (fig. 8 , 9.00-10.22; fig. 7 , 9130-11.27; fig. 14 , 9.30-11.27). While these movements are occurring in the head region dorsal closure is completed in the trunk.

A ventral movement begins at this time, which continues throughout the involution process, bringing the boundary between head and thorax (fig. 14 , T) anteriorly, so that the former is finally enclosed in the latter. The movement of ventral material into the embryo, where it forms the floor of the pharynx, also carries the common duct of the salivary

/glands

glands into this region.

The ectoderm moved in form the dorsal surface forms the dorsal wall of the pharynx, and the thick layer of mesoderm underlying it forms the pharyngeal musculature. The turning in of the pharyngeal material results in an extreme thinning of the material remaining at the surface (fig. 67 , page 169). The invagination of the pharyngeal material causes the anterior end of the embryo to become blunt, and retracted from the vitelline membrane anteriorly (fig. 8 , 13.13; fig. 7, 13.02; fig. 14 , 11.35-13.15).

The next process is the formation of the frontal sac. The description by Poulson (Poulson, 1950) does not make it clear that the most conspicuous part in this process is played by the forward movement of the thoracic segments dorsally, and that it is very different from the invagination of the pharyngeal material. The frontal sac is formed by the movement of the thoracic body wall over the dorsal head material of which it is to be composed. At the edges of this advancing thoracic material, head material is turned in, and the sac-like structure is achieved (fig. , page ; fig. , page ; fig. , 14.05; fig. , 13.27-15.37). At the conclusion of the process,

/pharynx

pharynx and frontal sac open to the exterior by a common "mouth". The structure of the completely involuted head is shown diagramatically in fig. 19, page. 50.

The end of the involution process comes with a very vigorous advance of the thoracic and anterior abdominal segments, especially on the dorsal side, so that the pharynx and frontal sac are completely enclosed by these segments, which tilt forward and form a hood over the mouth opening.

Involution in the mutant embryo:

Thus, normal involution of the head is seen to consist of two principle elements:

1. The invagination of the pharyngeal material, which involves only the head segments;
2. The formation of the frontal sac, and the enclosure of the enclosure of the head within the thorax which depend upon movements of the thoracic and abdominal regions.

In the mutant, the movements of the first class are possible up to a point, but those of the second class are precluded by the presence of the constriction, which in fact acts like a ligature tied round the anterior part of the embryo. The usual result is

/illustrated

illustrated in fig. 33. The pharynx is not perfectly formed, but the material of its roof has mostly been invaginated, and the pharyngeal muscles have developed though there are rather few of them. The material of the floor of the pharynx is partly invaginated, but much of it is still external, showing as a thick wall where there would normally be a thin layerof attenuated cells. The second class of movements has been prevented; there is no frontal sac formed, and the head material remains isolated in front of the constriction. The thoracic segments have advanced as far as possible, causing a further constriction of this neck region. In most cases there is a compression of segments together at this region. Three embryos occurred in which the anterior furrow was much shallower than usual, and in these a rudimentary frontal sac was produced, and the head was partly enclosed in the thorax.

Some of these events may be followed in the filmed embryo (fig. 44). At8.55 the first head segment is visible; it is withdrawn in a sequence of abortive involution movements. At 12.40 the head has retracted from the anterior end, owing to the invagination of pharyngeal material, and there has been a corresponding thinning of more posterior

/tissue.

tissue. This has been inflated by the pressure of the embryonic haemolymph to form a protruberance, in which loose yolk granules appear. The tip of this protuberance appears to break off, but it is difficult to say how this can have happened. The movement forward of the thoracic segments is delayed, but their crowding behind the constriction is evident in the final photograph (19.20).

Organ formation:

The gut:

1. The fore-gut:

The fore-gut does not develop far beyond the extent described in the account of involution. A cuticle is laid down in the lining of the pharynx but no cephalopharyngeal skeleton is formed; nor are the mouth hooks or the median tooth.

The oesophagus develops normally, as a narrow tube leading into the proventriculus, but its path is deflected by the disarrangement of the parts of the gut.

2. The mid-gut:

The proventriculus is formed more or less normally, and when dorsal closure has occurred its position is

/not

not greatly disturbed. Where closure has not occurred, and there is no constricted neck of body wall at the furrow, it is often pressed forward anteriorly against the the head material.

The rest of the mid-gut develops abnormally and becomes very complicated. The later stages of mid-gut development are difficult to follow even in the normal, owing to the complex coiling which it undergoes. The coiling of the abnormal gut of the mutant makes its later stages almost impossible to sort out, but the nature of its early divergence from the normal condition is clear.

In the account of shortening it was described how the arms of the anterior mid-gut rudiment grow backwards under the yolk in the normal way, while the proctodaeum, and often the posterior mid-gut rudiment, is pressed forwards into the yolk. The yolk itself not including the mass which is often isolated in the head region, is enclosed in a primitive gut (compare figs. 31 and 32).

The growth of the anterior endoderm rudiment continues normally, and soon after dorsal closure it has enclosed its part of the yolk dorsally. The deep columnar cells of the rudiment become cuboidal as they come to form the wall of the gut.

/Owing

Owing to its position, the posterior endoderm rudiment cannot enclose its part of the yolk normally nor unite properly with the arms of the anterior mid-gut rudiment (compare figs. 31 and 32). The large cells which are normally found at the tips of the growing posterior mid-gut rudiment, and which are incorporated in the middle part of the mid-gut, have not been clearly seen in any of the mutant embryos; they are possibly present in some (fig. 31), but definitely not in most cases. Poulson (Poulson, 1947) considers these to be pole cells which do not become included in the gonads as germ cells. Whether or not this is the case (for a further discussion, see page 153), it is difficult to see why these particular mid-gut cells should be absent. The abnormal position of the posterior mid-gut rudiment might entail that the pole cells should emerge from it in such a position that none could be included in the mid-gut. In this case they might be included in the gonads as extra cells. But the gonads are usually formed normally. In some embryos only a single gonad is distinguishable, but in none are there noticeably large gonads which might indicate the presence of extra germ cells.

Some sort of union, usually only partial, is established later between the anterior and posterior

/parts

parts of the mid-gut, but some of the yolk usually escapes into the body cavity. The precise bucklings and changes of form of the normal mid-gut sac into first two chambers, then four which buckle and elongate to form the complicated but regular tangle of the definitive mid-gut, are never achieved. There are all grades of abnormality, depending upon the degree to which the original union of the two rudiments to form the sac-like early mid-gut has been disturbed. For instance, if the gut remains open along one side, the contraction into half-chambers may occur on the other.

Differentiation of the cells of the posterior part of the mid-gut is never complete, and they remain cuboidal, or even columnar, rather than flattening and elongating as normal mid-gut cells do. Much yolk remains undigested in the lumen of the mid-gut, as does that which has escaped into the body cavity. The mid-gut caecae are variable in their development, but their forward extension is always prevented by the presence of the furrow, and usually they remain short. The malpighian tubules are formed, but are abnormal; instead of becoming extremely elongate tubes with a narrow lumen, they remain short, and often the lumen becomes expanded, and the walls

/attenuated.

attenuated.

3. The hind-gut:

The difficulty of determining the extent of the hind gut has been mentioned above. In some embryos it is distinguishable at the beginning of the gut differentiation, but as neither its own cells, nor those of the mid-gut with which it is continuous, differentiate into their charactersitic final forms, the increasing complexity of the gut makes it more difficult to identify in later stages. At the end of th the development it lies among the complications of the mid-gut, instead of arching over it as in the normal; its walls remain thick, and do not acquire the wavy margin which in the normal is formed by the protruding inner margins of the fully-differentiated cells.

The splanchnic mesoderm spreads over the walls of the gut during its development and forms a layer of visceral muscle.

The yolk:

The bulk of the yolk is included in the primitive gut and is partly enclosed by the gut. As the primitive gut is absorbed, the yolk escapes into the

/body

body cavity wherever the gut wall is defective. The granules become distributed between the organs in the haemolymph, and are ingested by wandering cells presumably phagocytic blood cells. The anterior mass of yolk which is usually isolated in the head region remains unabsorbed. Often, yolk breaks out of the embryo at the furrow, and comes to lie between it and the vitelline membrane (fig. 36).

The body wall:

Posteriorly to the furrow the body wall is formed almost normally. Where dorsal closure has been defective there is a break in this region, but elsewhere it resembles the normal in essentials. Except for the occurrence of this break in some embryos, the hypoderm encloses the tissues completely. It is divided into segments, though these are often distorted owing to the general disarrangement of the embryo. There are internal apodemes intersegmentally, to which are anchored the muscle fibres, which have arisen quite normally (figs. 31 ,33 and 36). Externally there is a cuticle, with chaetae. The body wall extends into the invaginated posterior tip.

Anterior to the furrow the case is quite different As explained above, little besides head material

/gets

gets into this region, but the body wall often extends into it along one side. The remaining tissues at the surface consist of so much of the pharyngeal wall as has not been invaginated, and of nervous tissue. The nervous tissue is not covered by hypoderm; it has not simply broken away at this region, but has actually never been formed (figs. 33 and 36).

The nervous system:

The chief peculiarity of the nervous system in the anterior region has just been mettioned, namely, that in the formation of the brain tissue only neuroblasts and their derivitives are formed, with no hypoderm cells enclosing them (figs. 33 and 36). In one instance, the same was noticed of the nervous tissue posterior to the furrow. That of the anterior region does not become a well-formed brain, and connectives with the ventral nerve cord are scarcely developed. Generally the nervous tissue fills the space not taken up by the pharynx or by the yolk mass (fig. 35).

In the posterior region the nervous system is relatively well formed, (fig. 33), though the nerve cord is disturbed by the distortions of the body segmentation. Its position is usually lateral

/with

with respect to the orientation of the chorion, indicating the rotation which the posterior region of the embryo undergoes. Ganglion cells and fibres are differentiated, and the disturbed arrangement of the fibres reflects the distortions of the segmental arrangement.

The nervous system is laid down along the whole length of the body, including the part which is invaginated posteriorly. Concentration of the nervous system occurs, which often has the effect of "spilling" the nervous tissue out anteriorly, since there is not an anterior wall at the furrow to hold back this accumulation of nervous material. Here, together with yolk granules which have also broken out, it fills up the space between the vitelline membrane and the point at which the embryo is constricted (fig. 36).

The tracheae:

The disturbances of the other systems distort the distribution of the tracheae very much. Their finer branches probably do not develop, but it would in any case be difficult to detect them. The most obvious feature of the tracheal system concerns the posterior tracheal trunks, which are larger than

/normal

normal, possibly because the tracheal material is not distributed so widely as in the normal. They open internally into the invaginated posterior end of the embryo (figs. 31 and 36).

The gonads:

These have been described in the section on the mid-gut. They are usually normal in appearance, and occur in about their customary position. Some embryos occur in which only one gonad is present. All parts of the gonad are present; the germ cells are enclosed in a gonad sheath, which is embedded in the fat body tissue.

The effect of constricting normal embryos:

Since many of the abnormalities described above appear to be caused by the presence of the deep furrow anteriorly, it was thought worth while to attempt to imitate it experimentally, to see whether any of the secondary abnormalities would be reproduced.

A normal Oregon K stock was taken, and embryos were ligatured, with a fine nylon thread, at about one third of the distance from the anterior end of the embryo, between the 4th and 5th hours of development, i.e. at the time and the place at which the

/furrow

furrow becomes deep in the mutant embryos. They were allowed to continue their development for approximately 24 hours, when they were fixed and sectioned.

Only a small-scale experiment was made, owing to the technical difficulty of ligaturing the embryos. These have been described by Pauli (Pauli, 1927), who ligatured Calliphora eggs shortly after fertilization in order to test their capacity for regulation. Their small size made manipulation difficult, and the high turgor pressure within the egg usually caused the vitelline membrane to burst when the ligature was tightened; only 18 out of 250 attempts were successful. In the case of Drosophila these difficulties are increased by the egg being only half the size of that of Calliphora, and in this study, out of approximately twenty attempts, only two embryos were constricted and brought to the completion of development. The method used was to place the egg on a small piece of food medium on a slide, with the nylon thread underneath it. When the food had dried, and the chorion stuck to it, the thread was knotted and pulled tight at the required region. It was possible to constrict the embryos perceptibly before the bursting point was reached.

The results were similar in both the successfully

/treated

treated embryos. Development in the posterior region of the body was normal, except for disarrangement of the organs. In the anterior region involution of the head had been prevented, and the pharynx region was small and grossly disorientated; the rest of the region consisted of nervous tissue, with no covering of hypoderm (fig. 40). Unfortunately, some degeneration had taken place here, and it is possible that the absence of hypoderm was secondary.

The ligatured embryos resemble the mutant ones in these two particulars; the significance of this result is taken up in the discussion.

Other abnormal embryos:

In tabular analysis of the sectioned material, it was noted that nine embryos occurred which were abnormal but were not of the characteristic mutant type.

Six of these were embryos in which the central material was that of the unfertilized egg, but in which there was also a variable amount of cellular material, distributed unevenly, but chiefly at the two extremeties. This consisted of small, rounded, undifferentiated cells. In the other three embryos, none of the material of the unfertilized egg remained; they consisted of partly differentiated cells with no structural organization.

/Discussion

Discussion

Expression of the lethal factor:

None of the embryos which are hemizygous for this factor survive beyond the embryonic stage. The characteristic abnormalities in their development are well-marked, with relatively little variation between individuals. The small proportion of abnormal embryos which does not fit into the general pattern show disturbances at the earliest stage of development, in the distribution of the cleavage nuclei, and little or no differentiation occurs subsequently. Rather similar disturbances result from the absence of the whole or half of the X-chromosome (Poulson, 1940), from ultrasonic treatment (Selman and Counce, 1953), and, in Calliphora, from centrifugation (Pauli, 1927). Similar abnormal embryos also occur in most of the other mutant stocks in the present study. It is possible that, under certain circumstances these early disturbances are caused by the Lff ll factor (the question of variation in expression is discussed later; page 212); but in this case it is equally likely that they have resulted from some other cause, possibly from another lethal factor segregating in the stock, or from occasional abnormalities in the formation of the egg in the female. Such a maternal origin has been found for

/somewhat

somewhat similar early abnormalities in the deep orange mutant (Counce, 1953).

Causal analysis of the mutant development:

The absence of much variation makes this mutant a favourable one for an analysis of the hierarchy of developmental abnormalities which culminate in the final aggregation of structural defects. The latter are the end effects of the factor; they are caused by preceding intermediate effects, which in turn are caused by preceding primary effects.

The relation of the end effects to the intermediate effects has been suggested above. Those in the anterior region probably are related to the presence of the deep constricting furrow; some experimental support for this is given by the result of the ligaturing experiment, in which similar abnormalities were produced in normal embryos simply by mechanical constriction. The absence of hypoderm over the nervous system is not easily explained, and will be discussed later.

The end effects on the region posterior to the furrow can be related to the disturbance of shortening and also, in some cases, to the failure of dorsal closure. These two processes are closely integrated, and the abnormality may be considered one of the general

/surface

surface movements, of which the disturbance of shortening is the most evident feature.

There is no obvious reason for the partial failure of shortening, but it is unlikely that it should not be causally related to the earlier development of the germ band. This occurs in its extension, when the posterior mid-gut rudiment invaginates posteriorly, and the germ band is also invaginated so that it comes to lie inside of the egg rather than along its dorsal surface. This abnormal postion appears to prevent its proper withdrawal later, in shortening, possibly because of the displacement of the mid-gut rudiment and proctodaeum at its tip.

Thus there are two abnormalities which may be considered the principle intermediate effects of the factor:

1) The precocious invagination of the posterior mid-gut rudiment posteriorly, and the consequent abnormal postion of the dorsal part of the germ band

2) The presence of the deep constricting furrow.

The question arises as to whether these can be related to a common cause.

It was suggested above (page 64), that the abnormality in the invagination of the posterior mid-gut rudiment and the germ band was a mechanical effect of the abnormal thinning of the dorsal region of the

/blastoderm

blastoderm at the time of gastrulation. This is the first observable effect of the factor, and may be considered its primary effect. It remains to consider whether the furrow may also be related to it.

The mutant furrow, the cephalic furrow, and the mechanics of gastrulation:

The invariable position of the constriction, one third of the distance from the anterior end of the embryo, coincides with that of the cephalic furrow. In some sense the mutant furrow appears to be a modification of the cephalic furrow, and if more were known about the latter it would be easier to say what processes the mutant factor had interfered with. But the cephalic furrow is a transient structure, of unknown significance. Poulson suggests that it is "the definitive boundary between the head and body regions", Sonnenblick that it "acts as a buffer during a period of extensive and vigorous cell movements" (Sonnenblick, 1950). Both may be right so far as they go, but their suggestions are too imprecise to serve as explanations. Certainly the furrow is part of a complicated rearrangement of the surface material, but whether it is a purely passive result of other surface movements, and, if so, what movements; or whether it initiates them; these

/cannot

cannot be decided until much more is known about the mechanics of the gastrulation process.

Little is known about the forces involved in early morphogenetic movements, even in amphibians, in which they are less complicated, and have been particularly studied. In Drosophila, early studies on the mechanics of gastrulation were only descriptive (Parks, 1935), and later work has resulted in a more adequate description, but not an explanation. The same is true for other insects. Some studies have been made on the forces involved in blastokinesis in grasshoppers (Andrewartha, 1943; Matthee, 1951), which may be relevant, since it has been suggested that all the changes in length and the movements of the germ band are related to this process (Johannsen and Butt, 1941). In particular, abnormalities in katatrepsis, which may be related to shortening, have been tentatively connected with abnormalities in the accumulation of amniotic fluid, which is held to initiate this process (Andrewartha, 1943).

Considerations of gastrulation must therefore be entirely speculative. There seems no way in which the mutant furrow could be caused by the dorsal thinning directly, but there are at least two possible ways in which it could be related ot it indirectly,

/through

through the abnormality of the posterior mid-gut invagination.

The first of these is suggested by the appearance of the furrow when it begins to deepen. At this stage it resembles the proctodaeal invagination; anteriorly it is bounded by embryonic membrane, posteriorly its columnar cells have the appearance of those of this structure in the normal embryo. Moreover, it is often difficult to make out a distinct proctodaeum between the posterior mid-gut and the germ band in the mutant. These two points suggest that the proctodaeal material may normally be invaginated from the lateral blastoderm, and that in the mutant, while the mid-gut rudiment is invaginated posteriorly, the proctodaeal invagination occurs separately at its usual position just behind the cephalic furrow. Isolated from its normal embryonic context, it merely becomes a deep groove of invaginated material. However, in its later later development it clearly shows itself to have been derived from potential hypoderm material, forming tracheal pits, and later apodemes to which muscles become attached, and secreting cuticle. Since there is no evidence for the transformation of proctodaeal material into hypoderm, this explanation must be abandoned.

This poses the problem why there should be an

/invagination

invagination of material at this point in the mutant. The invagination of the material of the posterior mid-gut and of the proctodaeum has taken place posteriorly and in the normal the cephalic furrow is at no time more than a shallow groove. A possible explanation suggests itself upon consideration of the probable nature of the gastrulation process.

The movement of the mid-gut rudiment over the dorsal side of the egg to the point of invagination anteriorly is very rapid, taking about half an hour. Sonnenblick suggests that it is brought about by the rapid mitotic division of the group of cells immediately behind the rudiment (Sonnenblick, 1950). But the movement seen in the film, even when allowance is made for the time-lapse magnification of the speed, suggests a sudden initiation of the movement, depending on physical forces rather than upon cell division. Moreover, in vertebrates, particularly in studies of amphibia, it has been found that the early morphogenetic movements are due to mass movements of the egg resulting from its submicroscopic structure and physical properties, and that its division into cells is irrelevant to them (Waddington, 1940a; Holtfreter, 1943 and 1944). Holtfreter showed that a pseudo-gastrulation occurs in unfertilized ageing frogs' eggs, without their division into cells.

/The

The morphogenetic movements which comprise "gastrulation" in insects and "gastrulation" in vertebrates are probably sufficiently analogous to warrant the supposition that the mechanisms of each are fundamentally similar, and that in insects as in amphibians they are movements of the whole egg mass. Three things support this supposition:

1. In making the film, certain of the eggs set up did not develop, and were presumed to be unfertilized. The film of these eggs revealed massive movements of their material, though there were no visible developmental changes.
2. Studies of the differentiation centre in Platycnemis (Seidel, 1936) showed that massive movements do occur in the yolk, contractions of which initiate the development of the germ band at a particular point.
3. In unfertilized eggs of Drosophila the contents - cytoplasm and yolk granules - coagulate after a few hours into large masses. In many of the unfertilized eggs which occur in the sectioned material (30 out of 67 in the present series) two main masses are formed, separated at about the region of the cephalic and the mutant furrows (fig. 39). This may be a coincidence, possibly caused by the

/presence

presence of the degenerating female pronocleus in this region acting as a condensation centre for the anterior mass. But it is also possible that it results from a pseudo-gastrulation of the egg substance, similar to that occurring in ageing frogs' eggs.

The film shows that in normal gastrulation, during the extension of the germ band, there is not simply a forward movement of the posterior mid-gut rudiment, followed by the germ band material; but there is a movement of material from both ends of the embryo, towards the region of the cephalic furrow, where the two streams appear to meet and move inwards. If it is the case that there are general movements of the egg material involved this may represent an expansion of the surface material comparable to the epiboly of the animal pole tissue in amphibians. Only the general direction of the movement can be seen in the film, not the dispositions of material before and after it has taken place. The cephalic furrow may represent the first result of the meeting of the two streams, and the pronounced inward movement normally becomes coincident with the proctodael invagination. As this deepens the furrow tends to straighten out, and almost merges with the latter dorsally.

Whatever the details of the process, some such

/mechanism

mechanism - a mass movement of the surface material, with a predisposition to invagination at this region - might account for the mutant furrow in the following way. The posterior mid-gut rudiment, the proctodaeal material, and the germ band are invaginated precociously, at the posterior end, owing to the mechanical circumstance of the blastoderm thinning dorsally. This would not interfere with the tendency of egg material to invaginate anteriorly, at the point where the rudiment of the proctodaeum would normally be turned in; in their absence the invagination merely produces a supererogatory furrow. Shortening does not affect it, since it has no connection with the germ band, and it becomes deeper as its cells proliferate, with the consequences that have been described.

The primary disturbance:

It has been explained that the primary effect of the factor is to cause an unusual thinning of the dorsal part of the blastoderm immediately prior to the forward movement of the germ band over the dorsal side of the egg. A similar process occurs in those parts of the blastoderm not incorporated in the embryo proper at a slightly later stage, to give the embryonic membranes. It is likely that the dorsal thinning in the mutant represents a precocious formation of this membrane material, and that

/the

the primary disturbance is of the time at which this process occurs. The precocious formation of tracheal pits shortly afterwards suggests that there is a general acceleration of the development of the ectoderm.

The absence of hypoderm over the nervous tissue anteriorly:

In this mutant the organization is disturbed but cell differentiation is relatively normal. The differentiation of the ectodermal material of the head is an exception, for here nervous tissue is formed, but apparently little or none of it gives rise to hypoderm and the nervous material remains uncovered. The result of the ligaturing experiment suggests that a similar disturbance of differentiation occurs when there is simply a mechanical constriction in this region. It is difficult to envisage how a constriction could have this result, though possibly it might restrict the supply of a necessary substance. The balance between nervous tissue and hypoderm appears to be a delicate one. In normal development the neauroblasts of the brain are said to differentiate at the surface, and to be only later covered by hypoderm (Poulson, 1950). Similar failure of the hypoderm over neru nervous tissue occurs in other mutants in this study, and it is a major element in the developmental pattern of X20 (page 179).

/Conclusions

Conclusions

Lff 11 is a recessive lethal factor on the 1st chromosome; its lethality is expressed in the embryonic stage in all cases. Abnormalities appear early, but the embryo survives through the whole of the normal period of development, though it does not hatch. It shows relaively few disturbances in the differentiation of cell types, but there are large disturbances in its organisation. A variety of end effects is caused by two principle intermediate effects: 1) the presence anteriorly of a deep constricting furrow, 2) abnormalities in the invagination of the posterior mid-gut rudiment and in the extension of the germ band in gastrulation. These intermediate effects are the consequences of a single primary effect, a disturbance in the time of formation of the embryonic membranes which leads to an abnormal thinning of the dorsal region of the blastoderm prior to gastrulation.

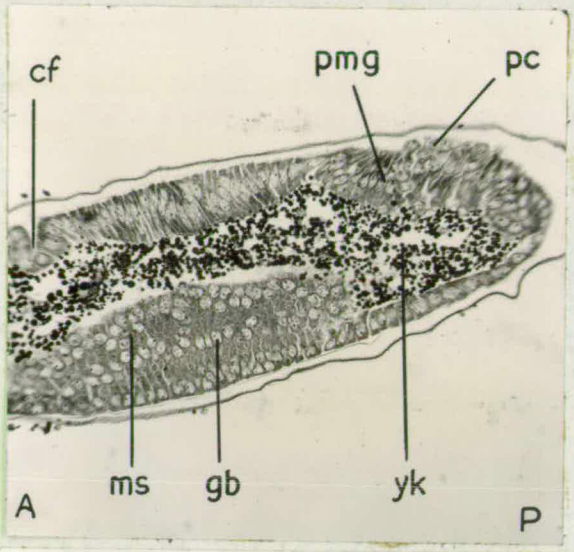
Fig.22. L.S. normal, 4-5 hours, x200.

Fig.23. L.S. normal, 4-5 hours, x320.

Fig.24. L.S. Lff 11, 4-5 hours, x325.

Key to figs. 22-44.

A, anterior.
amg, anterior mid-gut rudiment.
bn, blastoderm nuclei.
br, brain.
cf, cephalic furrow.
ch, chorion.
chf, chorionic filament.
em, embryonic membrane.
fs, frontal sac.
gb, germ band.
gnso, sub-oesophageal ganglion.
gon, gonad.
hg, hind-gut.
hy, hypoderm.
ilb, inner limit of blastoderm.
lig, position of ligature.
mc, mutant constriction.
mf, mutant furrow.
mg, mid-gut.
mic, micropyle.
mmg, middle mid-gut.
ms, mesoderm.
mt, malpighian tubule.
mus, muscles.
nbl, neuroblasts.
nca, nuclear area.
nt, nervous tissue.
oes, oesophagus.
P, posterior.
pc, pole cells.
ph, pharynx.
pi, proctodaeal invagination.
pmg, posterior mid-gut rudiment.
pmus, pharyngeal muscles.
pr, proctodaeum.
pv, proventriculus.
sg, salivary gland.
spm, splanchnic mesoderm.
st, stomodaeum.
tr, trachea.
trp, tracheal pit.
uyk, unenclosed yolk.
vm, vitelline membrane.
X, fixation puncture.
y, yolk granules.
yk, yolk.
ykcy, yolk and cytoplasm.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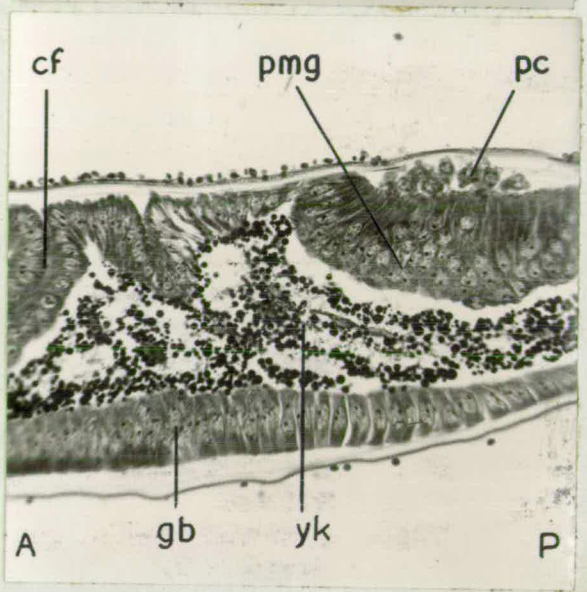
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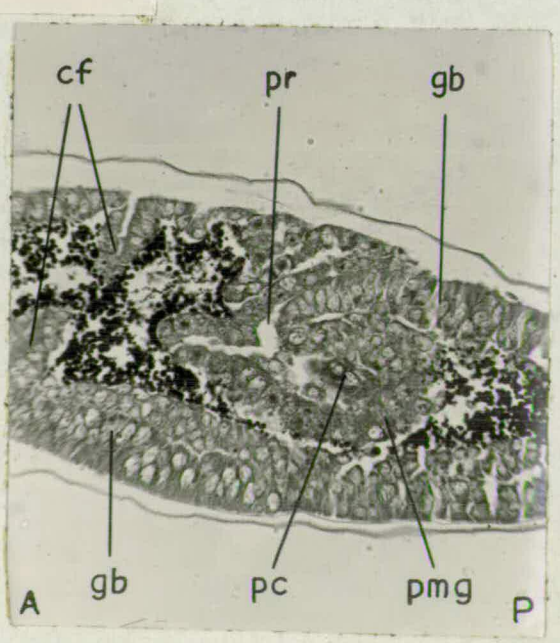
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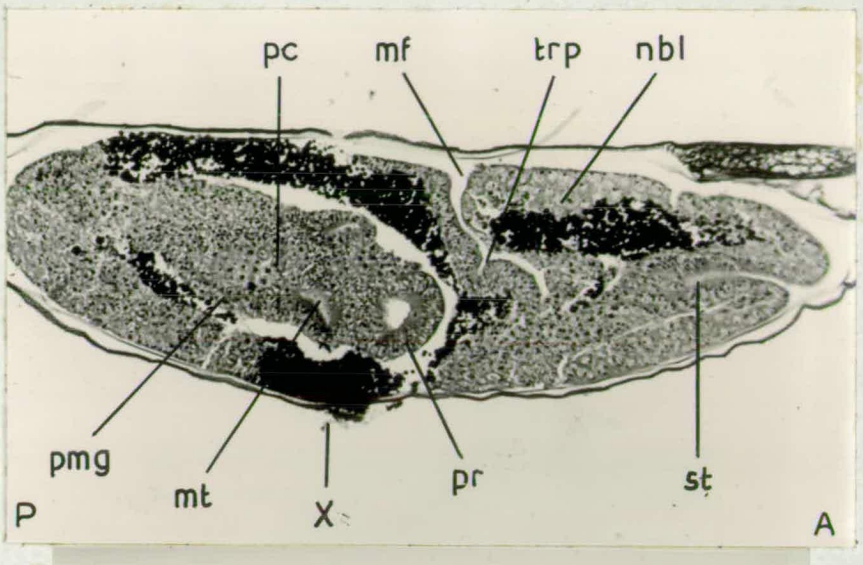
Fig.25. L.S. Lff 11, 5-6 hours, x300.

Fig.26. L.S., the same embryo, x320.

Fig.27. L.S. normal, 5-6 hours, x320.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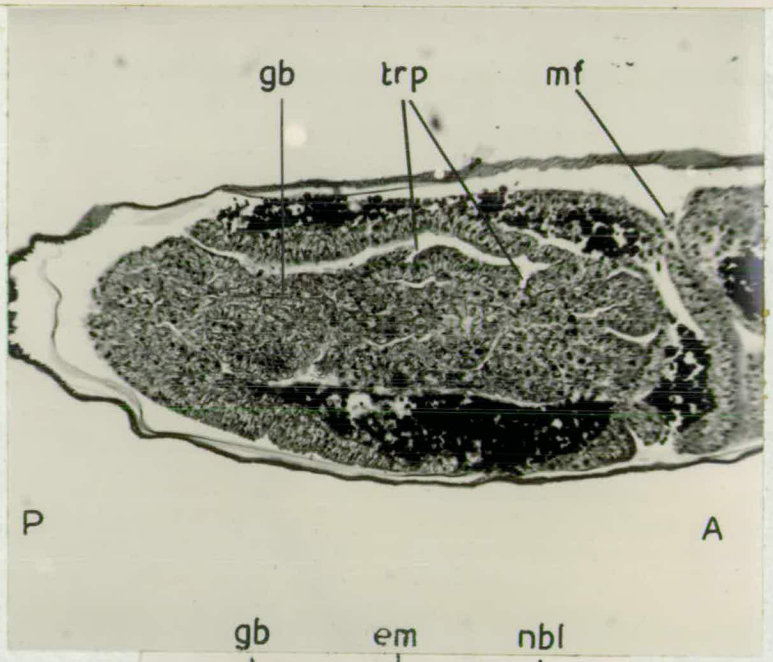
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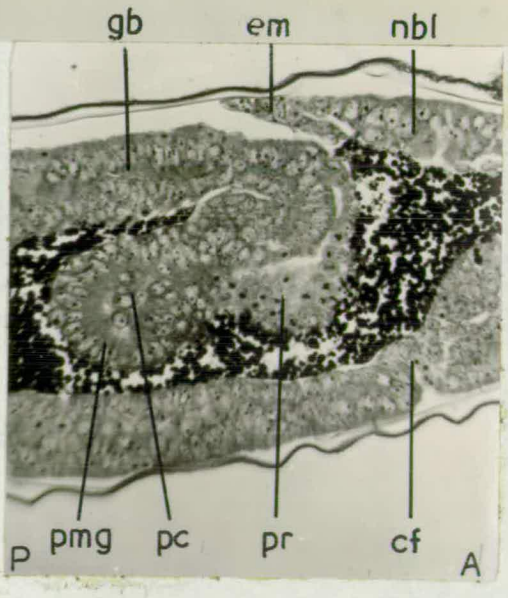
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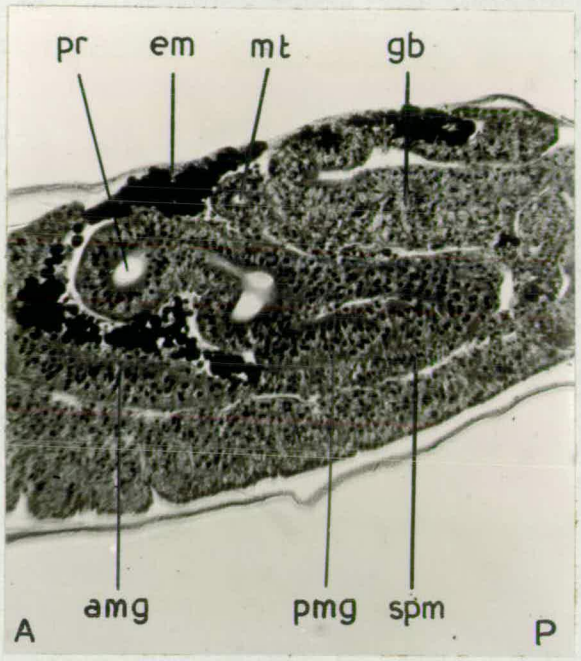
Fig.28. L.S. Lff 11, 9-10 hours, x375.

Fig.29. L.S. normal, 8-9 hours, x320.

Fig.30. L.S. normal, 10-11 hours, x320.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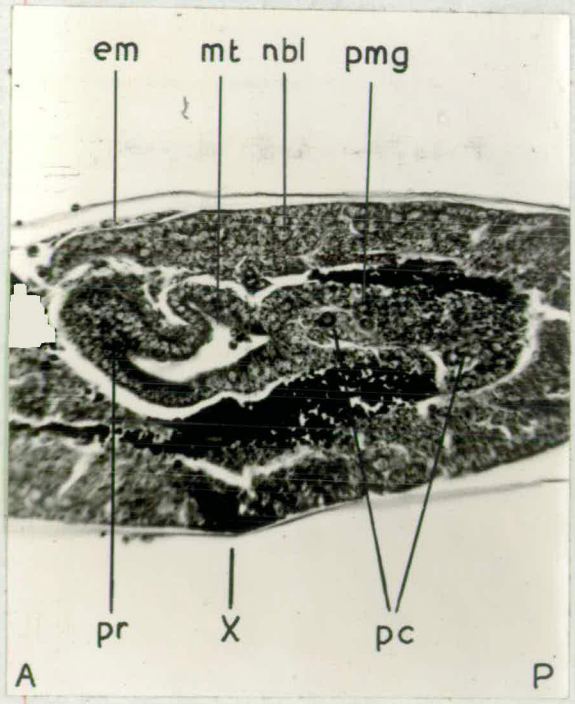
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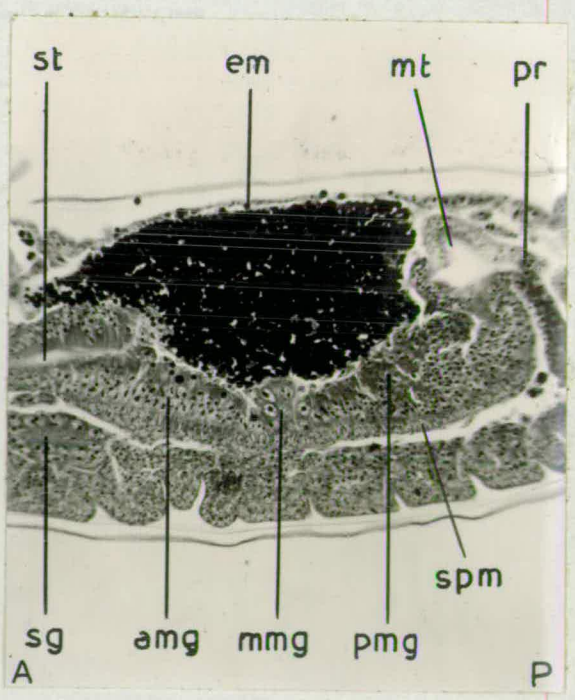
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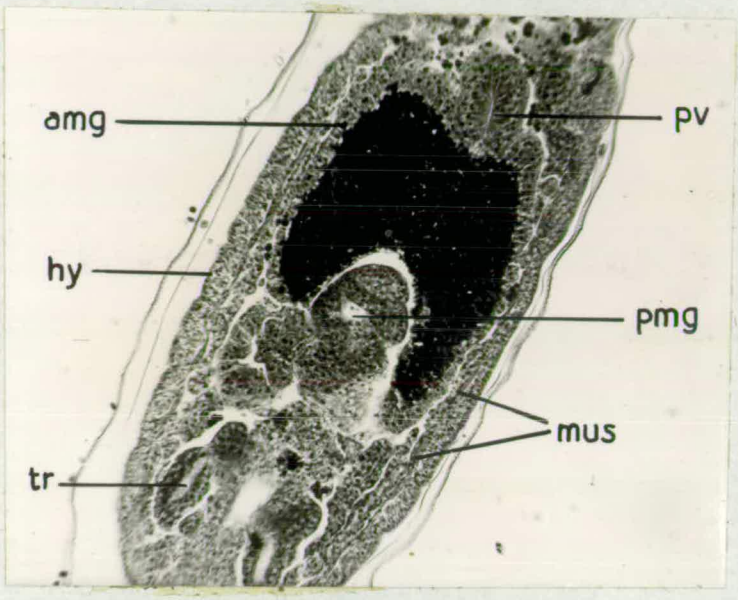
Fig. 31. H.S. Lff 11, 12-13 hours, x320.

Fig. 32. H.S. normal, 10-11 hours, x320.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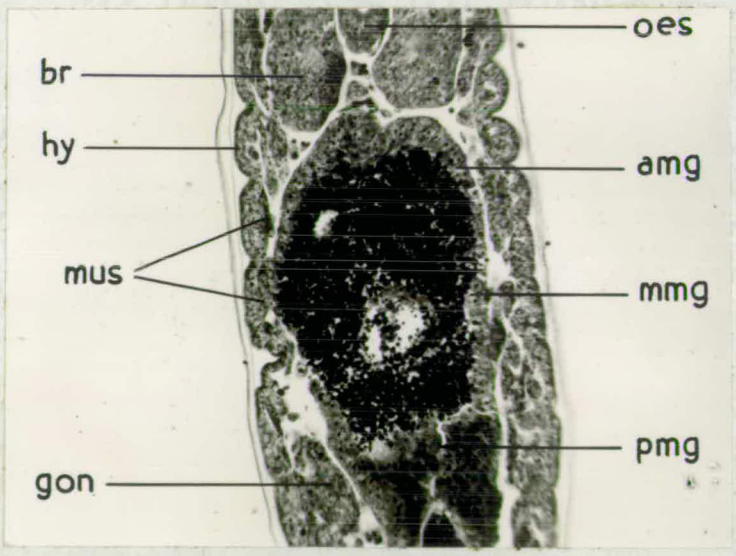
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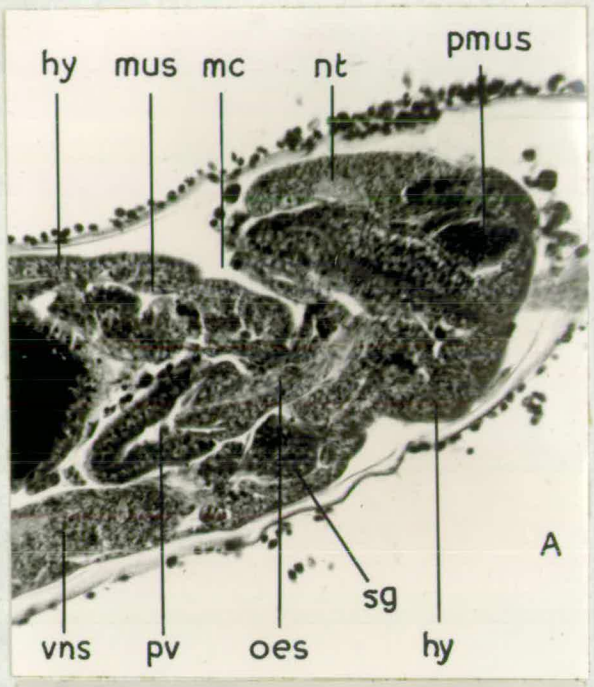
Fig. 33. L.S. Lff 11, 14-15 hours, x375.

Fig. 34. L.S. normal, 12-13 hours, x260.

Fig. 35. H.S. Lff 11, 14-15 hours, x 260.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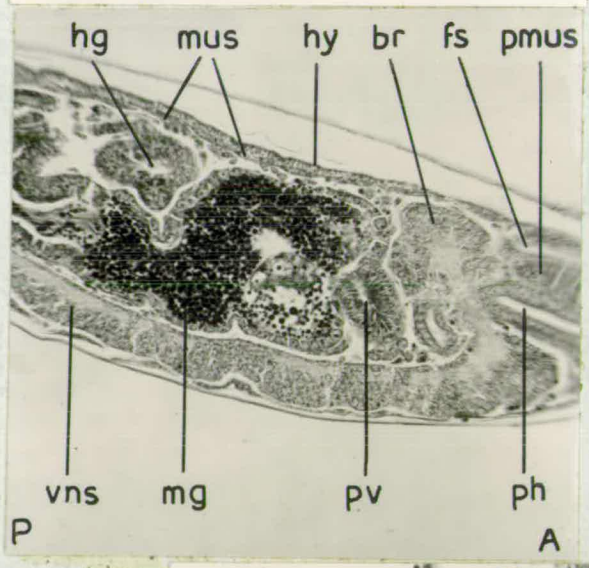
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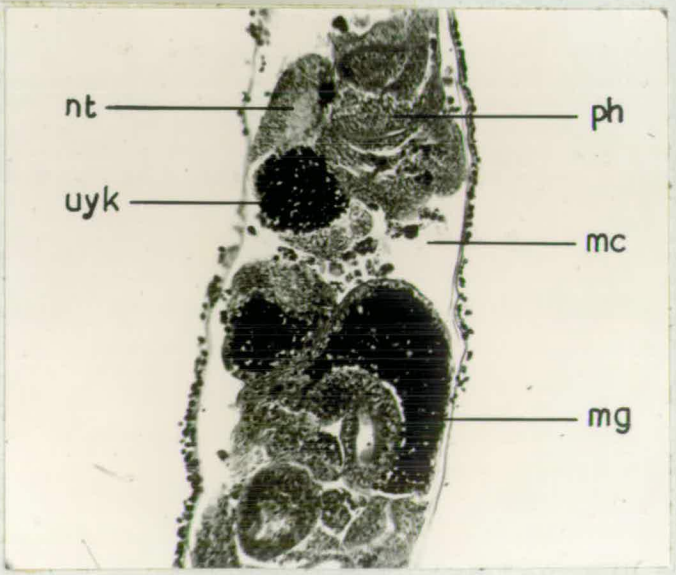
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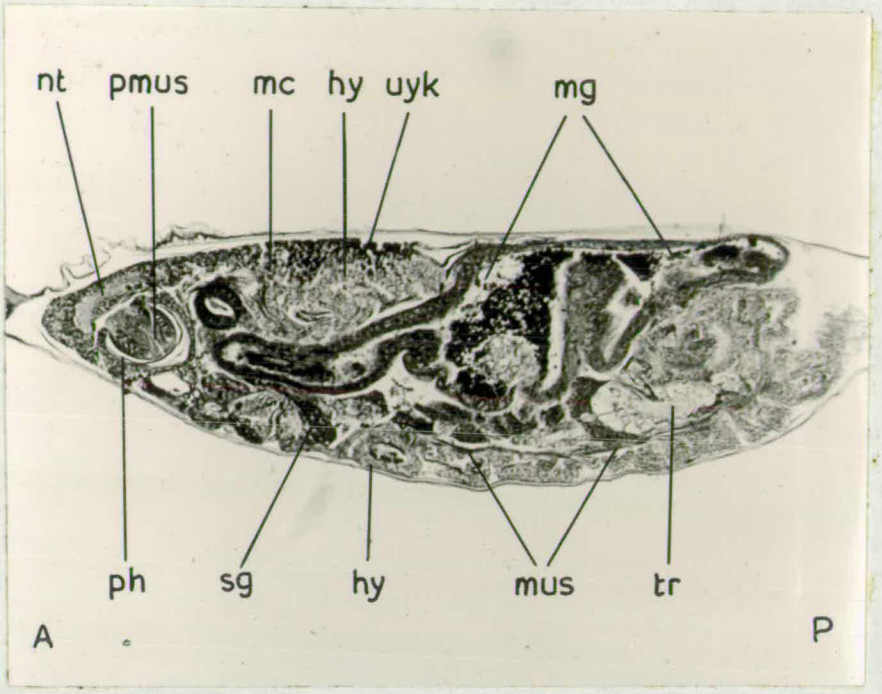
Fig. 36. L.S. Lff 11, 25-26, x300.

Fig. 37. L.S. normal, fully developed embryo, x300.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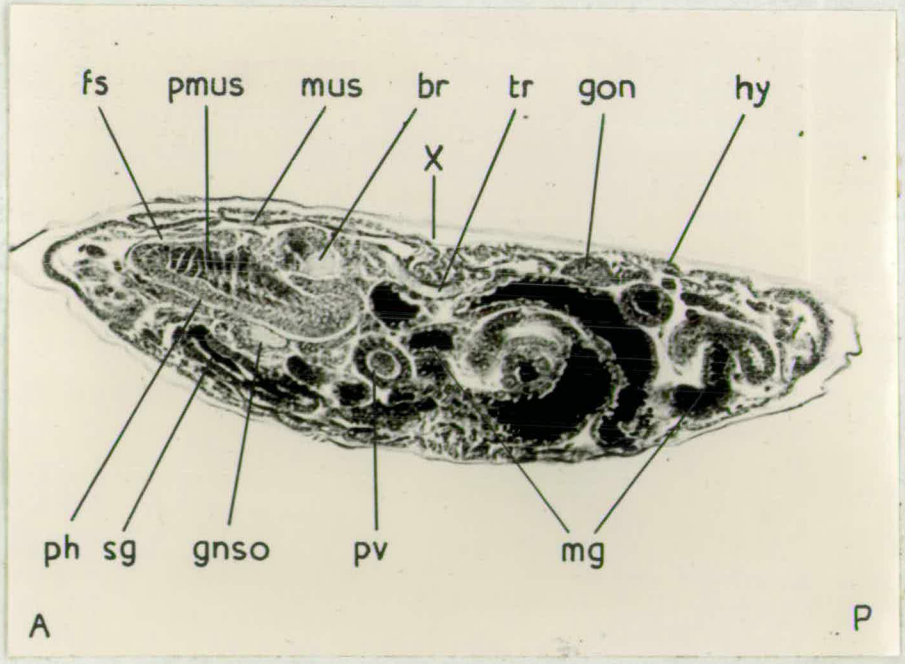
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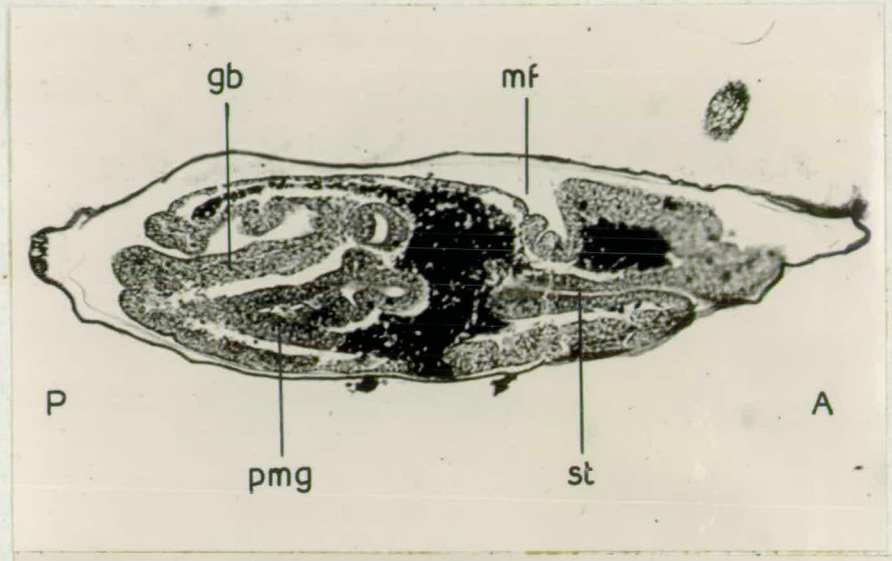
Fig. 38. L.S. Lff 11, 6-7 hours, x300.

Fig. 39. L.S. unfertilized egg, 5-6 hours, x300.

Fig. 40. H.S. embryo ligatured at 4 hours, 26-28 hours, x320.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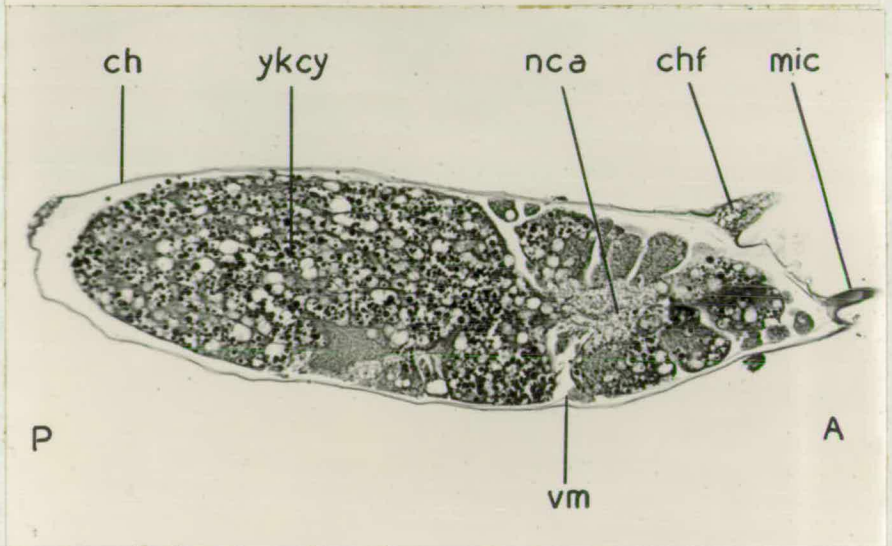
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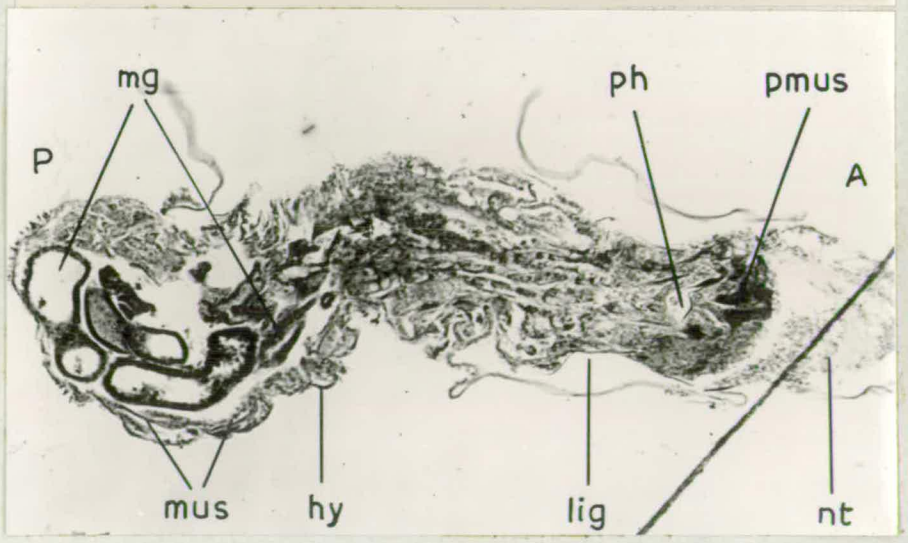
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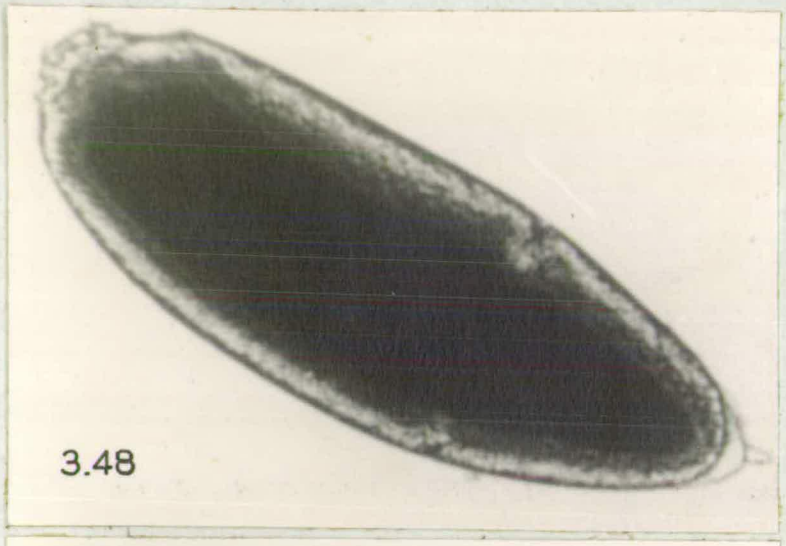
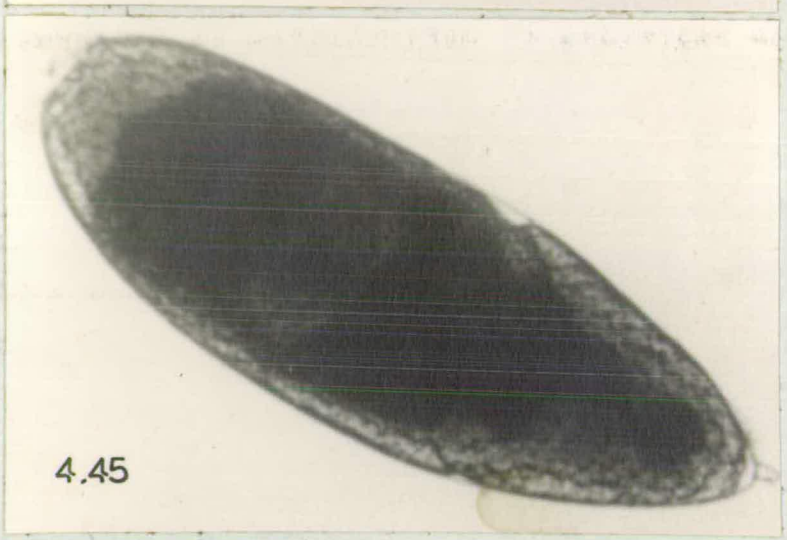
Fig.41. Lff 11: film sequence, from the beginning of gastrulation to 6 hours.


3.48


4.45

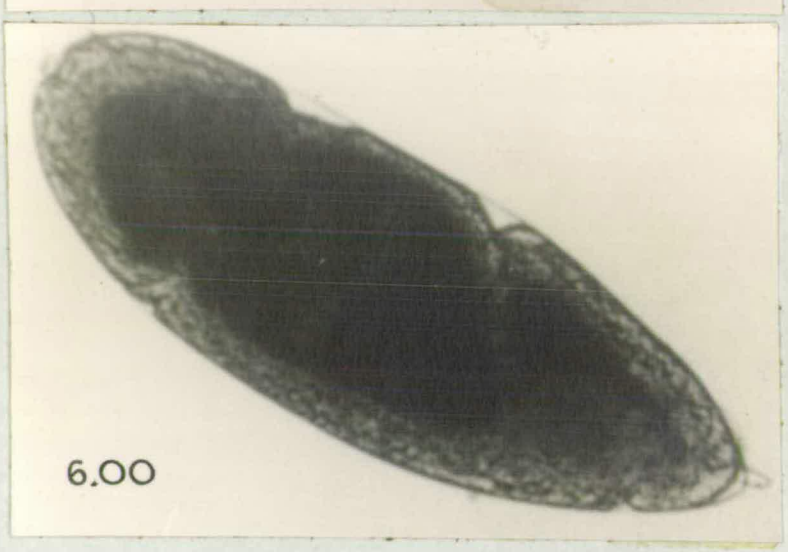
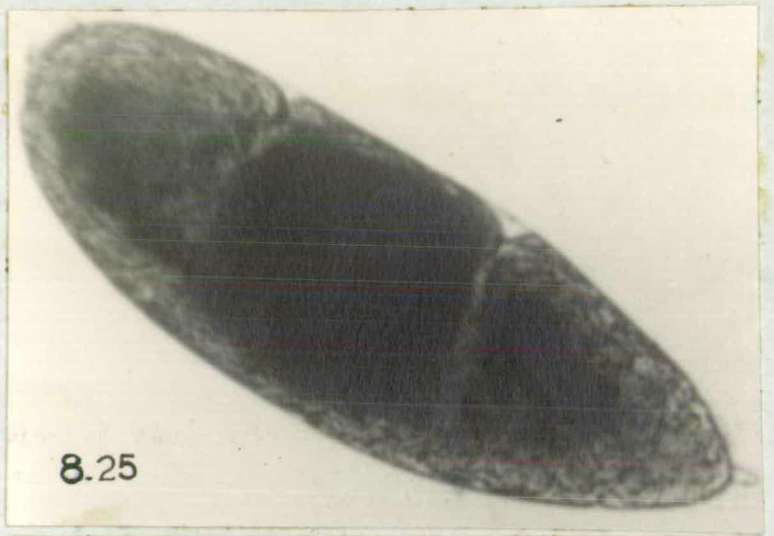
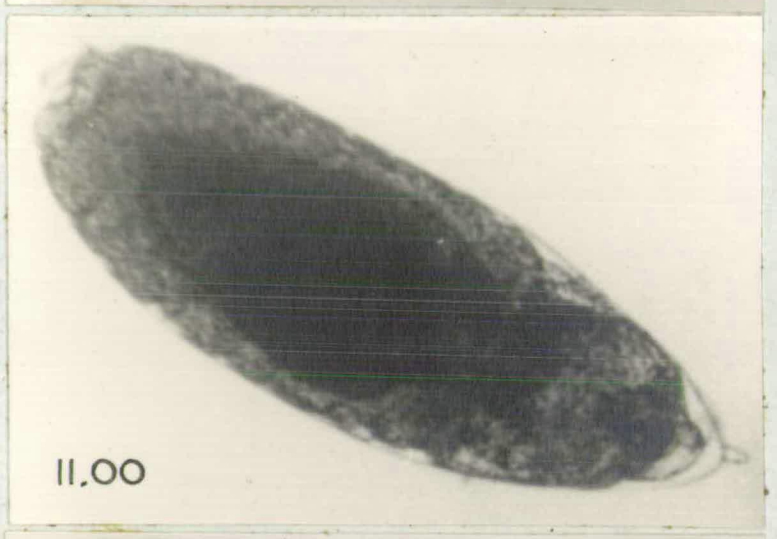

6.00

Fig.42. Lff 11: film sequence, from 6 hours to the end of development.


8.25


11.00


17.40

Fig. 43. Lff 11: film sequence, from fertilization to 6 hours.

For corresponding photographs see fig. 41.

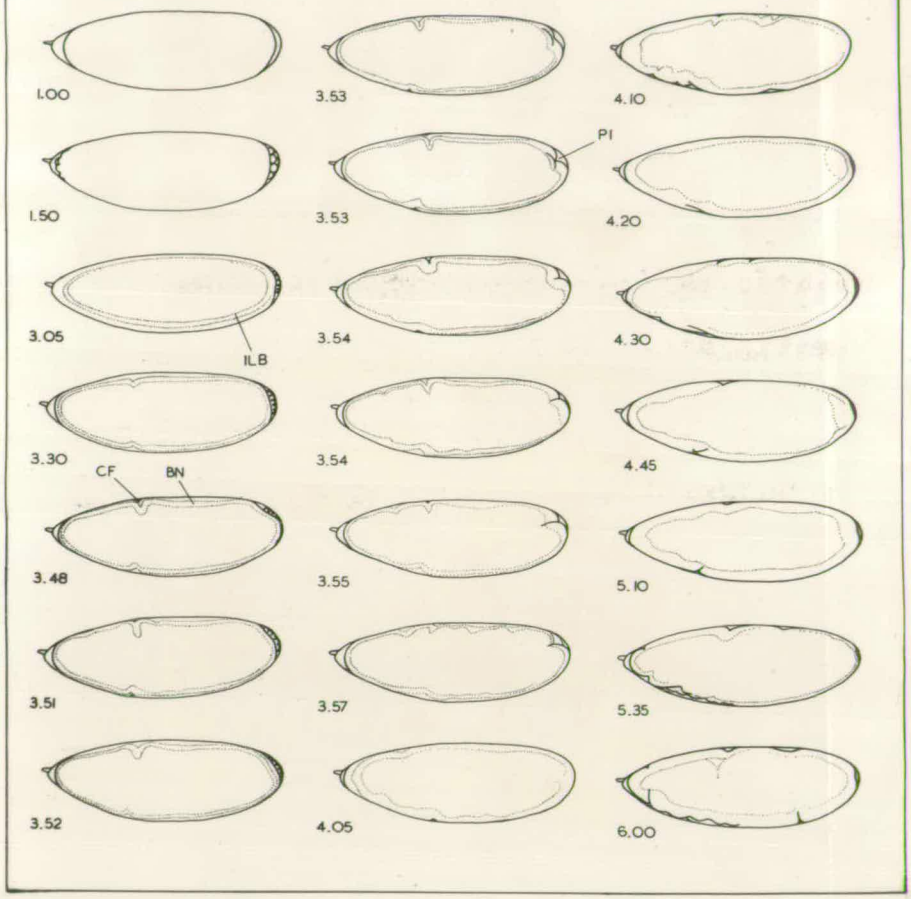
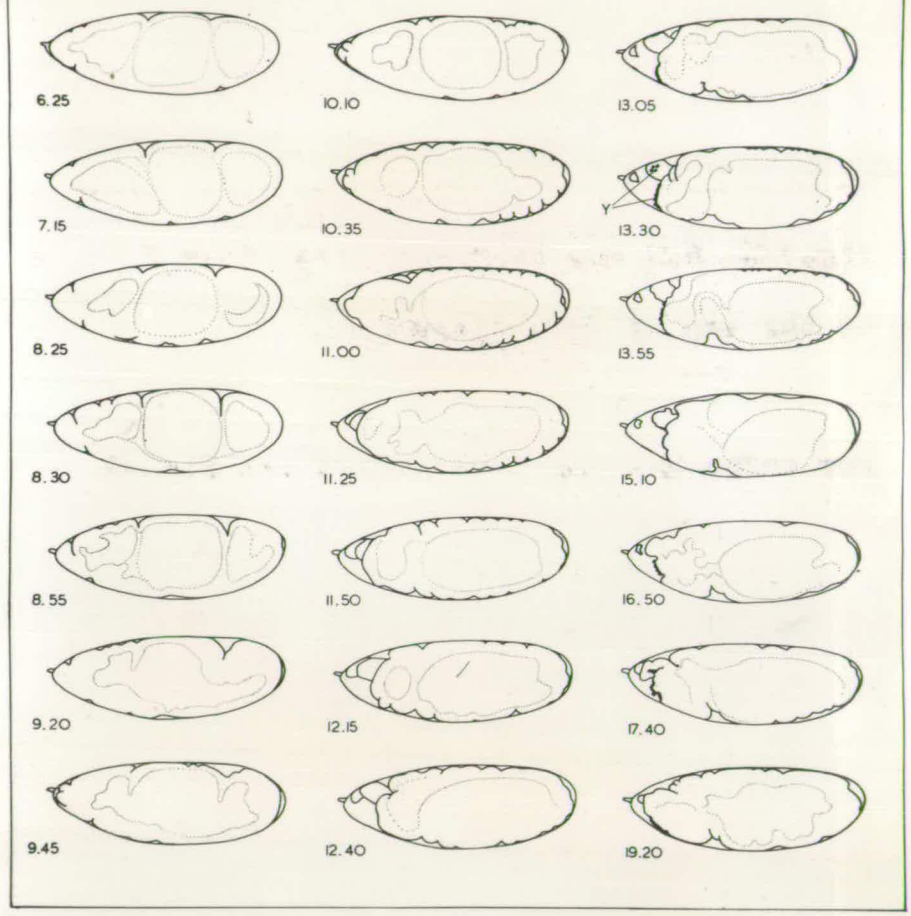

1.00
3.53
4.10
PI
1.50
3.53
4.20
3.05
ILB
3.54
4.30
3.30
3.54
4.45
CF
BN
3.48
3.55
5.10
3.51
3.57
5.35
3.52
4.05
6.00

Fig.44. Lff 11: film sequence, from 6 hours to the end of development.

For corresponding photographs see fig.42.


6.25
10.10
13.05
7.15
10.35
13.30
8.25
11.00
13.55
8.30
11.25
15.10
8.55
11.50
16.50
9.20
12.15
17.40
9.45
12.40
19.20

Mutant X2

Material:

Observations were made upon living embryos, and one was filmed. The description of development is based chiefly upon sectioned material.

Preliminary data:

The factor was induced by X-irradiation of sperm, and a preliminary salivary gland chromosome analysis shows that there is no large rearrangement, and that it is located near the forked locus (56.7) (H.Slizynska, unpublished).

Analysis of sectioned material:

Period	Unfert-ilized	Unident-ifiable	Normals	Mutants
0-5hrs.	10	17	97	14
5-18	28	-	195	69
23-30	3	-	-	23
Totals	41	17	292	106

In the period between 5 and 18 hours, when mutants are distinguishable and none of the normals have hatched, 69 out of 264 fertile eggs - approximately a quarter - produced mutant embryos. The

/factor

factor may therefore be considered as being expressed completely in the embryonic stage.

Final Appearance of the Embryo

No mutant embryos hatch, but they may be seen moving actively inside the vitelline membrane at the time when the normals are doing so. The embryo is then at an advanced stage of development, with the cuticle and chaetae well developed on the segments. But the latter are disarranged and the whole embryo is distorted. The hypoderm is often incomplete, and the nervous tissue exposed in parts; parts of the gut appear externally at both the anterior and posterior ends; there are often accumulations of undigested yolk between the embryo and the vitelline membrane. Some of these featured may be seen in the final shot from the film (fig. 54, 25.00). Cell degeneration begins at about 25 hours.

Development of the Embryo

Events up to gastrulation:

In the majority of cases the development of the mutant appears not to differ from that of the normal

/up

up to the time of gastrulation. The embryo which was filmed showed completely normal blastoderm formation (fig. 53, 3.15, and compare fig. 55, 1.00-3.30 with fig. 9, 1.30-3.30). But two sectioned embryos showed a striking abnormality which has not occurred elsewhere. In these a complete syncytial blastoderm was formed, with the nuclei at the periphery as usual; but the nuclei were not uniform with respect to size or stage of mitosis. Over some areas the nuclei were of normal size and in resting phase, and were crowded t together so that they tended to buckle upon each other. In other areas resting phase nuclei were only about half the size, and in other there were crowded mitotic figures (fig. 45). In one of these embryos pole cells occurred, but not in the other.

In the normal embryo mitoses are synchronous up to the formation of cell boundaries. In these abnormal cases there must have been a disturbance of the factor controlling nuclear division, which probably occurred in some of the cleavage nuclei during their migration to the periphery. This would account for the patchy distribution of the irregularities, each patch representing the descendants of the early cleavage nucleus whose divisions had got out of phase. In several other embryos there appeared to be less

/striking

striking irregularities in the blastoderm, but it was not possible to classify them as certainly abnormal. Probably disturbances in the control of nuclear division represent the primary effect of the mutant factor; but the time at which the factor comes into action varies. Usually its effects are not apparent at this stage, but in the two abnormalities described they appear unusually early.

Gastrulation:

There is no disturbance of the mesoderm in later development which would suggest that there is ever a failure of the first stage of gastrulation, in which the mesoderm material is turned in at the ventral furrow. In the filmed embryo the next stage, the formation of the cephalic furrow, is accomplished, and there is the buckling of the dorsal cells of the blastoderm which normally precedes the third stage, the extension of the germ band. But the extension itself does not take place (fig. 53, 3.50 and 4.20; fig. 55, 3.35-4.20). A similar sequence of events probably occurs in most of the mutant embryos; in some the disturbance may occur earlier, but in none does it occur later, than during the last stage of gastrulation.

/The

The film shows the posterior mid-gut rudiment as a shallow depression, arrested in its forward movement not far from the posterior end of the embryo (fig. 53, 4.20). Sections of other embryos show that its cells do not take on the elongate flask-shape normally characteristic of it; instead the rudiment forms as a depression of the blastoderm, whose cells tend to be cuboidal rather than columnar. Pole cells are apparently often reduced in size and number and it becomes difficult to follow them in subsequent development.

Instead of the normal continuation of gastrulation in the process of extension, the mutant embryo becomes characterized at this time by the appearance of a number of deep furrows. These consist of blastoderm mterd material, chiefly of the ectoderm germ band and its underlying mesoderm, and they extend deeply into the interior of the egg. They vary in number; in some cases only a single one has been found to occur, but in most cases thereare several, so that the egg becomes entirely dissected by them (fig. 46). There seems to be no regular pattern in their arrangement, but the most common is fan-wise from the dorsal side. They do not show well in the profile view of the filmed embryo until rather later in development (fig. 58, 6.00).

The

The invaginations which occur in normal development — the posterior mid-gut invagination, the proctodaeum, stomodaeum and the anterior mid-gut rudiment, either do not occur or remain shallow. The material which would normally be turned in during their formation remains at the surface.

There are two principle abnormalities here:

1. The failure of the invagination of the ectodermal and endodermal material of the gut,
2. The formation of the furrows.

It is obvious that the presence of the furrows greatly increases the surface area of the egg, and hence the amount of ectoderm. This cannot be accounted for as consisting partly of the uninvaginated gut material, because later development shows that this is confined to the anterior and posterior ends, whereas the furrows are chiefly in the middle region. Nor can it be said that the germ band, being prevented in some way from extending over the dorsal side, simply elongates and buckles inwards; in other mutants (Lff 11, X27) extension is inhibited, and the film of Lff 11 shows a slight buckling of the germ band in consequence (fig. 4/, 6.00), but there is nothing corresponding to the fantastic furrow development of the present mutant. It is very difficult to make accurate counts

/of

of the cells at this stage, but it is evident that there are are many more than normally. This may probably be accounted for by the disturbance inthe control of mitosis mentioned above. There it was pointed out that in two embryos the blastoderm contained areas in which nuclei were smaller and more numerous than usual. It is likely that in most of the mutants an abnormal proliferation of cells occurs somewhat late during gastrulation rather than blastoderm formation. It was further noted that there was a tendency for the cells to be crowded and to buckle upon one another; this might initiate the buckling leading to the formation of the furrows in late gastrulation . It is possible that the furrows grow inward as the cells proliferate, but their formation must be very rapid, as sections rarely occur with shallow furrows which might be in the process of developing. It seems more likely that an excessive proliferation of cells at an earlier stage has resulted in the germ band consisting of many more cells than it normally does. It has been already suggested (page 96) that the extension of the germ band takes place too suddenly to be accounted for merely by cell division. The expansion of individual cells might play a considerable part and if this were so the expansion of the very crowded cells in the mutant embryo could very well account for the sudden appearance of the furrows

/just

just at the time when the germ band would normally be extending.

At the end of the gastrulation period the embryo presents an extremely characteristic appearance; the cephalic furrow persists and deepens, contributing with the other furrows tothe "dissected" appearance of the embryo. This appearance is particularly noticeable in the yolk which is ocmpressed between the furrows. The posterior mid-gut invagination is rudimentary, and the pole cells are frequently hidden in the crevices produced by furrowing posteriorly. There is no proctodaeal invagination, and there is at most a small stomodaeal invagination anteriorly. The dorsal layer of cells becomes thin forming the embryonic membrane over the dorsal side of the egg.

Differentiation between 5 and 9 hours:

Ectoderm:

That part of the ectoderm which gives the hypoderm and nervous tissue in the normal embryo differentiates in the same way in the mutant, except that in many cases no hypoderm is developed over the differentiating neuroblasts. The hypoderm becomes segmented laterally. Salivary gland rudiments are formed, but their invagination is often prevented by the distortion of the surface in

/their

their neighbourhood. Tracheal pits are also formed, in many cases within the furrows. The cells of the proctodaeum and the stomodaeum undergo differentiation externally at the posterior and anterior ends of the embryo respectively.

Mesoderm:

The mesoderm is difficult to follow at this stage, but myoblast cells become visible towards the end of it. Splanchnic mesoderm never becomes more than sparsely visible, probably simply because of the absence of well-formed gut to which it could attach itself.

Endoderm:

There is a great deal of mitotic activity at the anterior and posterior ends of the embryo during this period. This is chiefly in the cells of the endoderm rudiments, which are either not invaginated at this stage or are just inside the embryo. Each forms a group of rounded, darkly staining cells. The yolk is split up by the furrows, and rounds off into masses which each become enclosed by primitive gut cells. In most cases there is no connection with the endoderm.

In general the differentiation seems to lag behind that of normal embryos.

/Shortening,

Shortening, involution of the head, and dorsal closure:

The distortion of the embryo is most marked in the period 5-9 hours. The great disturbances inthe arrangement of the tissues, and in the shape of the embryo, naturally interfere profoundly with the general mass movements considered here. But they are not prevented altogether, and have the effect of "straightening out" the embryo to a surprising extent. A slowing of the differentiation of cells was mentioned above, and a similar lagging occurs in these movements, which even in their modified form are considerably slowed down.

Since there was no proper extension of the germ band there cannot be any shortening of it in the strict sense. But there is a concentration of the material of the ventral side, which has the effect of compacting the furrows and making them difficult ot distinguish externally (figs 47 and 48; fig. 54, 11.25 and 13.05). Also, the yolk is often concentrated into a single mass, covered by embryonic membrane dorsally. Yolk not concentrated in this way is distributed inother parts of the embryo or comes to lie outside of it against the vitelline membrane.

Yolk which escapes usually does so before dorsal closure. The latter is always accomplished, and encloses any yolk which still remains in the embryo. A typical

/condition

condition after dorsal closure is shown in fig. 47 (detail, fig. 47). The body walls of each side have united dorsally, where segmentation is distinct but very irregular. Furrows do not occur in this newly formed region, but they persist ventrally. The ventral nervous system is now distinct from the hypoderm, and both follow the lines of the furrows. Between the nervous system and the dorsal body wall is a mass of yolk, unenclosed by endoderm. The endoderm cells, and the ectoderm of the fore and hind guts, form a mass at each end of the embryo.

Involution of the head is generally entirely prevented by the disarrangement of the parts invdved in it. The head material is not sufficiently organized to allow of its invagination, and the irregularity of the body wall of the middle region prevents the thoracic material from enclosing the head. However, in the filmed embryo, which is less markedly abnormal than most, involution movements of a sort can be seen (fig. 56 , 10.10 - 13.22). At first a distinct anterior head segment is visible; then there is a rounding off and withdrawal of the head material from the vitelline membrane (fig. 56 , 13.05). The forward movement of the thoracic segments appears to be occuring as far as the general disorganization will allow it. This never results in the enclosure of the head, but there is an aggregation of the segments behind it and

/consequently

consequently to a constriction of the embryo in this region. This is seen in the film (fig. , 25.00), and in sectioned embryos (figs. 49 , 50 , 52).

Organ formation:

The gut:

1. The fore-gut:

Up to the time of shortening the cells of the stomodaeum are uniform and rounded, forming a compact group at the anterior tip of the embryo. From 10 hours they undergo differentiation, together with those of the pharyngeal material, but since no invagination occurs, the fore-gut develops "inside-out". A typical embryo is shown in fig. 49.; there is a very shallow invagination with oesophageal material extending outside of it. Beyond that is the "dorsal" pharyngeal region, with the pharyngeal muscles attached to it. There is no suggestion of a frontal sac. One of the salivary glands has developed in a distorted way behind the fore-gut material, but since involution has not occurred its duct has not been carried forward. A cuticle is secreted by the fore-gut cells, and there is sometimes a sclerotized portion which represents an abortive cephalopharyngeal apparatus.

2. The mid-gut:

/The

The extent to which the mid-gut develops varies according to the degree to which embryos are divided by the furrows after gastrulation. Where these are very extensive the proliferation of the cells of the mid-gut rudiments is restricted to the anterior and posterior ends of the embryo, and the yolk remains in the middle region without any mid-gut forming around it. Usually, in such cases, scattered endoderm cells find their way into the middle region, but remain undifferentiated and do not form any sort of mid-gut (fig. 50).

More often, a fair amount of endoderm material is moved into the middle region after the "straightening out" of the embryo which, as mentioned above, tends to occur during this process. It is mostly posterior mid-gut material, possibly because the formation of the const constriction behind the head prevents any belated invagination of gut material such as occurs posteriorly. Those endoderm cells which reach the middle region spread over the yolk masses and form mid-gut walls around them; they are very thin, since there are insufficient cells to cover the yolk properly. The nuclei are large, as is characteristic of the mid-gut cells, but the cytoplasm connecting them is attenuated. The endoderm which remains posteriorly differentiates externally, as can be seen in the film (fig. 54, 13.05 and 25.00; fig. 58, 11.22 - 25.00), apparently into a coiled gut, even though no yolk is

/present

present (fig. 51); but it is difficult to distinguish the mid-gut material from that of the proctodaeum, and it is possible that the coils so developed represent hind-gut only. Defintie malpighian tubules have not been seen.

Anteriorly a proventriculus is usually formed but, being unable to expand in the restricted space available, it remains a compressed structure, with walls of relatively undifferentiated cells (fig. 51).

3. The hind-gut:

The proctodaeum usually begins to extend into the embryo from about 12 hours, as its cells begin to differentiate. But its forward extension is always restricted, and the change in shape of its cells results most often in the formation of dilated hind-gut, since its inability to increase in length has been compensated for by an increase in breadth (fig. 52). Cell differentiation is not accomplished sufficiently to make it possible to distinguish the hind-gut from the mid-gut with certainty.

The visceral muscle is very poorly developed; probably the splanchnic mesoderm cells, finding no gut rudiments to which to attach themselves, simply persist as undifferentiated cells in the middle

/region.

region.

The yolk:

Whether or not the yolk is enclosed by mid-gut cells, it diminishes in the course of development so that finally as little remains undigested as in normal embryos.

The body-wall:

The body-wall is formed in the middle region of the embryo, but not at the extremities, where the gut material develops externally. In the middle region it is complete dorsally, but it is often absent ventrally, where hypoderm has failed to appear under the nervous system. Where it occurs the tissues of the body wall are differentiated normally, but the arrangement of them is naturally disturbed by the abnormalities mentioned. Segmentation is clearly apparent, but irregular, and the segments are crowded together (figs. 50, 51, 52). This crowding causes the intersegmental divisions to be deeper than normallythey are, and the muscles which are attached to the apodemes, and extend between them, hang loosely instead of being stretched taut (fig. 50). The segments are frequently so disturbed by the

/furrowing

furrowing that the muscles have become attached to apodemes at one end, but not at the other, so that groups of muscles form fan-shaped bunches, attached tothe hypoderm at only one point (fig. 51). Where the area of hypoderm is very much reduced by its failure to develop under nervous tissue, the muscles become crowded together on such hypoderm as is available in the dorsal region. This phenomenon is considered further in the section on mutant X20(page 89). Cuticle is secreted by the hypoderm cells.

The nervous system:

Ganglion cells and fibres differentiate and become compacted into a distinct nervous system, which is however grossly disarranged by the general spatial disorganization. The brain tissue is formed as a shapeless mass in the head region, and is included within the body wall. The ventral nervous system is laid down along the lines of the furrows (fig. 48). but as it concentrates in later development the furrows tend to be obliterated, and inturned hypoderm to be compressed andto degenerate (fig. 50). As explained above, the differentiation of the neuroblasts frequently takes place without any corresponding differentiation of hypoderm cells in the same region,

/so

so that the nervous tissue is exposed (fig. 52).

The tracheae:

The groups of tracheal cells come together to form the main tracheal trunks, which wher their cuticular lining is secreted become visible as tortuously twisted dorso-lateral tubes. Their openings at the posterior end are interfered with by the disturbances in this region, and the spiracles have not been found.

The gonads:

The pole cells are difficult to trace even in the early stages, and it is not clear what happens to them. The fate of the pole cells is obscure in normal embryos, and to trace them in such a radically disturbed organization as the present one is even more difficult. In one of the embryos with disturbed blastoderm formation there were no pole cells, but no other embryos were found inwhich they were absent, although their numbers might be reduced. The pole cells disappear form view when furrows are formed, probably becoming hidden in the posterior ones. In late embryos they are sometimes visible as isolated cells, and in some

/cases

cases a single normal gonad is formed (fig. 50); two gonads have never been found. In most embryos the pole cells apparently become lost in the confusion of gut tissue at the posterior end.

Discussion

The expression of this factor varies slightly in degree, but very little in its developmental pattern. Most of the cell types are differentiated, and the organ systems, though distorted, are formed; but all parts of the embryo at the end of its development are involved in a gross general disorganization. The particular features of this may be called the end effects of the factor, and in the description it was shown that all of them, with the possible exception of the absence of hypoderm in places, could be ascribed to displacements of the organ forming materials, and disturbances in their subsequent development, brought about by the spatial and mechanical conditions, arising during gastrulation.

These conditions result from two things:

1. The partial or complete failure of the endoderm rudiments and the proctodaeum and the stomodaeum to invaginate,
2. The presence of deep extra furrows over the whole of /the embryo.

the embryo.

These are the intermediate effects of the factor. They are not obviously related; there is no direct reason for the furrows preventing the invaginations though they would inevitably distort them. The question arises as to whether they represent different aspects of one effect, or arise from a common cause.

If similar abnormalities are sought for in other organisms, the exogastrulation phenomena in amphibians and in echinoderms and molluses suggest themselves, for if material which is normally invaginated in Drosophila were to "attempt" to turn outwards instead of in inwards, the vitelline membrane would prevent its bulging and this might result in a buckling of other material to form the extra furrows. It has been suggested recently that in Limnaea, where exogastrulae can be induced by treatment with lithium chloride, that the exogastrulation is a tertiary effect caused by a disturbance of the respiratory mechanism; the latter being a secondary effect which results from the primary effect of lithium on the submicroscopic structure (Raven, 1954). This is the sort of hierarchy of events which might also be expected in the case of genetic abnormalities, and in fact disturbances of respiratory metabolism have been demonstrated in

/embryos

embryos deficient for the X-chromosome (Boell and Poulson 1939; Poulson, 1945; Poulson and Boell, 1946). But the shallow beginnings of invaginations inwards occur and reasons have been given above, in the description of the development of this mutant, for believing that the relationship between the furrows and failures of invagination is not such a simple one; the material turned in in the furrows arises from the presence of an abnormally high number of cells in the germ band. If this is the case, the furrowing might be the earlier effect, which disturbs the general surface movements of gastrulation.in some way which inhibits the proper invaginations. It is extremely probable that the two abnormalities are related, but again absence of fundamental knowledge of the mechanics of the gastrulation process precludes an explanation.

A way in which the furrows might arise from an excessive number of cells in the germ band has been suggested above, and also the possibility that this in turn arises from a disturbance of the rate of cell division. The mitotic rate is presumably under genetic control; growth rates certainly are, but it is usually difficult to say how far growth depends upon cell division. One of the few established cases in animals is in rabbits, in which it was shown that the developing

/eggs

eggs of a large race were made up of more blastomeres than eggs of a small race at the same period of development (Castle and Gregory, 1929). The primary effect of the factor could be to increase the rate of cell division in the germ band, leading to an excessive number of cells in this region, and a buckling inwards on their expansion, assuming this to occur, in the last stage of gastrulation.

One objection to this is that the rate of mitosis, during the early cleavage divisions, when it can be measured, at least, is very high (Sonnenblick, 1947). An increase in such a rate would require that less than ten minutes should elapse between each division which would be unlike anything now known.

It is also difficult to explain, in the case of the two embryos in which the abnormality appeared in the blastoderm stage, why all the nuclei did not divide at the same rate, even if this were more rapid than usual. It is established that the cleavage nuclei in Platycnemis are identical and that the differences in their fate are due to differences residing in the cortical cytoplasm of the egg (Seidel, 1932). Such experiments as have been done suggest that the same is true for the Diptera (Reith, 1925; Pauli, /1927)

1927), and the observations of Parks on Drosophila lead to the same conclusion (Parks, 1936); so that if the chromosomal factor affects division, it should affect the nuclei in the same way.

In these two embryos, not simply the rate of division is affected; the observable effect is upon its synchrony. Although their blastoderms are still syncytial, synchronous cleavage occurs only within groups of nuclei. Evidently, whatever factor is responsible for the usual integration of the cleavages, it is overridden by the mutant factor. A synchrony was found to occur in embryos from gametes of X-rayed adult flies (Sonnenblick, 1940), but there were also many other irregularities in mitosis, such as asymmetrical distribution of chromosomes to daughter nuclei, multipolar spindle configurations and chromosomal clumping. Similar abnormalities havebeen found in many disturbances of development: in X-deficient embryos (Poulson, 1940), in ether treated embryos (Corrman, 1944), in ultra-sonic treated embryos (Selman and Counce, 1953), and in the female sterile mutant deep orange (Counce, 1953). But all these affected the distribution of the cleavage nuclei also, and therefore blastoderm formation. In the present case, the disturbance of synchrony was the only disturbance observed. The primary effect of the

/factor

factor therefore must be regarded as not well established but as probably affecting the rate of cell division in the formation of the germ band.

Conclusions

X2 is a recessive lethal factor on the first chromosome, not attributable to any large chromosomal rearrangement, and located close to the forked locus (56.7). Its lethality is always expressed in the embryonic stage. Development proceeds through the whole of the normal period, and there is no fundamental disturbance of differentiation. The arrangement of the organ systems however is quite abnormal, and the organs themselves are malformed. The most distinctive end effect of the factor is the absence of gut in the middle region of the embryo, and its abortive and partly external development at the anterior and posterior extremeties. The end effects are directly traceable to spatial and mechanical disturbances caused by two intermediate effects which appear at gastrulation:

1. The rudiments of the gut fail to invaginate properly,
2. A number of deep furrows divide the egg.

The primary effect of the factor is not certainly established, but it is likely that a disturbance in the rate of cell division results in the germ band containing

/an

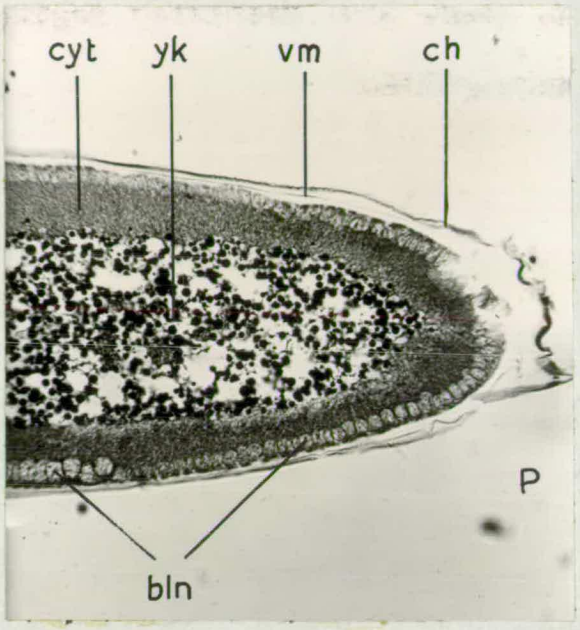
an excessive number of cells, with the consequence that in gastrulation a general buckling occurs in it instead of the normal dorsal and anterior extension.

Fig.45. L.S. X2, posterior region, 3-4 hours, x350.

Fig.46. L.S. X2, 5-6 hours, x300.

Key to figs. 45-56.

A, anterior.
amg, anterior mid-gut rudiment.
bln, blastoderm nuclei.
bn, blastoderm nuclei.
cb, cell boundaries.
cf, cephalic furrow.
ch, chorion.
chf, chorionic filament.
cyt, cytoplasm.
ect, ectoderm.
em, embryonic membrane.
en, extensions from nuclei (see page 226).
gon, gonad.
hg, hind-gut.
hy, hypoderm.
ilb, inner limit of blastoderm.
isg, irregular segmentation.
mes, mesoderm.
mf, mutant furrow.
mg, mid-gut.
mmg, middle mid-gut.
mus, muscles.
nt, nervous tissue.
P, posterior.
pc, pole cells.
ph, pharynx.
phmt, pharyngeal material
pmg, posterior mid-gut.
pmus, pharyngeal muscles.
pr, proctodaeum.
sg, salivary gland.
sm, segmentation.
tr, trachea.
uyk, unenclosed yolk.
vm, vitelline membrane.
vns, ventral nervous system.
yk, yolk.
ykg, yolk granules.


cyt
yk
vm
ch
P
bln

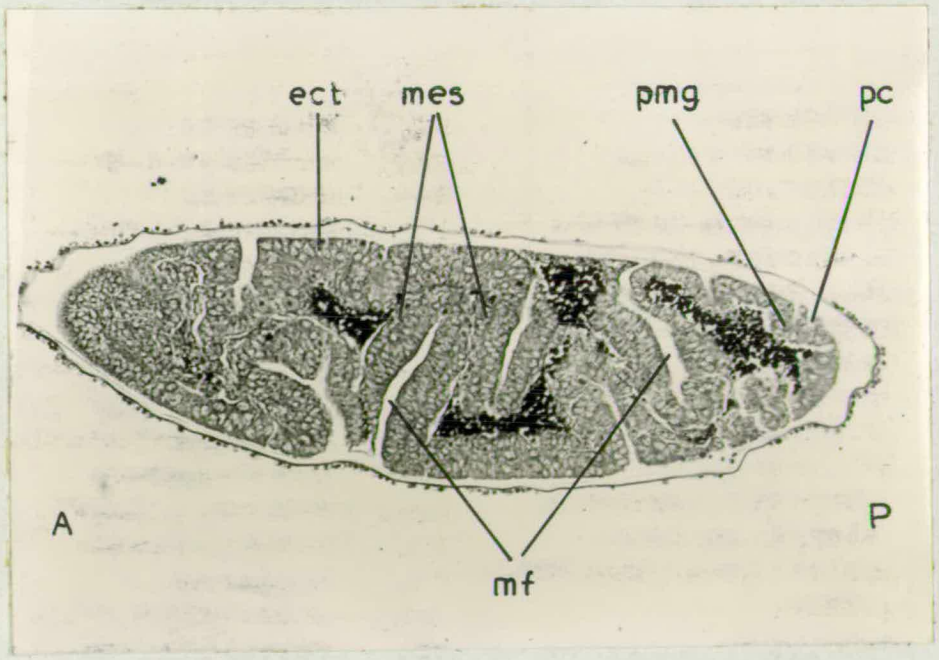
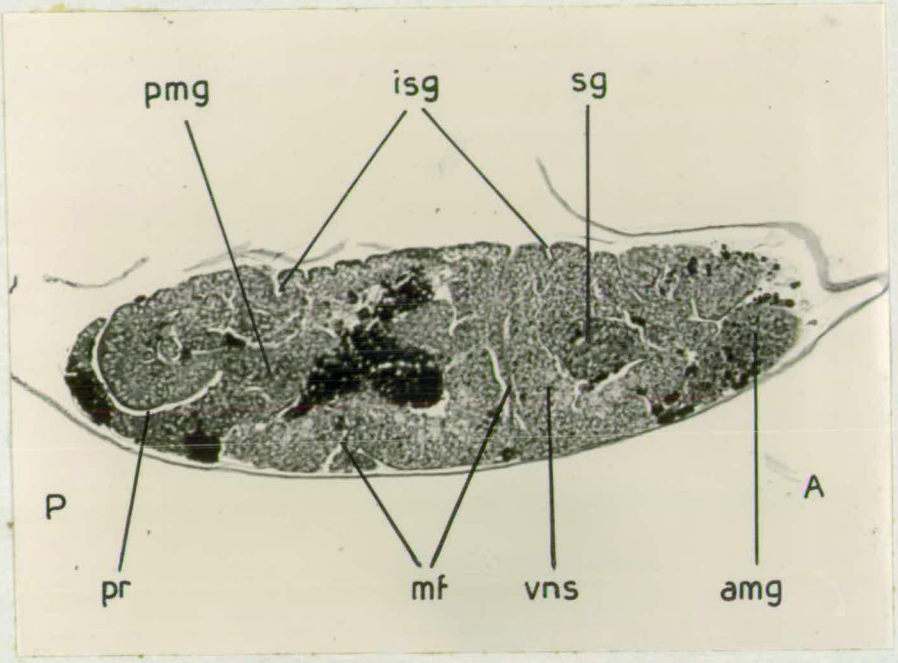

ect
mes
pmg
pc
A
P
mf

Fig. 47. L.S. λ2, 10-11 hours, x300.

Fig. 48. The same section; detail of anterior region, x350.


pmg
isg
sg
P
A
pr
mf
vns
amg

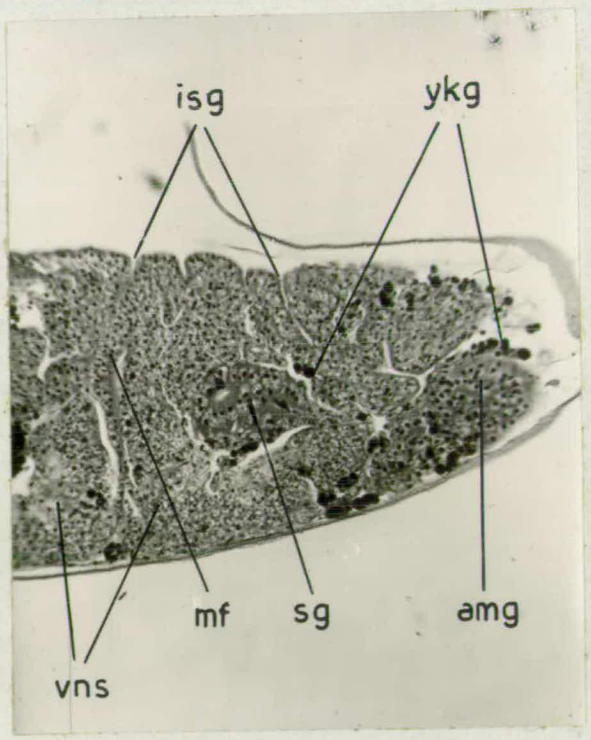
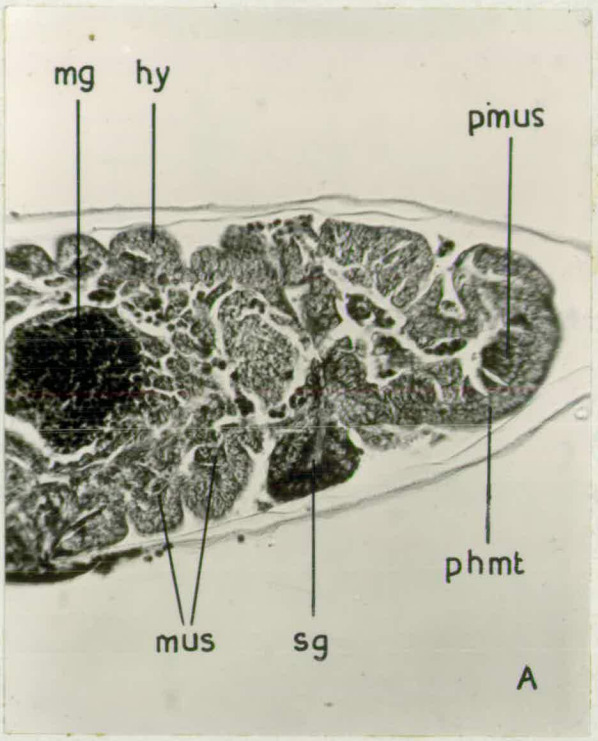

isg
ykg
mf
sg
amg
vns

Fig.49. L.S. X2, 11-12 hours, x250.

Fig.50. L.S. X2, middle region,
15-16 hours, x350.


mg
hy
pmus
phmt
mus
sg
A

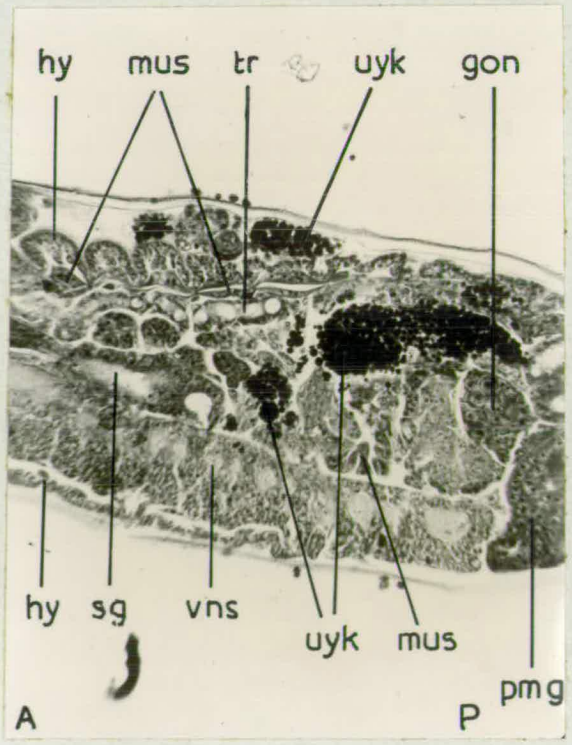
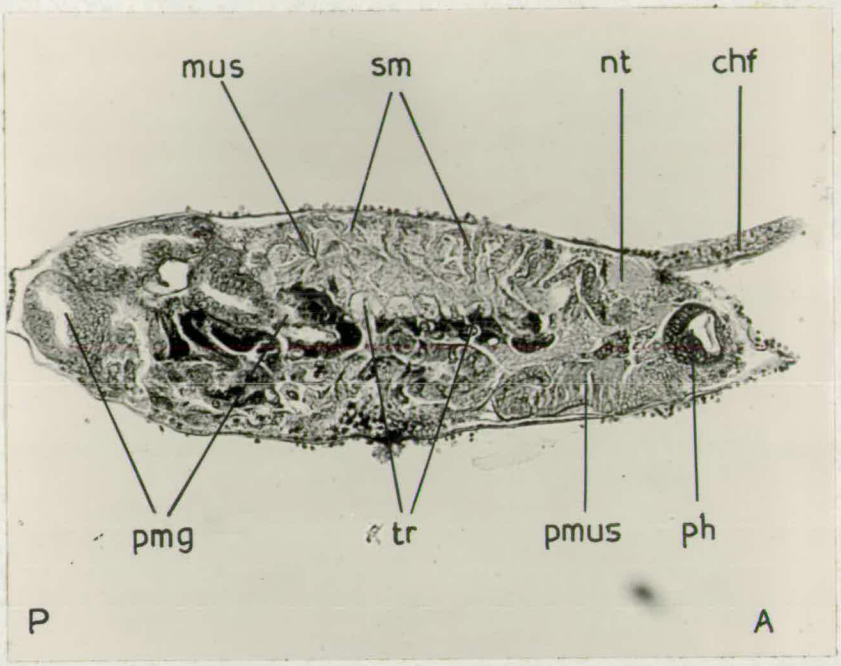

hy
mus
tr
uyk
gon
hy
sg
vns
uyk
mus
pmg
A
P

Fig. 51. L.S. X2, 23-24, x300.

Fig. 52. L.S. X2, late embryo, x320.


mus
sm
nt
chf
pmg
tr
pmus
ph
P
A

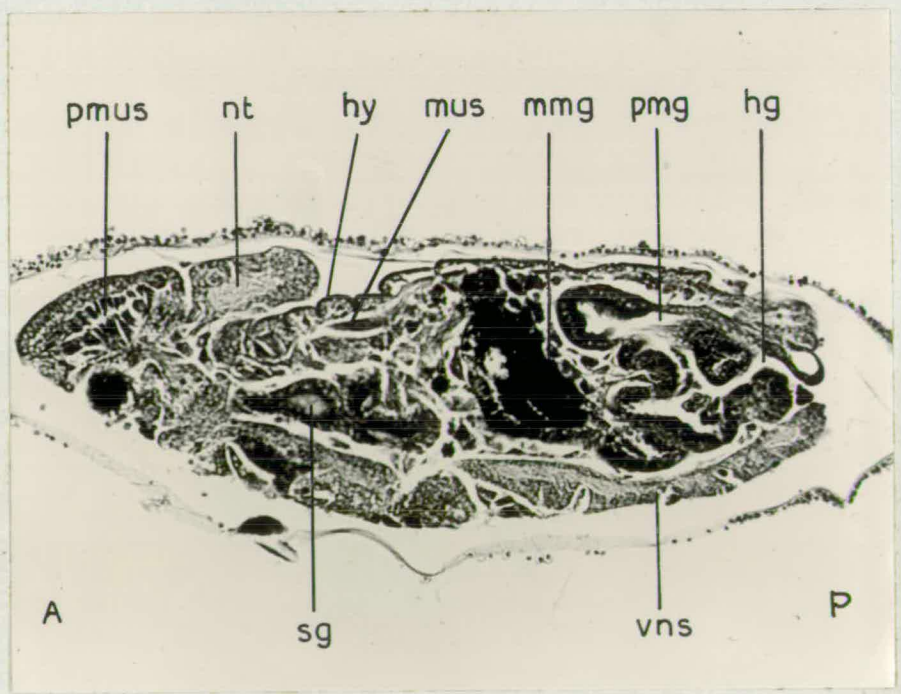
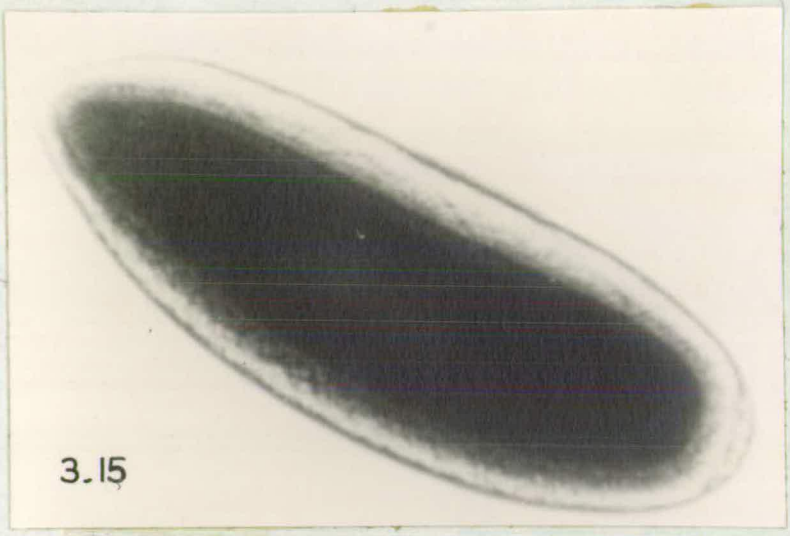
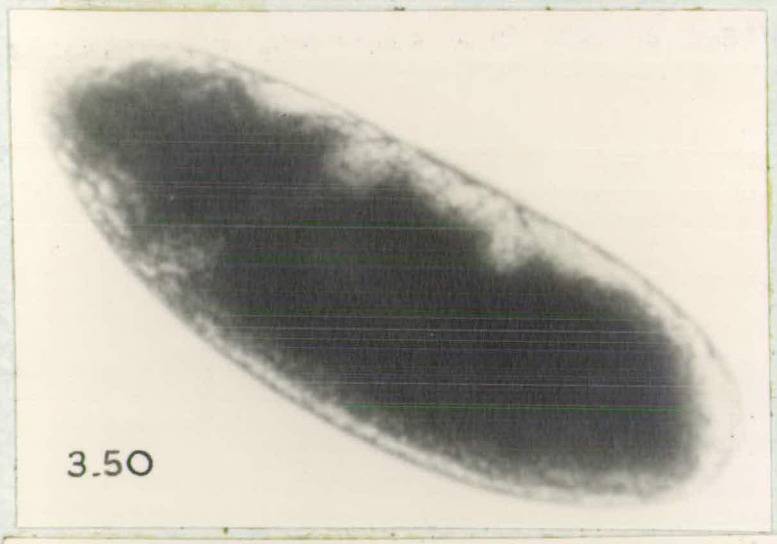

pmus
nt
hy
mus
mmg
pmg
hg
A
sg
vns
P

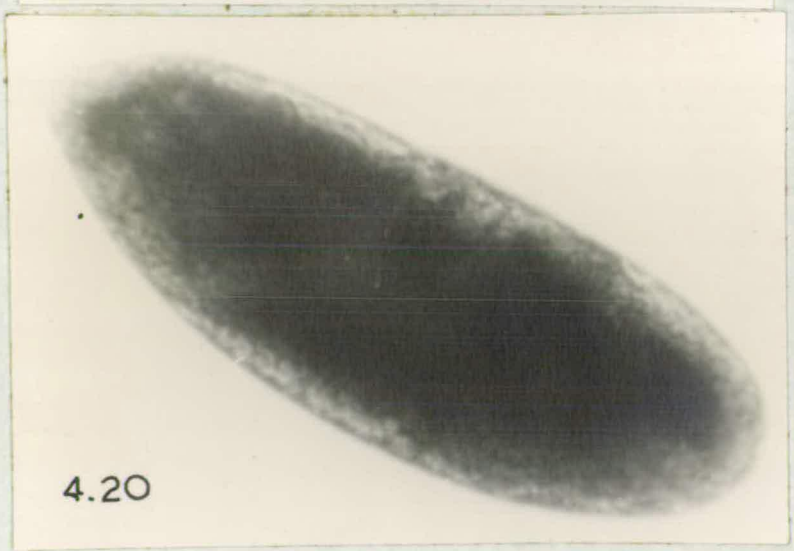
Fig. 53. X2: film sequence, gastrulation.



3.15



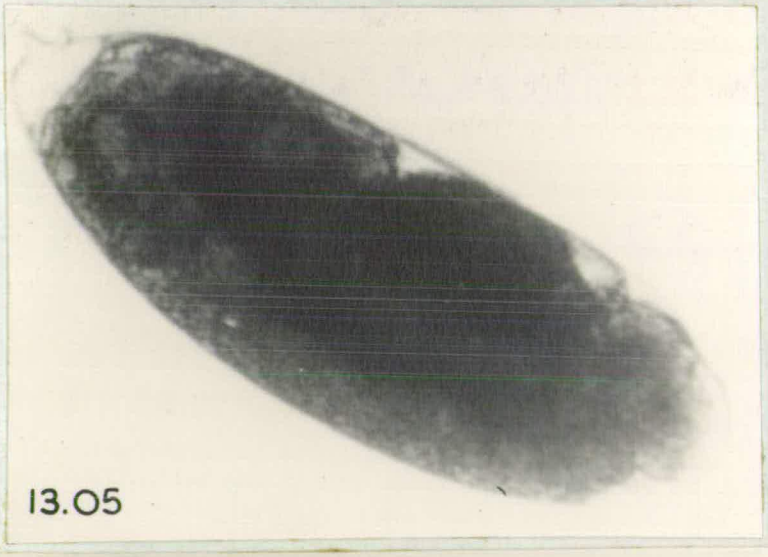
3.50



4.20

Fig. 54. X2: film sequence, continued from fig. 53 to the end of development.


11.25


13.05


25.00

Fig. 55. X2: film sequence, from fertilization to gastrulation.

For corresponding photographs see fig. 53.

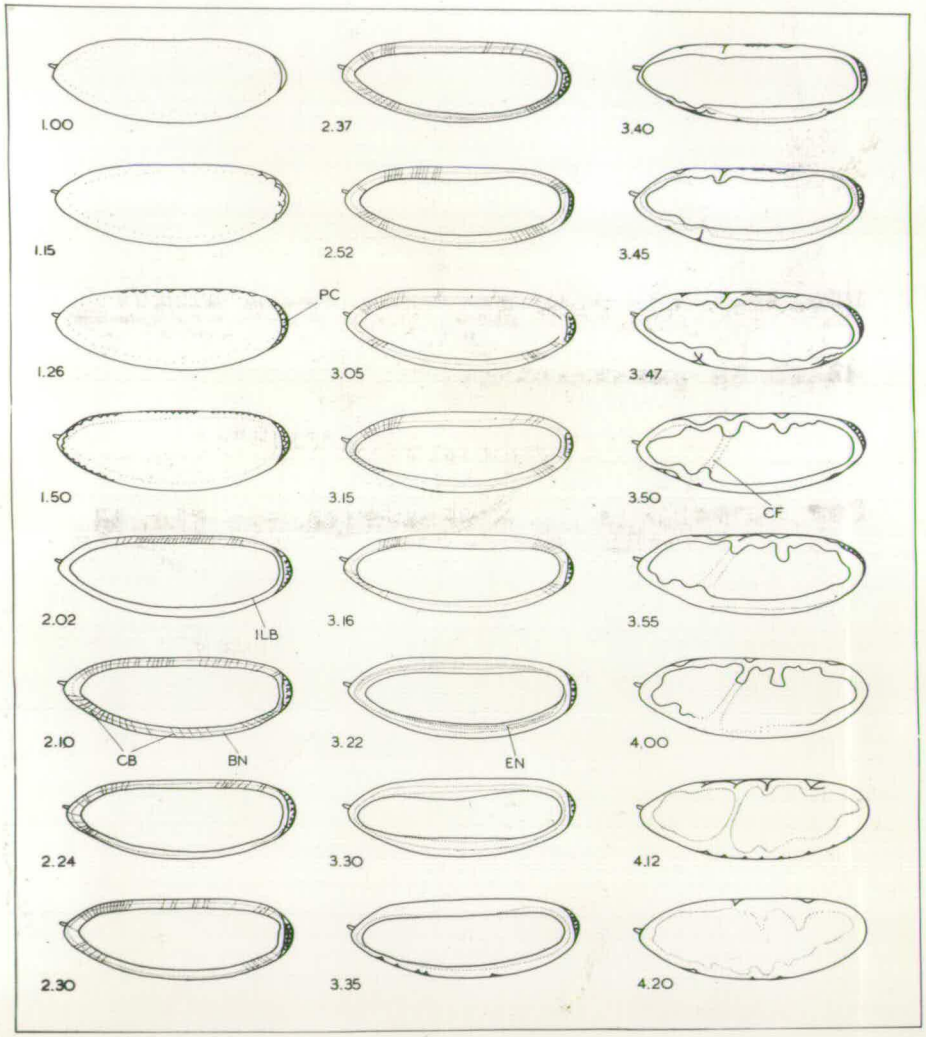
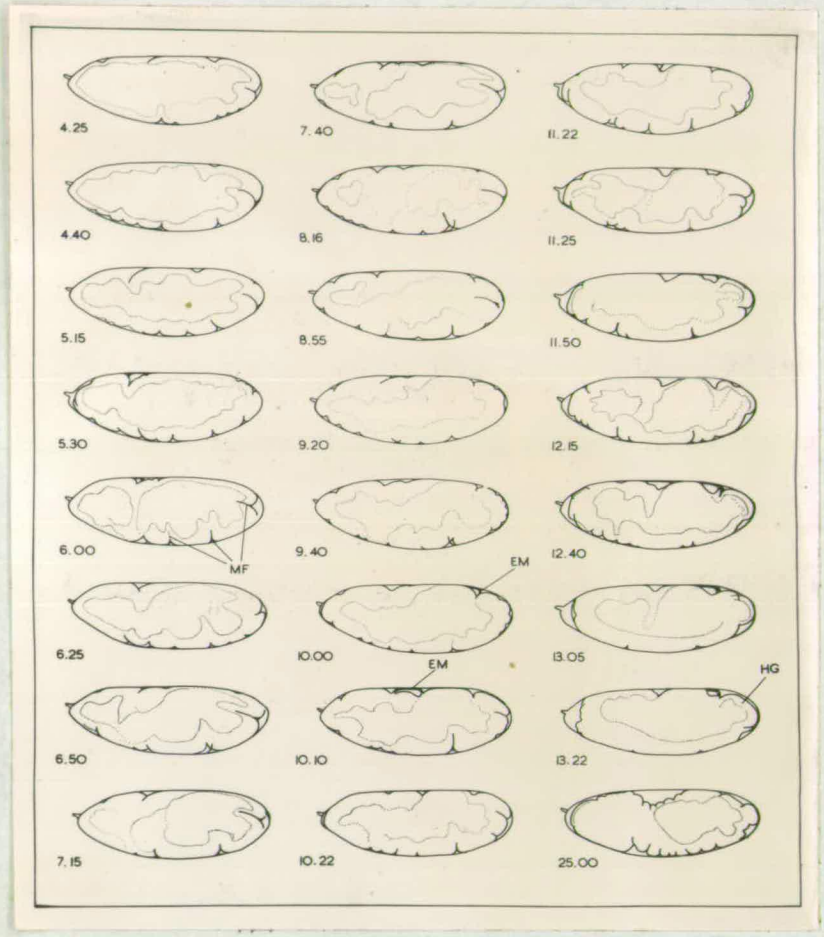

1.00
2.37
3.40
1.15
2.52
3.45
PC
1.26
3.05
3.47
1.50
3.15
3.50
CF
2.02
ILB
3.16
3.55
2.10
CB
BN
3.22
EN
4.00
2.24
3.30
4.12
2.30
3.35
4.20

Fig. 56. X2: film sequence, from gastrulation to the end of development.

For corresponding photographs see fig. 54.


4.25
4.40
5.15
5.30
6.00
MF
6.25
6.50
7.15
7.40
8.16
8.55
9.20
9.40
10.00
EM
10.10
EM
10.22
11.22
11.25
11.50
12.15
12.40
13.05
13.22
HG
25.00

Mutant X27

Material:

Embryos sectioned at all developmental stages provided most of the information. Living embryos were also examined.

Preliminary data:

The factor was induced by X-irradiation of sperm and a preliminary genetic analysis has shown it ot be located to the right of the carnation locus (62.5), at about 63.4 (H. Slizynska, unpublished).

Analysis of sectioned material:

Period	Unfert-ilized	Unident-ifiable	Normals	Typical Mutants	Other Abnormals
0-10hrs	14	42	134	15	6
10-19	11	8	134	67	35
19-30	5	3	-	22	4
Totals	30	53	268	104	45

The expression of the lethal factor:

Two distinct sorts of abnormal enbryos occur in eggs from this stock: the "typical mutants" and the "other abnormals". The latter are characterized by proliferation of embryonic cells, without accompanying

/differentiation

differentiation. Cleavage nuclei are formed which migrate to the surface of the egg, where they divide to give a mass of small cells which encroaches upon the yolk. Finally, the cells occupy most of the egg, and the yolk is reduced to a sphere in the centre. The cells are quite uniform, except for the occurrence of a number of larger ones, which are polyploid (fig. 57).

The question arises as to whether both sorts of abnormal embryo, or only one, are caused by the factor under consideration. It is possible for a single factor to have different forms of expression (discussed on page 212), but in this case it is likely that the "other abnormals" result from some other factor which is segregating in the stock. In the period of development between 10 and 19 hours, when abnormal embryos can be quite clearly distinguished form normal ones, 67 out of 236 fertile eggs gave "typical mutant" embryos. This is not significantly different from the expected lethal fraction of 25%. Including the "other abnormals" gives 102 out of 236, which far exceeds it. The factor K27 may therefore be considered to express itself uniformly and completely in the embryonic stage. The other abnormal embryos are not considered further.

Final Appearance of the Embryo

/The

The embryo is living at the time when normals are hatching, but it exhibits very little movement because the muscular system is extremely poorly developed. The general features of a typical late embryo are shown diagramatically in fig. 74. The hypoderm is complete, and there is a cuticle, but it is unsegmented. The musculature of the body wall is scanty, and the only well developed muscles are those of the pharyngeal pump. The fore-gut is relatively normal, but the mid-gut is sac-like, and contains much undigested yolk; the hind-gut has partly failed to invaginate, and opens dorsally. The nervous system is irregularly developed and the ventral nerve cord is interrupted in the region of the large mid-gut. The tracheal system is represented only by short isolated lengths of the tracheal trunks. Cell degeneration begins at about 25 hours.

The Development of the Embryo

Events up to gastrulation:

Blastoderm formation is normal; subsequent events suggest that irregularities in the cellular detail of the blastoderm and the yolk nuclei might be shown by further investigation, but there is no disturbance of the general features.

/Gastrulation:

Gastrulation:

Spatial disarrangements in the development of the present mutant are less obvious than inthe two preceding o ones; the abnormalities consist as much in disturbances of cellular diffenentiation, whichare less easy to follow in early development. Gastrulation is disturbed from its earliest stages, leading to characteristic abnormalities of the post-gastrulation stage embryo (figs. 58, 60). The most striking histological featureis the great irregularity in thickness of the germ band (fig. 60); the most striking spatial disturbance is in the position of the proctodaeal invagination, much posterior to its normal one (figs. 60 and 72).

These abnormalities are shown in a tranverse section of the middle region of the embryo (compare figs. 61 and 62). The opening of the proctodaeal invagination is distorted, and some of its material remains uninvaginated. The nature of the disturbance of the germ band is shown ventrally, where a vestige of the ventral furrow remains, long after it has completely disappeared in the normal embryo(see also fig. 72).

The disturbance of the germ band appears to be the primary effect of the factor. The persistence of a vestige of the ventral furrow shows that the invagination of the ventral cells is incomplete, and it is this that

/accounts

accounts for the irregularities in the germ band. It is extremely likely that these inhibit its proper extension in the last stage of gastrulation (compare figs. 58 and 59), and, as will be seen later, its shortening also. In a smaller number of cases, extension is complete, but shortening is prevented.

The cephalic furrow is formed normally (fig. 72). The explanation suggested for the abnormal deepening of this furrow in mutant Lff 11, connecting it with the precocious invagination of the posterior mid-gut rudiment, suggests tha possibility of a similar consequent abnormality in this mutant, where invagination of the mid-gut rudiment is also posterior to its normal position. In fact, a persistent constriction has been observed in this region in a few embryos, but in the majority of cases the cephalic furrow disappears normally later. The resemblance to the disturbance in Lff 11 is possibly quite superficial.

Apart from its invaginating more posteriorly than usual, the posterior mid-gut rudiment is normal, and contains the pole cells. Its unusual position forces the yolk anteriorly, and consequently the yolk appears rounded in tranverse sections (fig. 72). The partial failure of invagination of the proctodaeal rudiment has already been mentioned. The germ band remains external;

/there

there is never any covering of it such as occurs in Lff 11. The anterior mid-gut rudiment and the stomodaeal invagination develop entirely normally.

Differentiation between 5 and 9 hours:

During this period many disturbances of cell differentiation become manifest, which cannot directly be attributed to the disturbances of gastrulation.

Ectoderm:

The irregularities in the germ band are reflected in the development of the neuroblasts. In the normal embryo they are evenly distributed over the brain region of the head and the whole of the ventral side of the embryo, but inthis mutant they are unevenly scattered, in clumps, presumably because of the disarrangement of the material of this region by the disturbance of the ventral furrow invagination.

The part of the ectoderm which remains external - the hypoderm, itself shows defects of differentiation. The most striking of these is its failure, complete or very nearly so, to become segmented. Tracheal pits are often not formed, and never to the normal extent. The salivary gland plates also show a partial or complete failure to invaginate, and often there is only one

/distorted

distorted salivary gland formed.

The stomodaeal invagination develops normally. The cells of the proctodaeum continue to develop in the abnormal situation, so that it comes to have a very large opening at the dorsal side (fig. 73).

Mesoderm:

A striking feature of the late embryo is the virtual absence of the somatic musculature. This, together with the abnormality in the ventral furrow, suggests that the mesodermal material may not have been invaginated. This is not the case; at least some, and probably all of the cells which give rise tothe mesoderm are invaginated, and form a layer between the gut and the hypoderm. But they are difficult to distinguish because their normal organization into segmental masses, and the subdivision of those into somatic and splanchnic parts, does not occur. They simply produce, by division, a mesenchyme of small round cells, a majority of which appear to become attached to the rudiments of the gut (figs. 60 and 61).

Yolk, endoderm and pole cells:

As was remarked above, the yolk is rounded in cross section, rather than flattened as in normal

/embryos

embryos (compare figs. 15 and 72). Also, it frequently contains far more cellular material than usual. This occurs in the walls of the primitive gut, formed at the end of gastrulation, and especially at the anterior and posterior ends, where the yolk merges with cells which are not sharply distinguished from those of the mesoderm (represented by heavy stippling in fig. 73). It appears likely that if the irregularity of the germ band has its origin in disturbances in blastoderm formation, these same disturbances may have resulted in more cleavage nuclei than usual remaining in the interior as yolk nuclei. The yolk remains very largely undigested. Much more of it escapes form the primitive mid-gut then inthe normal, and the free granules are found particularly among the irregular clumps of ventral nervous tissue. Each granule becomes engulfed by a phagocytic cell, as in the normal embryo.

The development of the anterior and posterior mid-gut rudiments is modified by the abnormalities of the yolk. This is so cumbrous that the proliferations of the endoderm rudiments are unable to grow out and under it normally. This is shown diagramatically in fig. 73, where the anterior rudiment is asymmetrical, and the posterior rudiment is not embracing the yolk at all, but partly owing to the shape and size of the yolk,

/and

and partly owing to its own displacement by the abnormality in gastrulation, it is situated entirely to one side of the yolk mass. However, the endoderm rudiments always do unite eventually, and form a complete mid-gut sac.

The embryo shown in this diagram is interesting because the displacement of the posterior mid-gut rudiment makes possible an observation bearing on the problem of the fate of the pole cells. As mentioned previously, the role of the pole cells which are invaginated in the posterior mid-gut rudiment is a matter of dispute. The most generally accipted explanation is that after gastrulation some of them migrate out of the rudiment between the endoderm cells, and eventually become incorporated in the gonads as the germ cells; others remain in the rudiment, and come to form the middle part of the mid-gut, whose cells are distinguishable by their larger size and cuboidal shape from the other cells of the mid-gut (Poulson, 1947). Poulson has more recently suggested that <u>all</u> of the pole cells in the mid-gut envagination become incorporated inthe gut, and that the germ cells arise from pole cells which migrate back into the interior of the embryo before the endoderm pocket is formed (Poulson, 1950). Another older explanation, which it is still thought may be

/correct

correct (Counce, 1953), holds that the middle mid-gut cells are simply specially differentiated cells arising from the posterior mid-gut rudiment, and that all of the pole cells escape into the body cavity to give the germ cells. In this particular embryo (fig. 73) the middle mid-gut cells are easily distinguishable at the tip of the endoderm rudiment, and they are clearly identical with the pole cells, forming a continuous group with those of the latter which have not yet emerged form the rudiment. This supports the first explanation, and does not discount the second; it would seem to invalidate the last.

Shortening, involution of the head, and dorsal closure:

The irregularity of the germ band always prevents shortening taking place. In the majority of embryos, where extension was also interfered with, this leaves the proctodaeal opening dorsal, but still posterior. In a smaller number of embryos, where the proctodaeal opening is carried enteriorly, it remains in this position, and differnetiation of the germ band goes on both dorsally and ventrally (fig. 65).

The failure of shortening rarely interferes with dorsal closure, which was found not to have occurred in only two embryos.

/It

It has been mentioned that some embryos are found in which the head region is constricted off from the rest of the embryo. In these, involution of the head is prevented, and its development is similar to that in Lff 11. But in most embryos the first stages of involution is normal, so that most of the pharyngeal material becomes invaginated; the distorted salivary glands, however, never come to open internally. The second stage of involution, in which the head becomes enclosed in the thorax, and in which the frontal sac is formed, is prevented. This may possibly be due to the absence of segmentation in the body wall, or of the musculature, or both. Sometimes the shallow beginning of a frontal sac occurs, but the movement is never completed.

Organ formation:

The gut:

1. The for-gut:

Except in the few embryos inwhich the head region is completely disturbed, the development of the for-gut is relatively normal. The position of the pharynx is usually disturbed (fig. 74), and the frontal sac is usually shallow or absent; but the pharyngeal muscles are well developed, even though the musculature

/of

of the body wall is quite undeveloped (fig. 69). Chitinization occurs to a varying extent, sometimes with the formation of the cephalopharyngeal apparatus. The oesophagus develops normally.

2. The mid-gut:

The mid-gut remains a sac-like structure, enclosing the large mass of undigested yolk. It is often almost spherical (fig. 63), but there may be some elongation and coiling (fig. 68). The differentiation of the mid-gut cells, which in normals distinguishes the posterior tubular coils of the gut from the more distended anterior parts, occurs in the mutant where there is some elongation (fig. 70), and even sometimes when the gut remains spherical, the posterior cells becoming relatively larger and cuboidal.

The proventriculus starts its development at the anterior end of the mid-gut, but the final differentiation of its structure is not achieved (fig. 66). Mid-gut caecae are not formed. The rudiments of the malpighian tubules appear at the junction of the posterior mid-gut rudiment and the proctodaeal invagination at the end of gastrulation, but they remain short and undeveloped.

3. The hind-gut:

/The

The hind-gut is formed from the proctodaeum with no change in its position. It is very distorted, and its uninvaginated material forms a broad opening which extends over a comparatively wide area of the postero-dorsal surface of the embryo. Its cells do not develop the protruberant inner borders which are characteristic of the normal hind-gut.

Though it is difficult to see it in many cases, the visceral musculature does appear to develop and to invest all parts of the gut.

The body wall:

The hypoderm is complete over the whole embryo; in many its thickness varies from place to place, seeming to indicate, even in the final embryo, a relic of the original irregularity in the thickness of the germ band. A cuticle is secreted. There is never more than a faint trace of segmentation, and often none at all. Apodemes are not developed.

The only well developed muscles in the body are those of the pharyngeal pump; those of the body wall are not developed at all, except for the occasional occurrence of clumps of myoblasts attached to widely scattered points on the hypoderm (figs. 69 and 70). In some embryos the somatic mesoderm remains as a

/mesenchyme

mesenchyme of small rounded cells distributed through the body cavity (fig. 65). In most cases, very little mesoderm appears at all in the final embryo, and it has presumably degenerated during its development.

The nervous system:

The early differentiation of the nervous system was described above, the chief peculiarity lying in the scattered distribution of the neuroblasts in the germ band. They appear to form clumps which lie loosely in the ventral body cavity, for they are displaced by the yolk-distended mid-gut sac; consequently, when the ganglia and fibres ofthe ventral nervous cord are formed, the cord not only reflects the irregularity of the distribution of of the original neuroblasts, but it is also interrupted in the middle region (figs. 63 , 68 , 74). The disorganization of the nervous tissue usually extends to that of the brain and the circum-oesophageal connectives.

The tracheae:

These are very poorly developed, and only traces of the tracheal trunks occur in the final embryo(fig. 74).

The gonads:

The gonads are rarely both found, though in some

/one

one of them is present, complete with the gonad sheath, but not embedded in fat body. In most embryos the gonads appear to be absent; in some, free cells which look like pole cells have been observed, and it is possible that usually they degenerate before the gonads are formed.

Discussion

Certain features of this mutant may be explained as arising through a sequence of events which has its origin in the first stages of gastrulation at latest and possibly in earlier blastoderm formation. These relate particularly to the hind-gut, and to the nervous system.

In the description of development, the distortion of the hind-gut was traced to the failure of the germ band to shorten, and to the incomplete invagination of the proctodaeal material; it was suggested that the cause of these abnormalities was the irregularity of the germ band, and that this was due to abnormalities in the formation of the ventral furrow. The disturbances of the furrow might have their origin in undetected disarrangements in the cells of the blastoderm.

The characteristic disturbance of the nervous system was also traced to the same source. The irregularities

/in

in the germ band were envisaged as resulting in a scattered distribution of the neuroblasts, which would explain the disorganization of the ventral nervous system. In this case another factor enters; the distribution of the neuroblasts is also affected by the pressure of the abnormally large mid-gut, which forces them anteriorly and posteriorly, so that the nervous system becomes interrupted in this region.

These two end effects resemble most of the terminal abnormalities in the mutants Lff 11 and X2m in that they may be reasonably explained as primarily mechanical consequences arising from disturbances of early embryonic movements. Both are traced to the same basic defect, an abnormality in ventral furrow formation, which is the primary effect of the lethal factor in this sequence of abnormalities.

But the other effects are abnormalities of cellular differentiation, and cannot be traced to any structural disturbance. Somewhat similar abnormalities have been described by Poulson as occurring in the development of embryos of three stocks, each of which was deficient for a section of the first chromosome containing the white locus (Poulson, 1945). He found the endoderm and the mesoderm both to be abnormal, the former giving rise to a sac-like gut, and the latter degenerating

/before

before the formation of any muscles, either somatic or visceral. The ectoderm is stated ot develop normally, but an accompanying photograph suggests that it is not segmented, and that the embryo resembles that of X27 very closely, at least in the condition of the hypoderm, musculature, and gut. No details are given which would make a closer comparison possible, and it is stated that the abnormalities do not become apparent until 10 hours. This suggests that the white deficiencies resemble the X27 lethals in the disturbances of differentiation, but not in the mechanical disturbances of gastrulation. The factors are not identical cytologically, as they are located at opposite ends of the chromosome.

Poulson concludes that a gene or genes in the region of the white locus is concerned in the development and differentiation of mesoderm and endoderm. Apart from the fact that it is likely that the ectoderm should also have been mentioned, this sort of statement, while too general to be contradicted, is at the same time too vague to be useful. More explicit suggestions as to the mechanisms involved are required, which have in them the possibility of being tested in the event of practicable experimental methods being devised.

A very low intensity of general metabolism in the embryo is reflected in the non-absorption of the yolk,

/which

which may be a secondary effect of the low level of differentiation activity in the cells, the reserve material simply not being required. Alternatively, failure of yolk metabolism may inhibit cellular differentiation. The mere mechanical presence of the persisting yolk mass may account for the sac-like nature of the mid-gut, whose form may be largely determined by the shape of the yolk mass which it has to enclose.

The question arises as to whether the abnormalities in the other two germ layers are simply parallel manifestations of a general metabolic disturbance, or whether disturbances in one result in secondary disturbances in the other.

Poulson has suggested, in connection with abnormalities in Notch deficiencies, that the mesoderm exerts an inductive action upon the ectoderm with respect to differentiation of the nervous system (Poulson, 1940, 1945). This is highly speculative, and evidence in other insects suggests that any inductions that occur are in the other direction, of ectoderm upon mesoderm. It was noticed several years ago that in Pieris (Lepidoptera) segmentation appeared in the ectoderm before appearing in the mesoderm (Eastham, 1930), suggesting a primacy in differentiation. More recently, clear experimental evidence has shown that in Chrysopa

/(Neuroptera)

(Neuroptera) the ectoderm is a self-differentiating system, and that it has an inductive action in organizing the mesoderm (Seidel, Bock, and Krause, 1940). + Haget It is therefore likely that if there is any inductive mechanism in Drosophila, the organization of the mesoderm should be affected by that of the ectoderm rather then vice versa.

It is striking that the defects in the ectodermal differentiation chiefly involve processes of invagination. With the exception of the stomodaeum, which develops normally, structures formed in this way - the ventral furrow, the proctodaeum, the tracheal pits, and the salivary glands - all fail to develop properly. It might also be suggested that the segmentation of the ectoderm comes about by the turning in of the hypoderm at the intersegmental regions; in fact, that it is a type of invagination. It is possible then, that there is some general factor inhibiting cellular invagination.

The absence of intersegmental regions, and therefore of the apodemes to which muscles are normally attached, may account for the failure of the musculature of the body wall to develop. No myoblasts are ever seen to differentiate into muscle cells without attachment to an apodeme, at least at one end; and where the apodemes are absent, it is reasonable to suppose that the myo-

/blasts

blasts will simply degenerate on this account. It is striking that well developed muscles occur in the pharynx; presumably, if this interpretation is correct, because the ectodermal sterial of the pharynx is normally developed and provides an organising centre for myoblast differentiation. Again, visceral musculature is present, presumably because, with respect to its serving as a base for the differentiation of the splanchnic mesoderm, the gut is sufficiently well developed. Further relations between the segmentation of the hypoderm and the development of the somatic musculature are mentioned under mutant X20 (page 189).

Conclusions

X27 is a recessive lethal factor located on the first chromosome at about 63.4. Its lethality is always expressed in the embryonic stage. The embryo lives through the whole of the normal embryonic period. Disturbances occur in the spatial arrangement of some tissues, especially those of the proctodaeum and the nervous system; these originate at the first stage of gastrulation in abnormalities in the ventral furrow. Other disturbances result from a partial failure of differentiation in certain tissues; in particular, the

/hypoderm

hypoderm does not become segmented, and mesoderm does not form segmental muscles. It is possible that the failure of the mesoderm is a direct, through an inductor mechanism, of the failure in the hypoderm. Thus a single primary effect is indicated for the structural defects, but these cannot, from the present study, be related to the defects in cellular differentation.

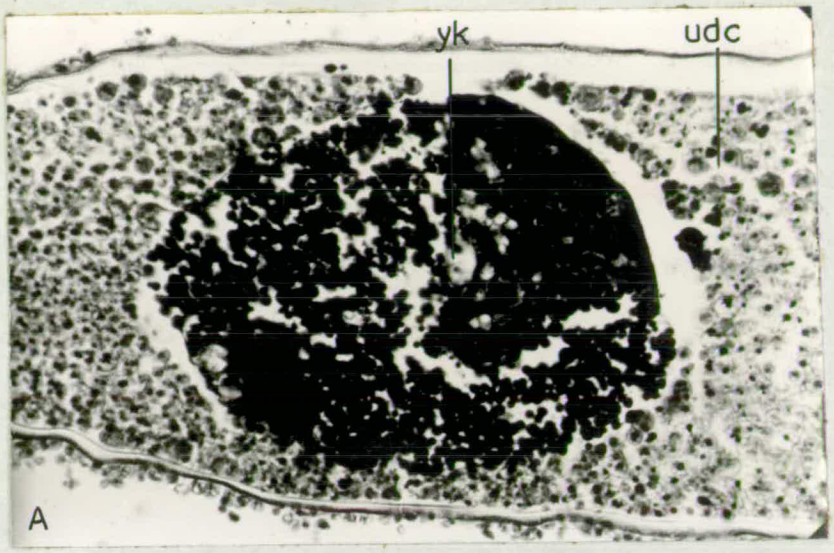
Fig.57. L.S. X27, abnormal embryo, 13-14 hours, x460.

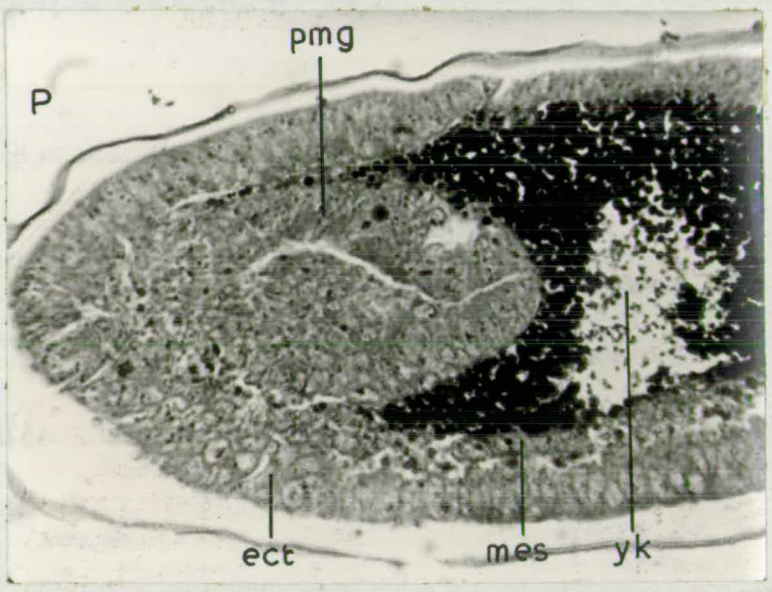
Fig.58. L.S. X27, 5-6 hours, x460.

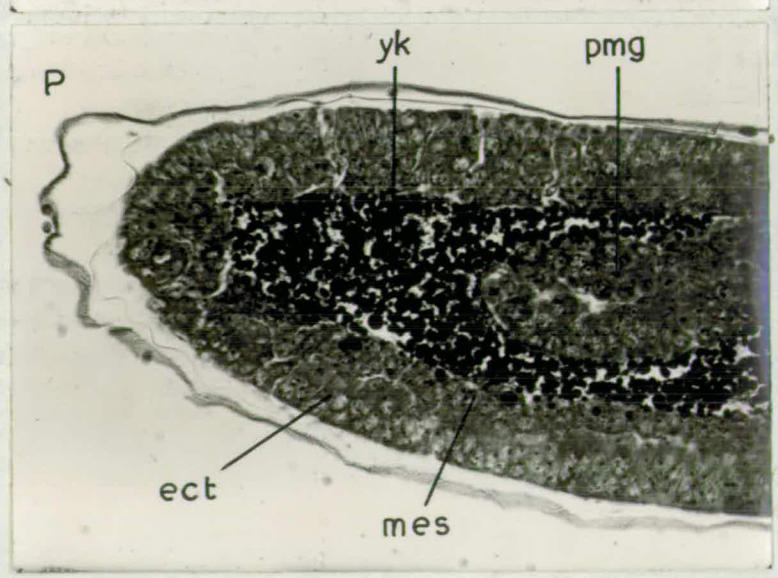
Fig.59. L.S. normal, 5-6 hours, x380.

Key to figs. 57-74.

A, anterior.
amg, anterior mid-gut rudiment.
apd, apodeme.
bnb, brain neuroblasts.
br, brain.
cf, cephalic furrow.
ch, chorion.
chf, chorionic filament.
ect, ectoderm,
em, embryonic membrane.
fs, frontal sac.
gnso, sub-oesophageal ganglion.
gon, gonad.
hg, hind-gut.
hgo, hind-gut opening.
hy, hypoderm.
igb, irregular germ band.
mcn, median cells of nervous system.
mes, mesoderm.
mg, mid-gut.
mmg, middle mid-gut.
msn, mesodermal mesenchyme.
mt, malpighian tubule.
mus, muscles.
myb, myoblasts.
nt, nervous tissue.
oes, oesophagus.
P, posterior.
pc, pole cells.
ph, pharynx.
pmg, posterior mid-gut rudiment.
pmus, pharyngeal muscles.
pr, proctodaeum.
pro, proctodaeal opening.
pv, proventriculus.
pvf, persistent ventral furrow.
sgi, salivary gland invagination.
st, stomodaeum.
tr, trachea.
udc, undifferentiated cells.
vf, ventral furrow.
vns, ventral nervous system.
yk, yolk.


yk
udc
A


pmg
P
ect
mes
yk


yk
pmg
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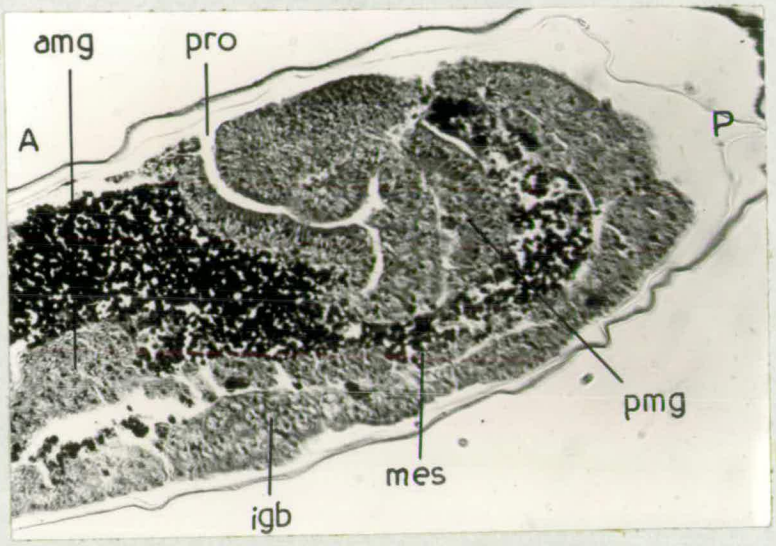
mes

Fig.60. L.S. λ27, 10-11 hours, x380.

Fig.61. T.S. middle region λ27, 7-8 hours, x380.

Fig.62. T.S. salivary gland region normal, 7-8 hours, x380.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pro
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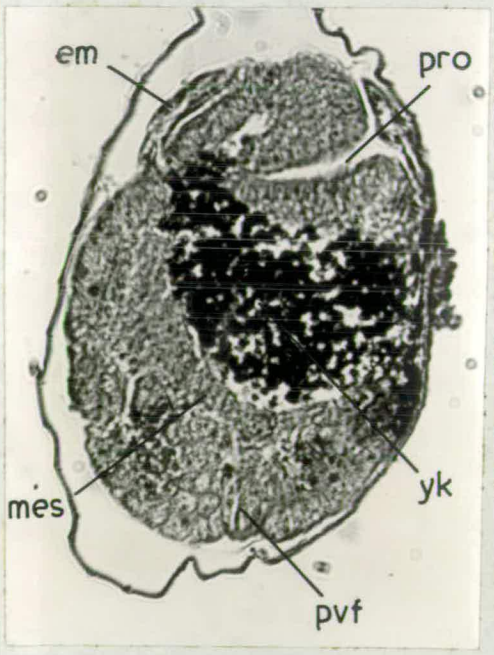
em
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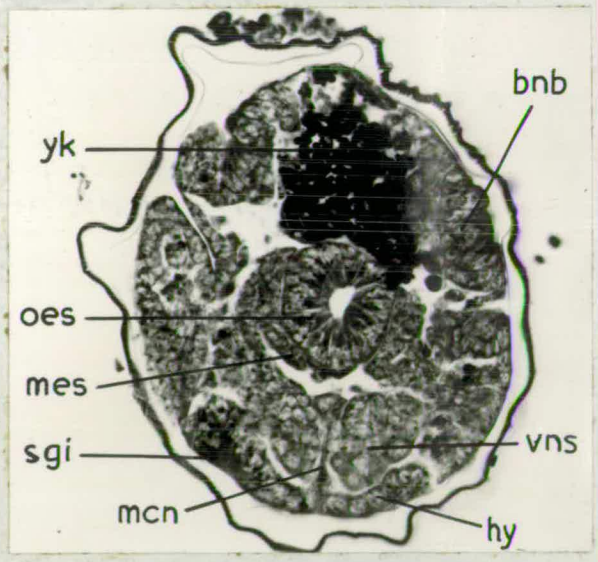
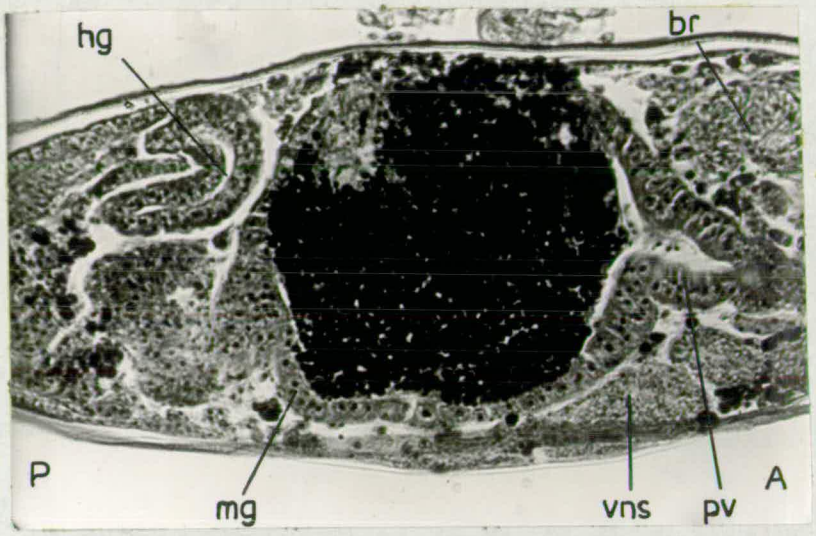

bnb
yk
oes
mes
sgi
vns
mcn
hy

Fig.63. L.S. X27, middle region, 11-12 hours, x430.

Fig.64. L.S. normal, anterior region, 12-13 hours, x500.

Fig.65. T.S. middle region X27, 13-14 hours, x460.


hg
br
P
mg
vns
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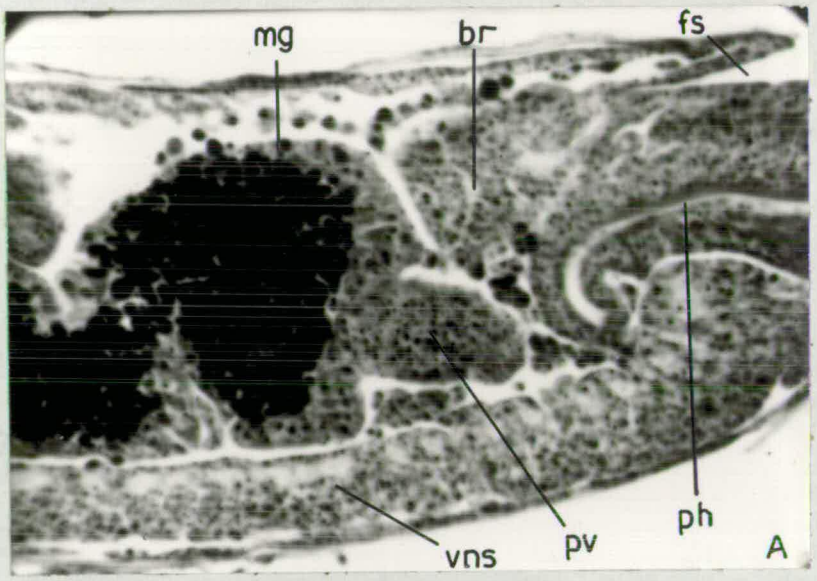
A


mg
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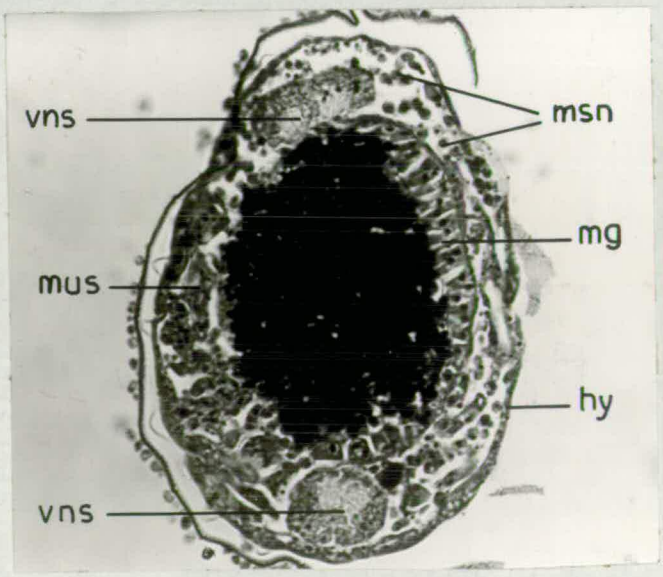
vns
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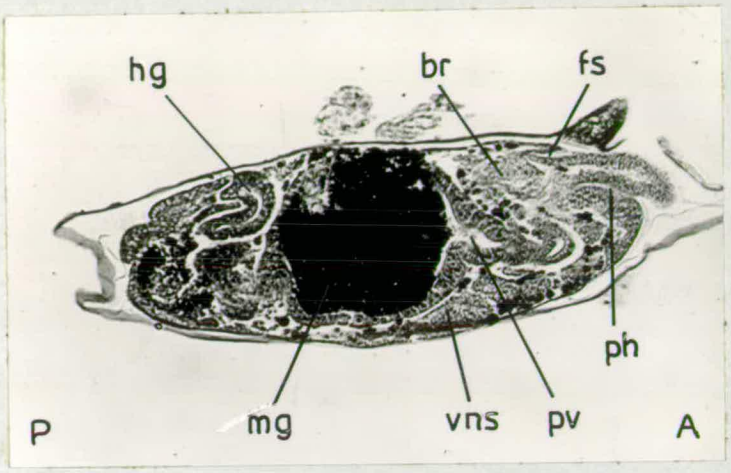
Fig.66. L.S. X27, 11-12 hours,
x180.

Fig.67. L.S. normal, 11-12 hours,
x200.

Fig.68. L.S. X27, 16-17 hours,
x200.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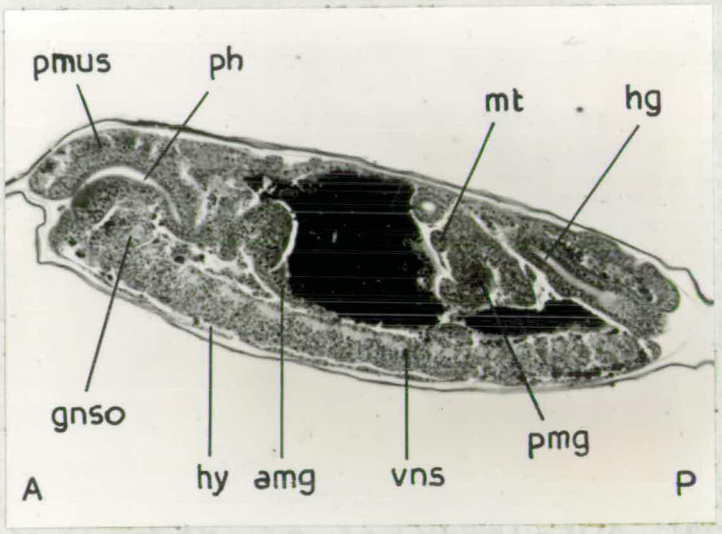
pmus
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gnso
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amg
vns
P

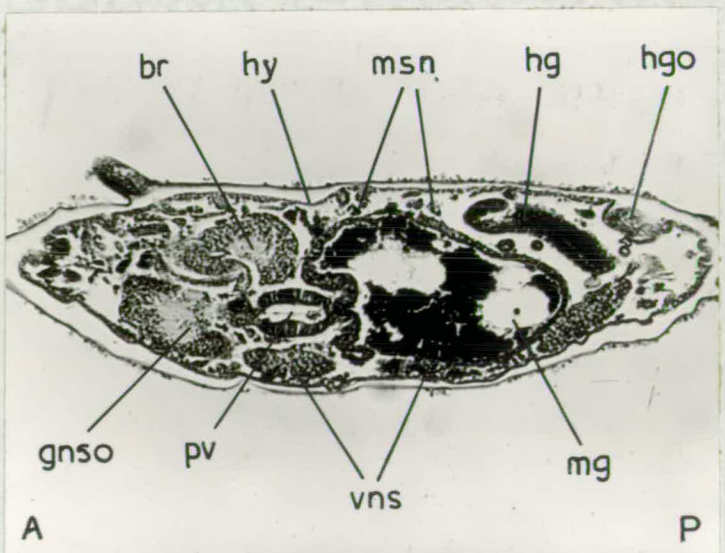
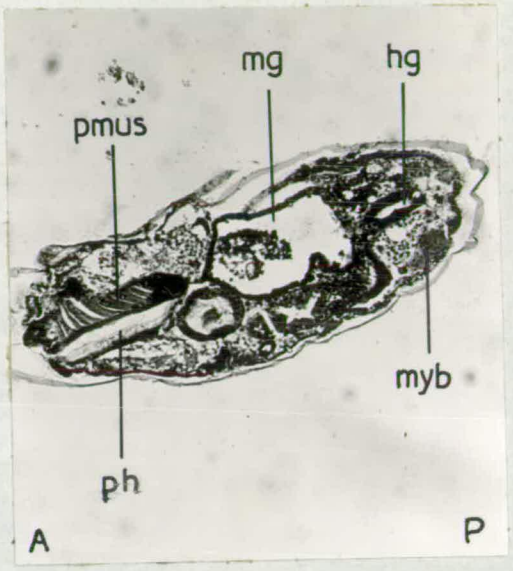

br
hy
msn
hg
hgo
gnso
pv
mg
vns
A
P

Fig.69. L.S. X27, late embryo, x160.

Fig.70. H.S. X27, posterior region, 16-17 hours, x460.

Fig.71. H.S. normal, posterior region, 16-17 hours, x500.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myb
ph
A
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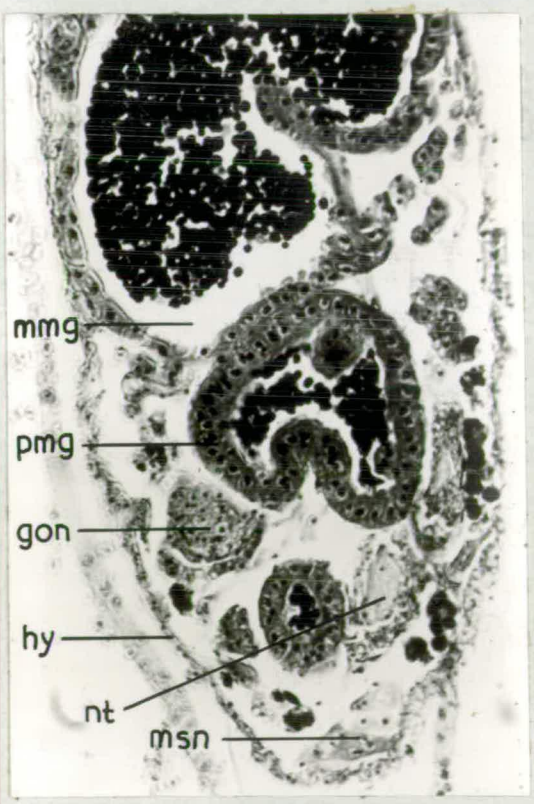
mmg
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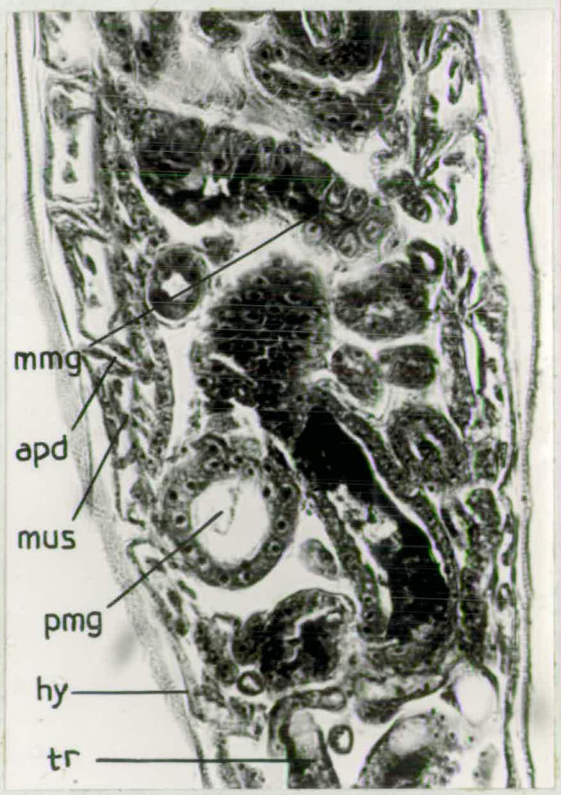
mmg
apd
mus
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hy
tr

Fig.72. Structure of typical X27 embryo at approximately 7 hours.

Key to shading in figs. 72-74.

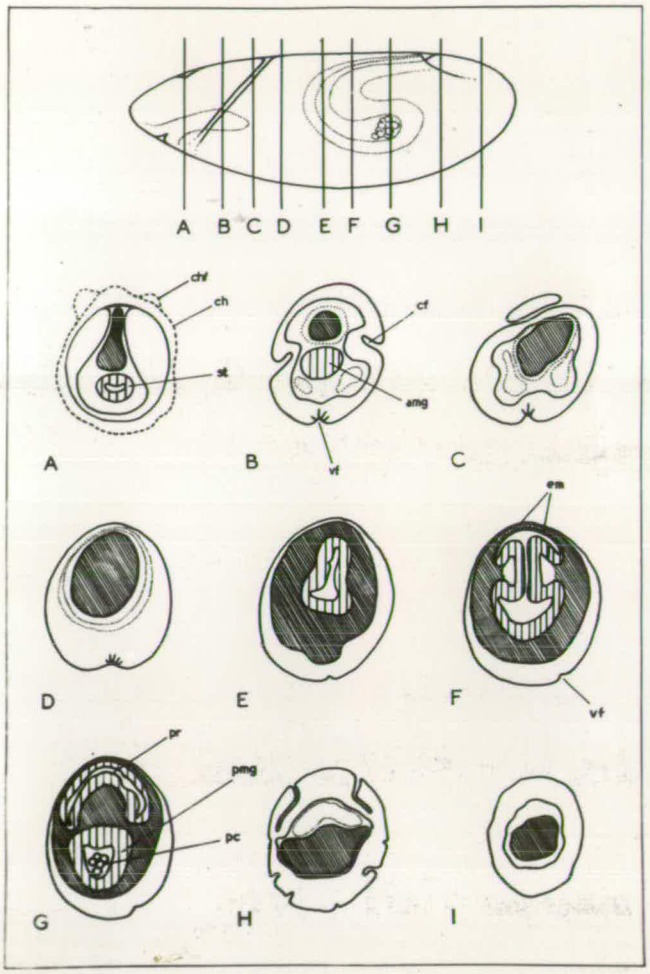
Diagonal lines - yolk.

Vertical lines - gut and gut diverticula.

Light stippling - nervous tissue.

Heavy stippling - mixed yolk and cellular material.

Dotted outlines - mesodermal tissues.


A B C D E F G H I
chf
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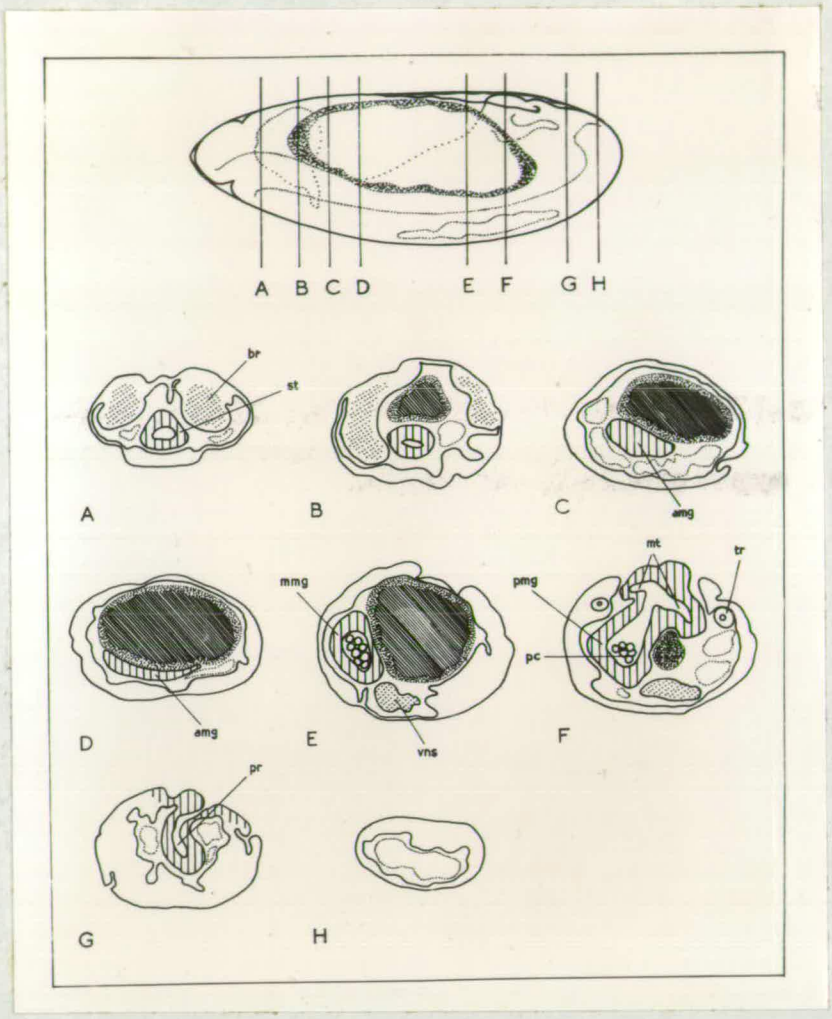
pc
G
H
I

Fig.73. Structure of typical X27 embryo at approximately 10 hours.


A B C D E F G H
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A
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amg
D
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mmg
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pmg
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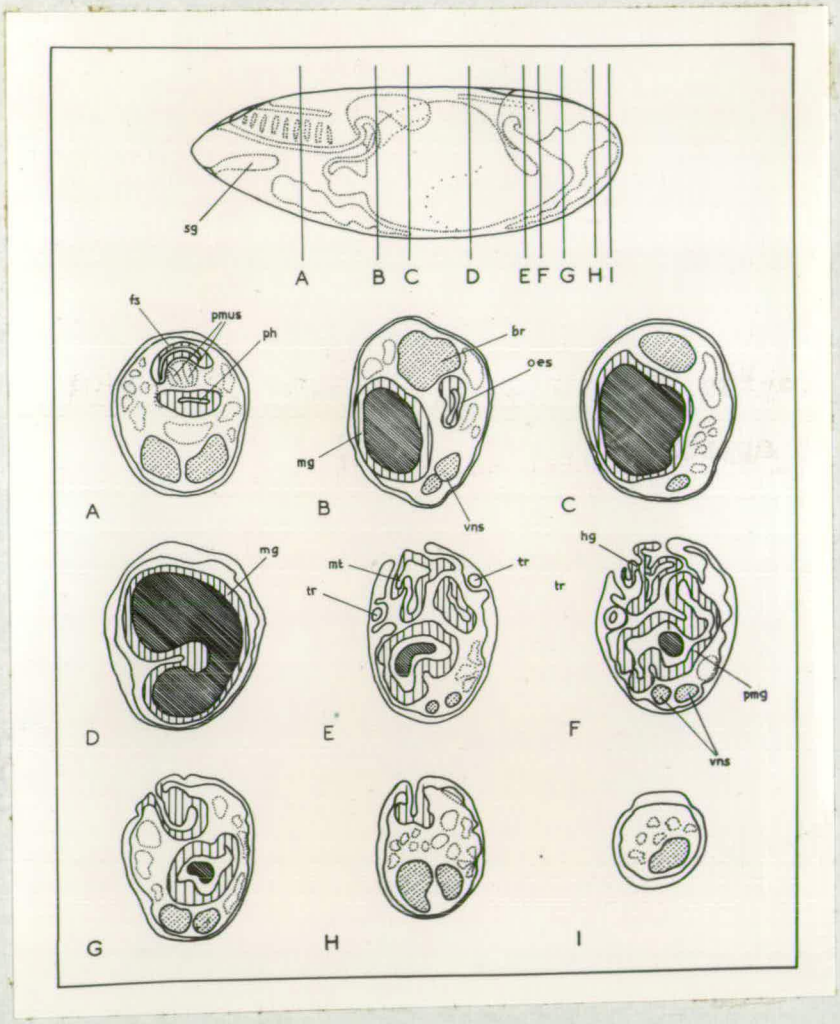
pr
G
H

Fig.74. Structure of typical X27 embryo at approximately 13 hours.


sg
A B C D E F G H I
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pmg
vns
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Mutant X20

Material:

Sections were made of embryos at all stages after 6 hours, which was the earliest at which useful information could be obtained. Living embryos were also examined.

Preliminary data:

1. The factor was induced by X-irradiation of sperm, and a preliminary genetic analysis has shown it to be located near the scute locus (0.0) (H.Slizynska, unpublished).

2. Analysis of sectioned material:

Period	Unfertilized	Unidentified	Normals	Abnormals			
				I	II	III	IV
6-10hrs	64	20	402	6	2	5	8
10-18	58	58	318	43	23	10	11
18-33	-	10	3	8	-	1	-
Totals	122	88	723	57	25	16	19

3. Failure of eggs to hatch in a collection from massed females:

(Eggs which could be recognized as unfertilized were not counted, and eggs containing active normal larvae

/were

were counted as"hatched").

Eggs laid	Unhatched at 30 hrs.	% unhatched
183	56	30.60

The expression ofthe lethal factor:

Four distinct types of abnormal embryo are found in this stock. All four are clearly recognizable between 10 and 18 hours; im this period their relative numbers are: type I - 4; type II - 2; type III - 1; type IV - 1. Types I and II are the most numerous and characteristic abnormal forms, and will be described in detail later. Types III and IV are described below:

Type III:

The blastoderm is malformed, and the germ band is extremely irregular. Gastrulation occurs, but little further development ensues, in form or in cellular differentiation (fig. 89).

Type:IV:

No blastoderm is formed, and there is no cellular differentiation. A varying number of small undifferentiated cells is produced, chiefly at the anterior end of the egg, less frequently at the posterior end also, and sometimes scattered between.

In the period 10-18 hours, the fraction of all the abnormal types taken together over the total fertilized

/and

and identifiable eggs is 87 out of 405, which is not significantly different from 25%; nor, in the hatchability test, is the fraction of unhatched eggs over the total laid. Therefore, it is reasonable to suppose that all the abnormal types are expressions of the same factor. Further evidence for the supposition was obtained from a study of batches of eggs collected separately from individual females. Male embryos in each batch therefore carried identical X-chromosomes. All four types of embryos were found among the progeny of each female showing that the variability is not caused by the presence of different sex-linked factors in the stock. Such variability in expression is considered further under mutant X10 (page 212). In one or other of these ways, the lethality is expressed completely in the embryonic stage.

There is an extremely high pupal lethality in the stock. At least 50% of the pupae become bright pink a few days after forming, and do not develop further. The expression of the factor under consideration is fully accounted for in the embryonic stage, and moreover the pupal lethality is far too high to represent that of a sex-linked recessive lethal factor. It will therefore not be considered further.

/Final Appearance of Types I and II

Final Appearance of Types I and II

Examinations of living embryos showed that in both types much yolk remains undigested, but that it is all enclosed in a gut. In type I the gut protrudes ventrally for in this region no hypoderm is developed. The hypoderm of the dorsal region is present, and extremely deeply segmented; attached to it is a well-developed musculature, which is active at this stage. The ventral nervous system is formed normally, but is displaced to one side by the gut.

In type II the gut is wholly internal, and the hypoderm is complete; the head is never invaginated, and the nervous system is almost entirely or totally absent.

Development of Types I and II

The strikingly contrasted characteristics of these two types makes it interesting to describe the development of both together. The early stages of the abnormalities are not easy to detect, and the difficulty is increased because each type is to be expected in only a small percentage of embryos. However, the main outlines of development are clear.

Events up to the end of gastrulation:

These appear to be entirely normal. In contrast

/to

to all of the previous mutants, the general embryonic movements of this stage are undisturbed. An exception to this was found in four type II embryos, which are described later.

Differentiation between 5 and 10 hours:

Ectoderm:

Differentiation in normal embryos:

Omitting the stomodaeum and proctodaeum from consideration, the most important events in the development of the ectoderm are:

1. The differentiation between hypoderm and nervous tissue,
2. The appearance of segmentation.

The nervous system is formed from neuroblast cells, which arise from the ectoderm of the ventral and head regions between 4 and 7 hours. A complete account is given in "The Biology of Drosophila" (Poulson, 1950). The essential events are the enlargement of certain of the ectoderm cells, their becoming flask-shaped, with the neck peripherally and the larger part internally, and their rounding off so that they are entirely cut off from the surface by hypoderm cells which replace them. They then undergo a number of mitotic divisions, in which the spindle is orientated at right angles to

/the

the surface of the embryo, and in which the innermost daughter cell is considerably smaller than the outermost one. The former, after another division, give the ganglion cells. The larger daughter cells continue as neuroblasts and produce more ganglion cells in the same way. Later, soon after shortening, nerve fibres are produced as outgrowths from the ganglion cells. Some of the neuroblasts innthe brain region are unusual in rounding off at the surface, and becoming covered by hypoderm cells only later, during shortening.

The hypoderm arises from the dermatoblast cells, which derive from the columnar cells of the blastoderm. As they proliferate they become more cuboidal, and the final hypoderm cells are extremely flattened. The inturnings at the intersegmental divisions appear at about eight hours, and are deep by the time of shortening. They are not present ventrally, where the hypoderm underlying the nervous system is extremely flattened (fig. 47). The lateral hypoderm of both sides is brought together in the median dorsal line at dorsal closure. The corresponding segmental regions of each side unite so that the intersegmental divisions become continuous dorsally.

Type I:

/The

The chief abnormality in early differentiation is the non-appearance of hypoderm in the region of the differentiating ventral nervous tissue. At first the difference from the normal lies only in the rounding off of neuroblasts at the surface, rather than their migrating inwards (compare figs. 75 and 29). Later, there is seen to be a complete absence of hypoderm cells (compare figs. 76 and 30), while in other respects the embryo is normal (fig. 77).

Type II:

Here almost the direct contrary occurs in differentiation; the hypoderm not only develops on the ventral side, but it becomes deeply segmented, though in the normal embryo segmentation never appears in this region (compare figs. 86 and 67). Nervous tissue, on the other hand, does not develop. The large cells which occur between the intersegmental divisions are presumably neuroblasts, but they do not produce ganglion cells, and probably degenerate later.

Mesoderm:

Mesoderm is formed in the normal way, but its subsequent development into the musculature is different in the two types, and will be described later.

/Endoderm:

Endoderm:

The differentation of the anterior and posterior mid-gut rudiments, and their union to form the mid-gut, is perfectly normal in both types.

Shortening, involution of the head, anddorsal closure:

Type I:

Shortening and dorsal closure are always accomplished, and so also, in many cases, is involution of the head (fig. 83). In others the head region is very disturbed. Fig. 80 illustrates an embryo in which the proventriculus has been displaced anterior to the pharyngeal pump, which has been forced posteriorly. Often the head region resembles that of Lff 11 embryos, in which much pharyngealmaterial remains uninvaginated, the nervous tissue uncovered by hypoderm, and the thoracic segments constricting it off behind instead of enclosing it (fig. 81).

Type II:

Again, shortening and dorsal closure are normal. Involution of the head, on the other hand, never occurs. The pharyngeal material remains uninvaginated, and the thoracic segments do not move forward to enclose the head.

/Four

Four of the type II embryos were found to differ from the others in that shortening had not occurred. In these the proctodaeal opening is dorsal and anterior (fig. 88); most of the yolk, enclosed in the mid-gut is posterior. The hypoderm follows the turn of the proctodaeum to some extent, so that the embryos appear to be doubled back upon themselves. This shaping of the hypoderm must have come about by an ingrowth rather like that of the abnormal furrow in Lff 11, and in fact such furrows are found in a few of the type III embryos (fig. 89; in this embryo there is also an extra ventral furrow, suggesting the abnormality of X2). Probably then, this "doubled-back" type of embryo resulted form the occurrence of an abnormal deep furrow just anterior to the proctodaeal opening. The accumulation of the yolk, and some formation round it of the mid-gut, at the posterior end, is also shown in a type III embryo, in which little except nervous tissue has differentiated (fig. 90). In this embryo such mid-gut cells as occur are growing towards the posterior mass of yolk. And here also there appears to be a furrow creating the "doubled-back" effect.

Organ formation:

The gut:

/1.

1. The fore-gut:

Type I:

The distortions of the head which sometimes occur, involving displacements of the pharynx, pharyngeal pump, and oesophagus, were described under the involution of the head. The structures themselves are normally formed and cuticularization occurs, with the formation, usually, of the cephalopharyngeal apparatus. In the other cases, the fore-gut is quite normal.

Type II:

Since the pharyngeal material is not invaginated, the ectodermal part of it is external (fig. 85); the pharyngeal musculature is well developed on its inner side. There is no frontal sac. Cuticularization occurs as if the pharyngeal ectoderm were part of the hypoderm. The oesophagus is developed normally. Salivary glands are formed, but retain their own independent openings to the exterior.

2. The mid-gut:

This is developed to exactly the same extent in both types, but in type I it displaces the nervous system to one side and protrudes ventrally. The division of the mid-gut sac into chambers occurs, followed by the

/first

first stages in its development into a convoluted tube (fig. 84). No further differentiation occurs, and it remains distended with undigested yolk. Anteriorly the proventriculus is well developed (figs. 80 and 81), but the mid-gut caecae are not formed. The malpighian tubules are formed normally.

In the "doubled-back" type of II, the mid-gut is quite abnormal, being formed around the mass of yolk which occurs at the posterior end (fig. 88). The embryo illustrated shows very clearly how the yolk serves as a mould around which the mid-gut forms. The proventriculus is formed anteriorly, then a section of mid-gut around a small mass of yolk: there follows a narrow solid section where there is no yolk, which then spreads around the posterior yolk mass.

3. The hind-gut:

This is developed normally in both types. All parts of the gut are invested by visceral muscle.

The body wall:

Type I:

Since no hypoderm is produced under the ventral nervous system, the body wall is limited to the anterior, dorsal, and lateral parts of the embryo (figs. 80, 81, 82, 83, 84).

/Sometimes

Sometimes it is also absent in the head region. The hypoderm is normal in structure, and is segmented, with extremely deep intersegmental divisions (figs. 80 and 81). A normal cuticle is formed.

In normal embryos the musculature of the body wall arises from the ventrolateral parts of the somatic mesoderm. The cells of this tissue are small and rounded at first, but elongate and fuse when the intersegmental apodemes appear. The muscles thus formed are attached to the apodemes, stretching the length of a segment from one to the next. Between 10 and 11 hours myofibrils become visible in sections, and the muscles are functional at 15 hours. In sections, a number of small cells are seen in each segment between the muscle and the hypoderm which are not referred to in the literature. These are not oenocytes - they are far too numerous - but are probably potential muscle cells which have not been used in muscle formation.

In type I the musculature is well developed, and the muscles are far more crowded on the apodemes than in the normal embryo. Where there is normally only a single strand of muscle between apodemes, here there may be up to six (compare figs. 82 and 20).

Type II:

/In

In this type the hypoderm is complete over the whole of the embryo, and the intersegmental divisions are of a normal depth (fig. 85). Apparently the deep intersegmental divisions seen earlier in the ventral hypoderm are reduced by its expansion in dorsal closure. The musculature is developed over the whole inner area of the hypoderm, and the muscles are finely drawn and slender. There appear to be none of the potential, unused, muscle cells between the hypoderm and the muscle sheet (compare figs. 85 and 21).

The nervous system:

Type I:

The development of the nervous system may be followed in figs. 80, 81 and 82; its clear demarcation from the regions where body wall is formed is seen clearly in horizontal sections (figs. 78 and 78a). It is quite normal, except where the whole head region is disturbed in which case the brain is distorted (fig. 81). It does not become concentrated at the posterior end, though the brain retreats normally during head involution. The posterior end remains at the posterior tip; it follows the curve of the embryo dorsally and often the body wall closes under it here (fig. 83). The movements of the developing mid-gut displace it slightly to one side.

/Type II:

Type II:

Sometimes the nervous system appears to be entirely absent. In other cases very small patches of nervous tissue occur, usually anteriorly (fig. 85).

The tracheae and the gonads:

These are developed normally in both types.

Discussion

Unlike the previous mutants there is in this one no primary structural defect which may be regarded as basic to the rest. The variability in the expression of the factor has been discussed above, but each of the different types represents a greater or lesser disturbance of differentiation; the basic morphogenetic movements and distribution of embryonic materials are unaffected. There are some exceptions to this; in the "doubled-back" type of type II embryos there is, as suggested above, an extra furrow, and a failure of shortening. The same configuration occurs in some type III embryos, without cellular differentiation. In types III and IV development is interrupted early, and there is little to say about them. The discussion which follows concerns types I and II.

In particular the disturbance in these is in the

/differentiation

differentiation of the ectoderm. The modifications of the mesoderm can be easily explained as resulting from the ectodermal abnormalities. The development of the endoderm is not so much abnormal as stopped short before the final structure of the mid-gut is achieved; the non-digestion of much of the yolk within it suggests a general slowing of metabolism.

The most striking abnormality in the development of the ectoderm is in the relationship between the hypoderm and the nervous tissue. Consideration of mutants Lff 11 and X2, and of the ligaturing experiment done in connection with the first, has suggested that there is a delicate balance between the tendencies of the ectoderm to produce one or the other, and that this balance is easily upset. The remarkable feature in this mutant is that the balance is easily upset in two contrary directions. Either, as in type I, a complete nervous system is produced without any covering of hypoderm overlying it, or else, as in type II, a complete hypoderm is produced, but not a nervous system.

This situation is difficult to explain. There is no question of an organiser disturbance brought about by the absence of a particular structure. It suggests that there is competition for a substance which is in some way necessary in the formation of

/both

both hypoderm and nervous tissue, and of which there is normally sufficient for each. In this mutant there would be only enough to allow the development of either hypoderm or nervous tissue, but not both. Whichever of the two was successful in cornering the supply would depend upon whichever was first to start using it in development, and this might involve a very narrow difference in time, so that chance variables would determine which tissue this happened to be.

The final differences in the musculature of the body wall of the two types may be explained as a result of the difference in the extent of the hypoderm. It was noticed in mutant XR that a diminishment in the area of the hypoderm led to an exaggeration of the intersegmental divisions, simply because the same degree of folding was concentrated into a smaller area; further the muscles became concentrated in this area, apparently developing wherever attachment to apodemes was possible. The same thing occurs in type I of X2O, but more clearly since the limited area of hypoderm is better defined. Myoblasts which would normally form the ventrolateral muscles in this case contribute to the dorsolateral musculature, which is consequently exaggerated.

The ventral area of hypoderm is normally devoid of muscles, the region being occupied by the nervous

/system

system; in type II, where the nervous system is absent the myoblasts distribute themselves over this area in addition to rest of the hypoderm. Consequently, the musculature appears less robust than normally, because the same number of muscles is distributed over a wider area. Furthermore, the unused potential muscle cells noted in the normals appear to be absent, which may mean that this reserve is used in these abnormal circumstances.

There is no obvious explanation for the invariable failure of involution of the head type II. It is possible however, that the invagination of its material depends upon the presence of nervous tissue in this region. It may be that some firm internal base is required on which forces can be exerted, or tractions anchored, and the anterior part of the nervous system is indeed the only large firm block of tissue in this region.

An interesting comparison may be made between the effects of this mutant, and those produced by subjecting normal embryos to ultrasonics at an early stage of their development (Selman and Counce, 1953; Counce, 1953). Counce reports that in addition to embryos whose development was stopped completely, and those in which only slight or no effects were produced three categories of abnormal embryos occurred, characterized by:

/1.

1. Proliferation without differentiation,
2. Differentation without organization (late gastrulation configuration was reached, and there was sometimes a persistent cephalic furrow),
3. Abnormal organization.

These categories correspond fairly closely to types IV, III, and I respectively in mutant X20.

The embryo in the photograph illustrating the category in which there is abnormal organization (Selman and Counce, 1953) is indistinguishable from a typical type I embryo, with the exaggeratedly segmented dorsal hypoderm, and the uncovered ventral nervous system. In fact, the ultrasonics treatment frequently results in the production of what are, in effect, phenocopies of this mutant. It is reported (Counce, 1953) that the hypoderm, in embryos of this category, is never complete, and that head involution is affected. Nervous tissue was always present, and it is clear that type II embryos were not produced. If the developmental mechanism of the abnormality is the same in the mutant as in the phenocopy, and if the balance between embryos of type I and type II is as delicate as the present study suggests, it is surprising that this should be so. However, only 23 of these abnormally organized embryos were produced, and embryos of type II might be found if a larger number was studied. It would also be interest-

/ing

ing to vary the times at which the treatment was given, in case the treatment at a slightly different time should upset the balance in the opposite direction, giving type II embryos.

The production of these phenocopies suggests a consideration of the primary effect of the factor. The categories of ultrasonics treated embryos in which there is no differentiation, or else no organization, may correspond to types IV and III without their developmental mechanisms being identical, since the effects are rather general. On the other hand the case for a close similarity in the mechanisms producing the particular effects of type I is fairly strong. In the treated embryos it seems clear that the original disturbance must be a chemical one, induced by the ultrasonic irradiation at a sub-microscopic level, possibly disturbing an enzyme system involved in the ectodermal differentiation. A similar chemical disturbance must be assumed as the primary effect of the mutant factor. The fact that the ultrasonics treatment was given at about 2 hours, at the syncytial blastoderm stage, establishes that the primary effect of the mutant factor may also come into play at this period; slight variations in the degree of the effect at this early period might account for the wide range of variability in its expression.

Conclusions

Conclusions

X2C is a recessive lethal factor located on the first chromosome at about 0.0. Its lethality is always expressed in the embryonic stage, but within this there is considerable variation. Four types of abnormal embryos occur: I and II, in which there are certain abnormalities in various organ systems; III, in which there is an irregular blastoderm, and no development after gastrolation; and IV, in which cell proliferation occurs but not differentiation. In types I and II, the abnormalities may be traced to disturbances in the differentiation of the ectoderm, but the disturbances in the differentiation of the ectoderm are of contrary kinds. The abnormal embryos of type I are identical with some which previous workers have obtained by treating normal embryos with ultrasonics. The primary effect of the factor must be upon a chemical system involved in early differentiation.

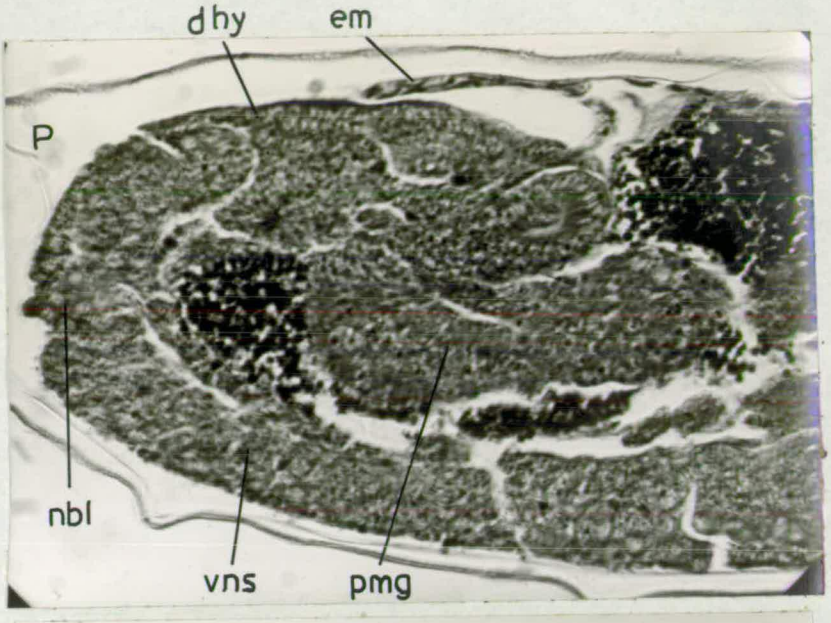
Fig.75. L.S. X20 type I, 9-10 hours, x500.

Fig.76. L.S. X20 type I, 10-11 hours, x400.

Fig.77. L.S. X20 type I, 11-12 hours, x400.

Key to figs. 75-90.

A,	anterior.
amg,	anterior mid-gut rudiment.
bl,	blastoderm.
br,	brain.
dhy,	dorsal hypoderm.
em,	embryonic membrane.
f,	furrow.
fs,	frontal sac.
gc,	gut cells.
gnso,	sub-oesophageal ganglion.
gon,	gonad.
hg,	hind-gut.
hgo,	opening of hind-gut.
hy,	hypoderm.
mg,	mid-gut.
mmg,	middle mid-gut.
msn,	mesodermal mesenchyme.
mus,	muscles.
nbl,	neuroblasts.
nt,	nervous tissue.
oes,	oesophagus.
P,	posterior.
pc,	pole cells.
ph,	pharynx.
pmg,	posterior mid-gut rudiment.
pmus,	pharyngeal muscles.
pv,	proventriculus.
sg,	salivary gland.
sti,	stomodaeal invagination.
tr,	trachea.
uyk,	unenclosed yolk.
vns,	ventral nervous system.
vshy,	ventral segmented hypoderm.
X,	fixation puncture.
yk,	yolk.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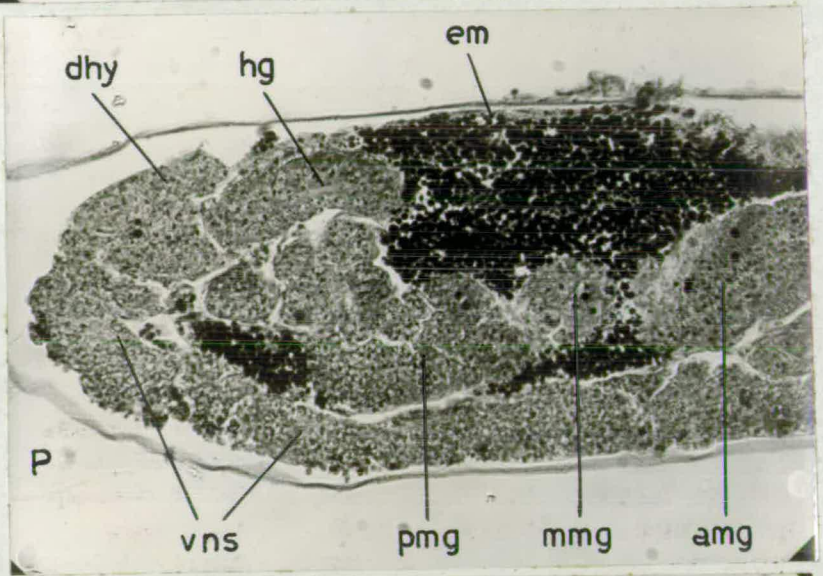
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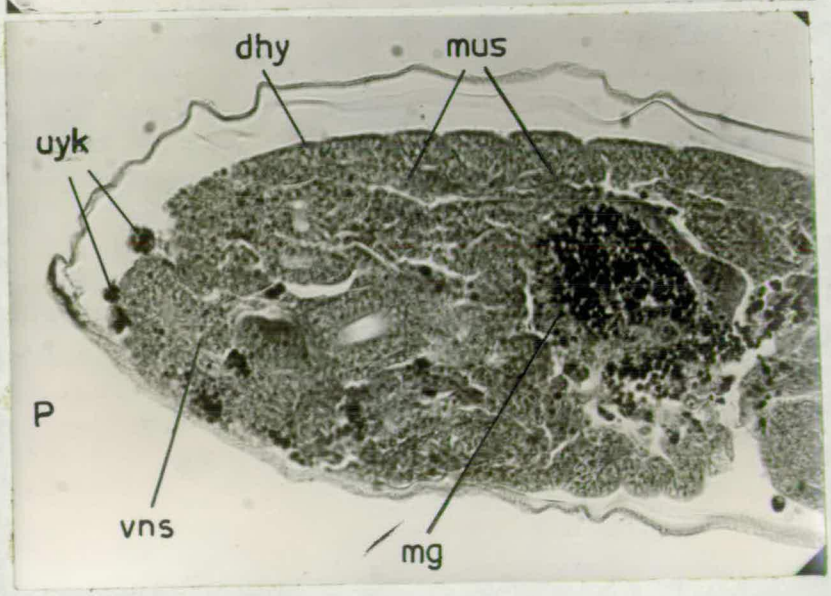
dhy
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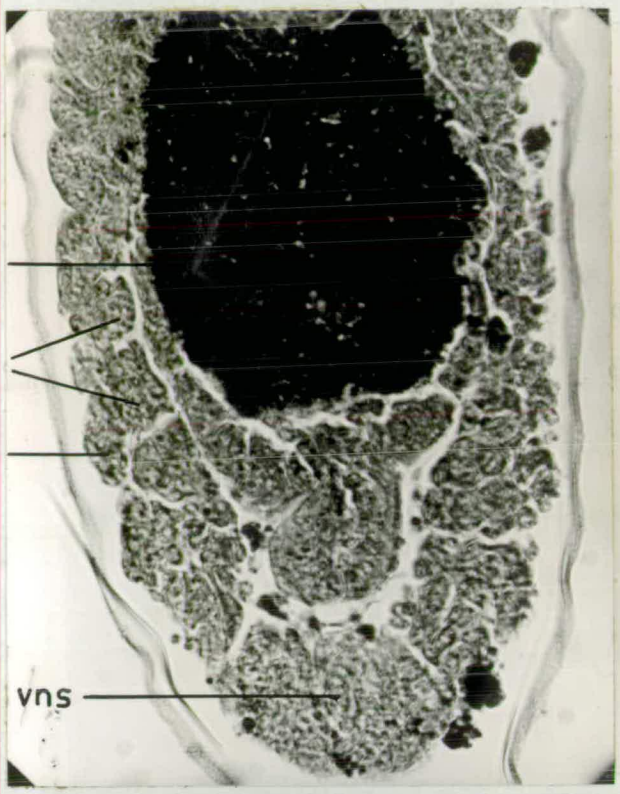
Fig.78. H.S. X20 type I, posterior region, 12-13 hours, x500.

Fig.78a. H.S. X20 type I, posterior region, 12-13 hours, x400.

Fig.79. H.S. normal, posterior region, 12-13 hours, x500.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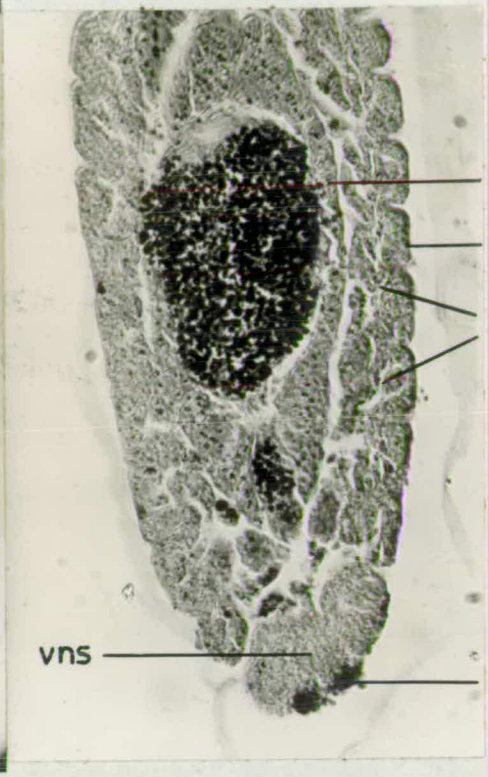
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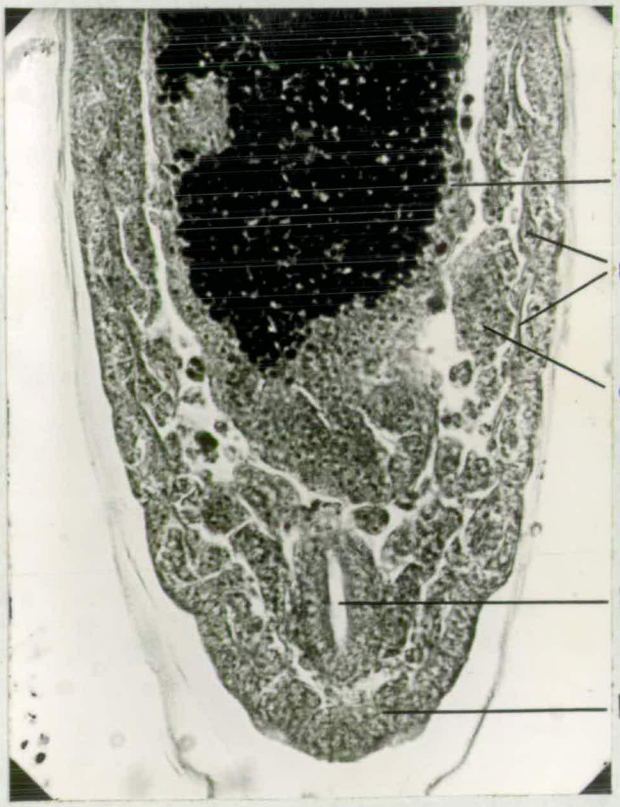
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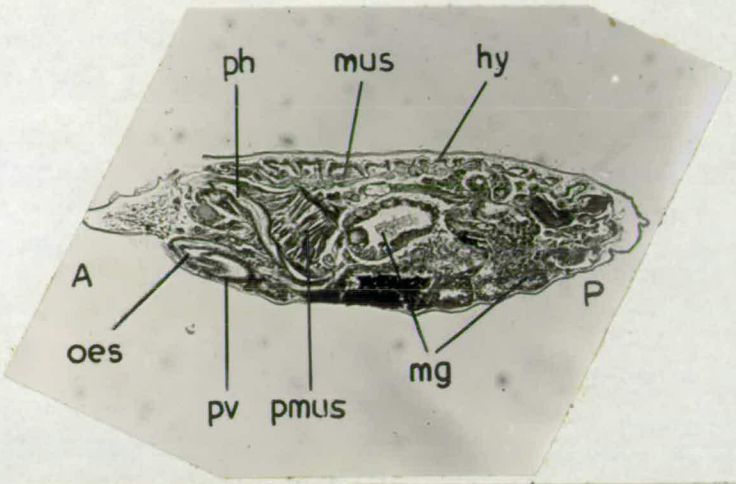
Fig.80. L.S. X20 type I, late embryo, x130.

Fig.81. L.S. X20 type I, anterior region, 16-17 hours, x500.

Fig.82. L.S. X20 type I, posterior region, 13-14 hours, x500.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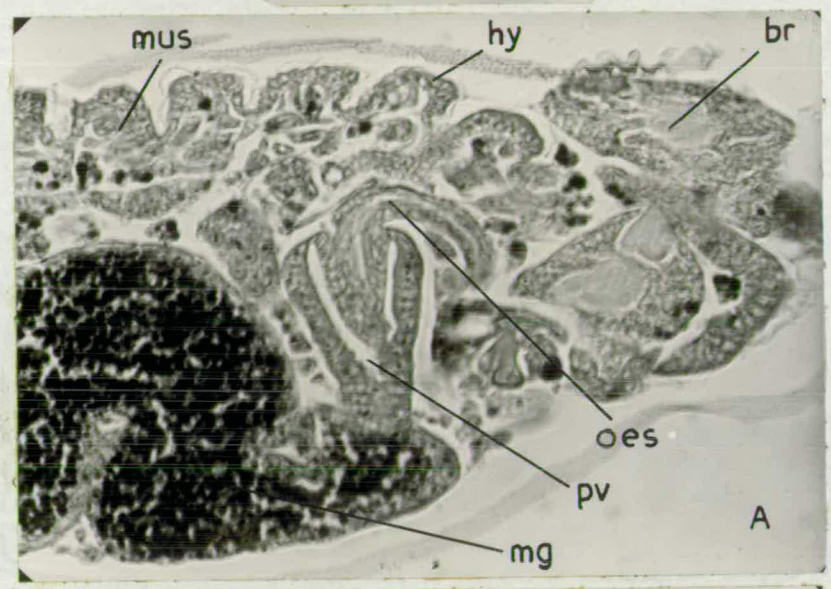
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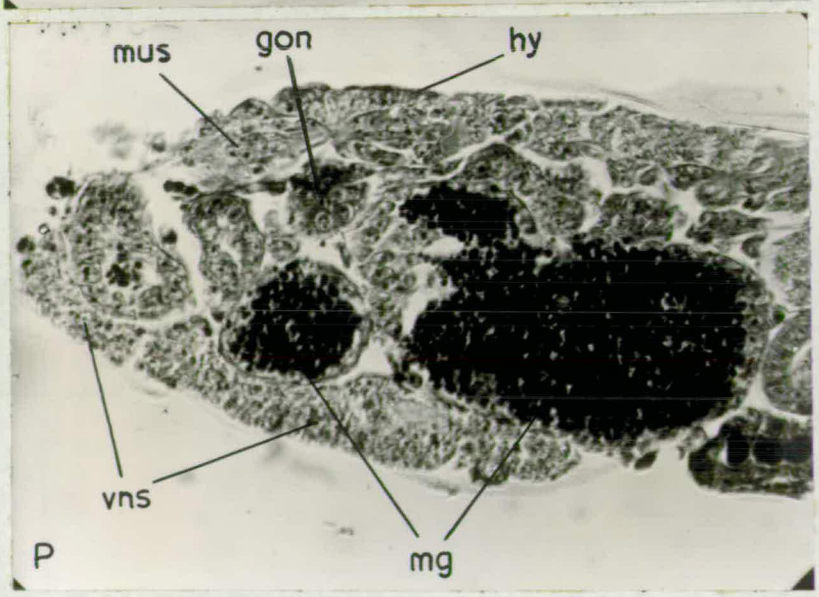
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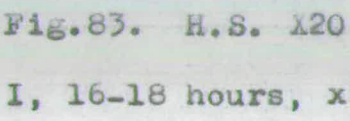
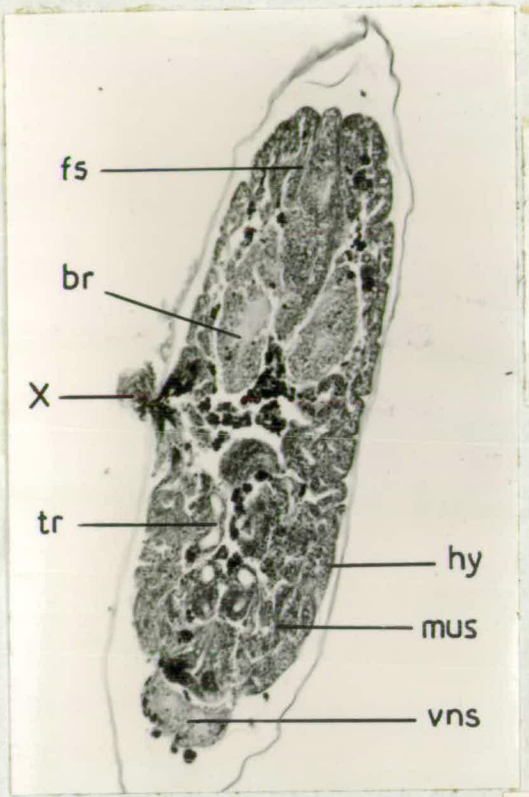


Fig.83. H.S. X20 type I, 16-18 hours, x200.

Fig.84. The same embryo; a more ventral section.

Fig.85. X20 type II, 16-18 hours, x200.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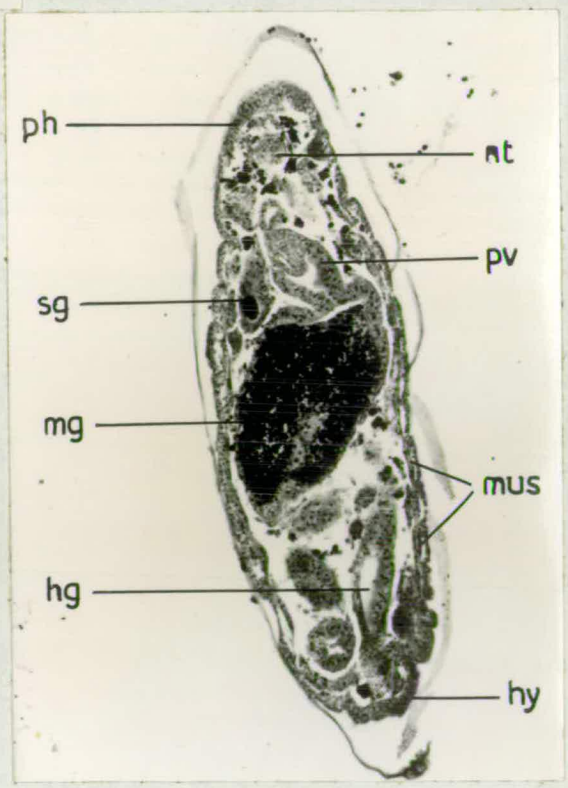
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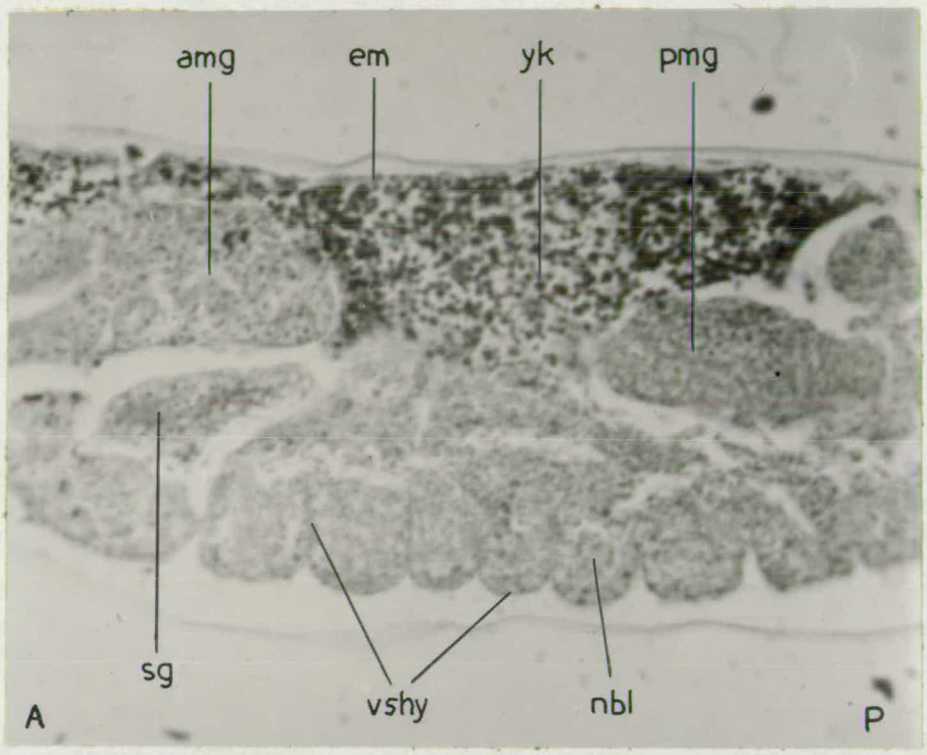
Fig.86. L.S. X20 type II,

10-11 hours, x600.

Fig.87. L.S. X20 type II,

11-12 hours, x400.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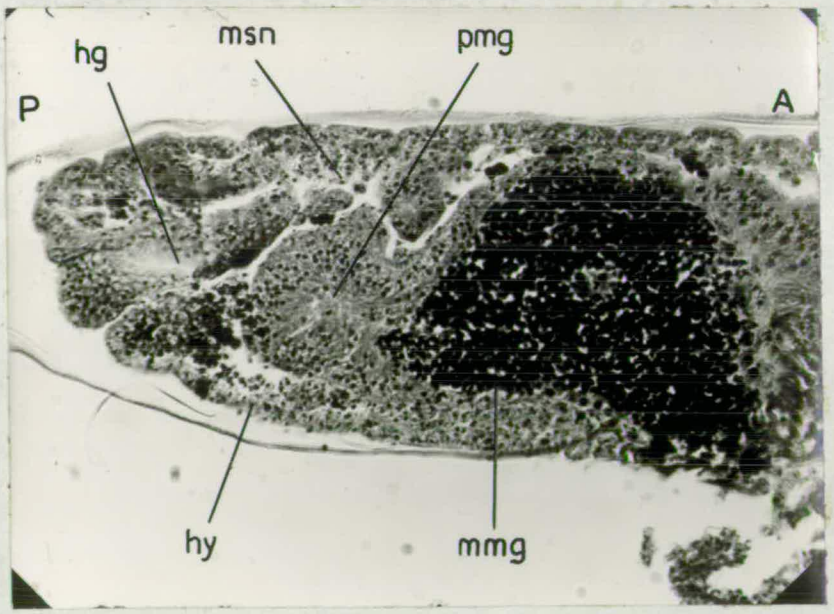
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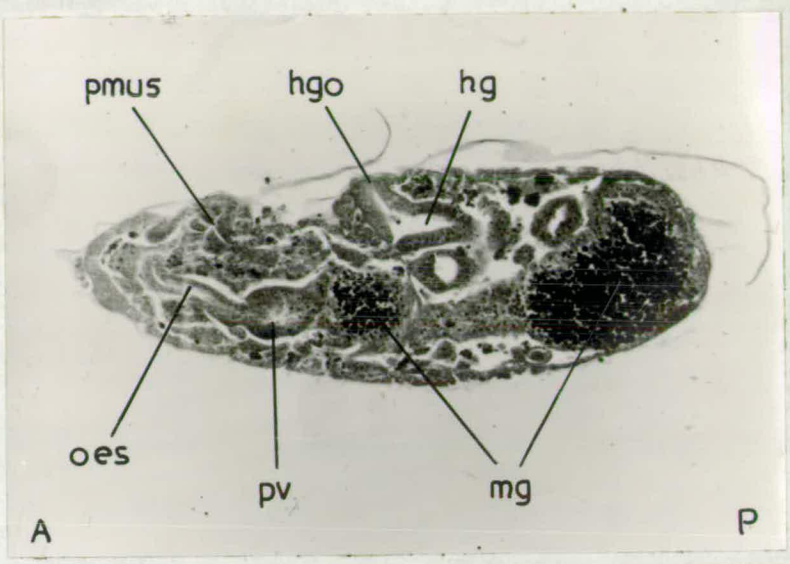
Fig.88. L.S. X20 type II, 16-18 hours, x200.

Fig.89. L.S. X20 type III, 6-7 hours, x200.

Fig.90. L.S. X20 type III, 14-15 hours, x200.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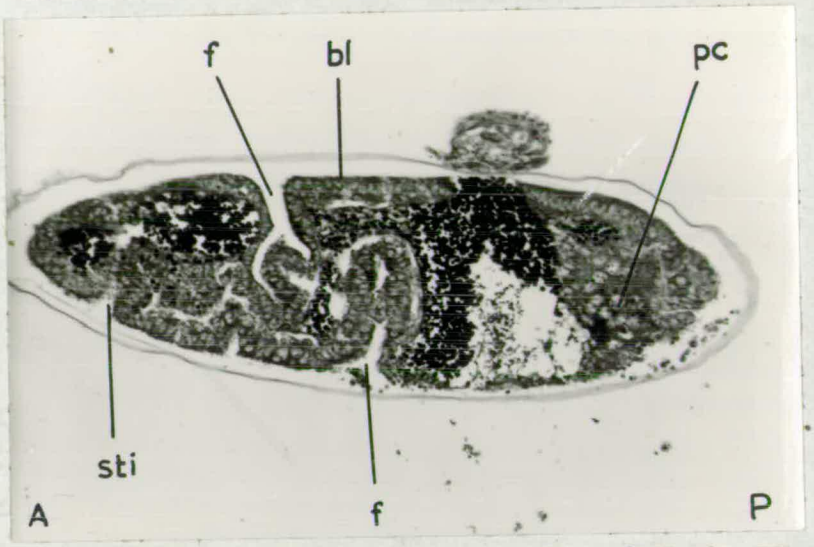
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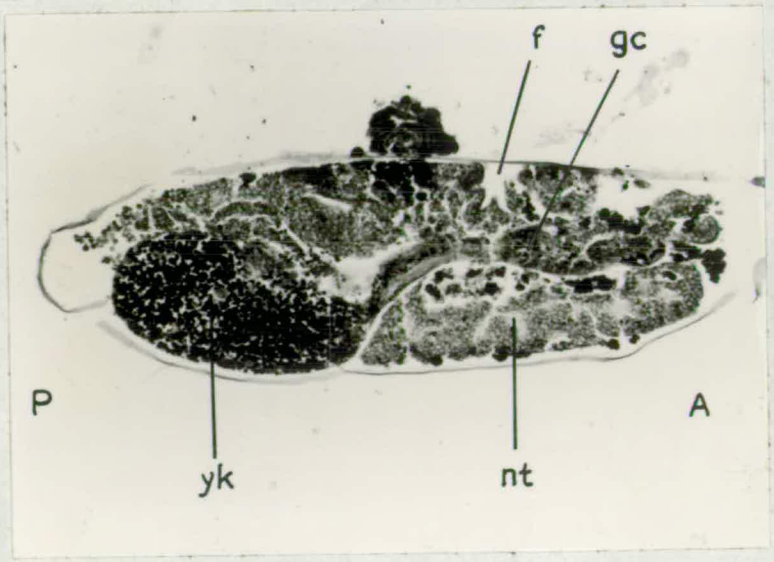
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Mutant X10

Material:

Embryos were sectioned at all stages after 4 hours. Living embryos were also examined.

Preliminary data:

1. The factor was induced by X-irradiation of sperm, and has been tentatively located, by genetic tests, close to the scute locus (0.0) (H. Slizynska, unpublished).

2. Analysis of sectioned material:

Period	Unfert-ilized.	Unident-ified.	Normals	Abnormals		
				I	II	III
4-12	25	6	107	9	6	3
12-15	35	13	128	15	1	10
17-20	12	14	103	25	3	20
22-30	51	1	24	15	9	30
Totals	123	34	362	64	19	63

The expression of the lethal factor:

Three types of abnormal embryos, described below, are found in this stock. Types I and II can be recognized in all the sectioned stages, and type III from 12 hours onwards. All types have been found in batches of eggs collected from single females, in which, therefore, embryos of each batch were identical with respect to their X-chromosomes. This shows that the presence of distinct abnormalities does not result from a mixture of sex-linked factors in the stock, but probably arises from differences in expression of a single factor.

The tabular analysis of the sectioned material suggests that the relative numbers in the different types are not constant. Moreover, the proportion of eggs which produce abnormal embryos, of any sort, appears to vary.

The first observations to be made were upon embryos in the groups 17-20 and 22-30 hours. In the first of these, in which normal embryos are still unhatched and represented, the fraction of abnormal embryos is 48 out of 151 fertilized and identifiable eggs. This is not significantly

/different

different from 25%. In hatchability tests, also, about 25% of eggs failed to hatch. It appeared that the lethality was expressed completely in the embryonic stage.

Approximately six months later embryos in the groups 4-12 and 12-15 hours were studied, in order to trace the development of the abnormalities. In the group 12-15, the lethal fraction was now significantly less than 25%; in the group 4-12 it could not be estimated because type III was unclassifiable, but the numbers of type I were considerably reduced. Hatchability tests were made upon twelve groups of females constituting the F_1 generations from single pair matings; in these the proportion of unhatched eggs, discounting apparently unfertilized ones, varied from 6.3% to 18.5%, with an average of 13%. It is possible that some lethal embryos were misclassified as unfertilized eggs, but it appears from these tests, and from the sectioned material, that the factor was sometimes not lethal in the embryonic stage.

These considerations suggest that the effect of the factor varies in its embryonic expression between the three types I, II, and III, and that in

/some

some cases the lethal effect does not occur in the embryonic stage at all. No red-eyed males, i.e. males carrying the chromosome with the lethal factor, have been found, and no abnormal pupal lethality has been observed; it is probable, therefore, that in such cases the lethality is expressed in the larval stage. There appears to have been a change, occurring between the earlier and the later series of observations, in the relative numbers of embryos falling into the various categories of expression of the factor, with a greater proportion of larval lethality in the later ones. This will be considered further in the discussion.

Embryos of Type I

The development of these embryos is abnormal from the time of cleavage, and there is no cellular differentiation or organization. The cleavage nuclei, with few exceptions, remain in the anterior region of the egg. They migrate to the periphery, but instead of forming a single layer, an irregular accumulation of nuclei is produced (fig. 91). Cells are formed by the division of the cytoplasm around these nuclei, which proliferate to form a cap of small undifferentiated cells which fills the

/anterior

anterior third of the egg. The remaining two thirds is occupied by yolk and cytoplasmic material, with a few scattered nuclei at the periphery. Occasional nuclei in both regions are polyploid, and may be recognized by their large size as well as by their high chromosome complement (figs. 91 and 92). Most of the cells are usually limited to the anterior cap, but in some cases there is a posterior cap also, usually linked to the anterior one by a peripheral strip of cells.

Similar pre-blastoderm disturbances of the cleavage nuclei have already been mentioned in Lff 11 and in type IV of X20. In both of these, however, the cells are less definitely limited to the anterior cap, and tend to be scattered irregularly through the egg. On the other hand, eggs deficient for the whole of the X-chromosome (Poulson, 1940), appear to be identical with this type.

One embryo was found in which the cap of undifferentiated cells was formed anteriorly, but in which cells at the posterior end had become differentiated and organized to form gut material (fig. 93).

Embryos of Type II

In these embryos the disturbance again appears before the blastoderm stage, leading to an irregular distribution of the cleavage nuclei. These do not become aggregated at the anterior end, but produce scattered groups of cells. In some, pole cells are differentiated (fig. 94); in a few cases an irregular blastoderm is formed, and there is a very small degree of differentiation (fig. 95); but most often, no blastoderm is formed, and the cells differentiate entirely into clumps of nervous tissue, with ganglion cells and fibres, while the remainder of the egg is partly filled with yolk granules and undifferentiated cytoplasm (fig. 96). Occasionally a type occurs which may be transitional to type III; in this there is a high degree of differentiation and organization, but there is an excess of nervous tissue, which takes the place of hypoderm over a large part of the embryo.

Embryos of Type III.

Final Appearance of the Embryo

Living mutant embryos are not easily distinguishable from normal ones. The hypoderm is /usually

usually complete, and segmentation is present, though irregular. Internally, all the organ systems are present, and are abnormal chiefly in their cellular differentiation. The differences will be described in the account of the development of the embryo. Cellular degeneration begins at about 25 hours.

Development of the Embryo

Early development:

The early developmental stages, including blastoderm formation and gastrulation, are normal. The differentiation which normally follows between 5 and 9 hours also occurs, but it is retarded. Up to approximately 10 hours the cells remain relatively uniform, and are not separated into distinct tissues. This is particularly evident ventrally, where hypoderm, nervous tissue, and mesoderm constitute a single mass of cells (fig. 97); the intersegmental divisions appear ventrally as well as laterally.

Separation into tissues has occurred by 12 hours, but differentiation in the form of the cells

/is

is far less advanced than normally. This is particularly evident in the gut, where the stomodaeum is still a simple tube of undifferentiated cells, and the endoderm rudiments are only beginning to surround the primitive gut (figs. 98 and 99). This may be compared with the normal embryo at this stage, the fore-gut is differentiated into pharynx and oesophagus, the proventriculus rudiment has formed, and the first constriction has appeared in the mid-gut sac (fig. 100).

The general embryonic movements of the next period are also slowed down. Shortening and dorsal closure always take place, but are not completed until approximately 14 hours. Involution of the head is never completed; the pharyngeal material is only partly invaginated, and the frontal sac remains shallow.

Organ formation:

The gut:

1. The fore-gut: Owing to the incompleteness of head involution, the pharynx is small. Some pharyngeal material remains externally, but the pharyngeal muscles are attached to the internal part

/of

of it, and extend between this and the material of the frontal sac, which is unenclosed. The oesophagus is displaced, but is otherwise normal. A chitinous lining is secreted, but not cephalopharyngeal bars. The salivary glands are small, and open separately and externally.

2. The mid-gut: The endoderm rudiments come to enclose the yolk completely, and the mid-gut sac thus formed undergoes some of the changes in shape which occur in the normal embryo. But it does not become coiled or tubular, and mid-gut caecae and malpighian tubules are not developed; a proventriculus is recognizable, but undergoes none of its characteristic cellular differentiation. The cells of the mid-gut wall remain cuboidal (fig. 101). A number of cells, some of which are probably pole cells, remain in the yolk.

3. The hind-gut: This is displaced, and its cells are poorly differentiated, but its external opening is in the normal place.

The gut is invested with visceral muscle cells, but they remain spherical and isolated instead of spreading over the gut wall.

The body wall:

In some cases there is no hypoderm formed over parts of the nervous system, especially anteriorly; but in general the hypoderm is complete, and a cuticle is formed. It is segmented, but the segmental divisions are irregular, and there is much buckling of the hypoderm within the segments. This appears to result from the abnormalities in the muscular system. In normal embryos the muscles are spindle shaped, and extend from one apodeme to the next; in this mutant the muscles are rounded, and are attached to the apodemes only at one end, so that they become arranged in clusters (fig. 102). Each muscle consists of a spherical cytoplasmic body, containing several nuclei, and there are no contractile fibrils. Sometimes there are short contractile filaments between apodemes, which are isolated from the muscle clusters (fig. 101). The shortness of these filaments, and the absence of tone resulting from the non-attachment of the muscles at one end, appears to be the cause of the concertinaring of the hypoderm (compare figs. 102 and 79).

The nervous system:

The nervous tissue of the brain region is often not covered by hypoderm (fig. 101), but the

/ventral

ventral nervous system always is. Ganglion cells and fibres are differentiated in the latter, but its form is irregular, and it lies deeper than in the normal embryo, with the intersegmental divisions and the abnormal musculature of the body wall extending under it.

The tracheae:

These are well developed, but the course of the main trunks is twisted by the concertinaring of the body wall (fig. 102).

The gonads:

This mutant provides some evidence concerning the relation of the pole cells to 1) the germ cells, and 2) the middle mid-gut cells, previously discussed on page 208.

1) No gonads are formed, and the pole cells may usually be found remaining inside the mid-gut. This strongly suggests that the germ cells normally arise from those pole cells which are carried in with the posterior mid-gut rudiment, and that in this case they are simply unable to migrate out of it into the body cavity.

2) In one embryo a number of cells, distinguishable by their large nuclei as pole cells, are partly incorporated in the gut wall, while others are free in the yolk (fig. 101). Those in the gut wall obviously correspond to the middle mid-gut cells, and are identical with the ones in the yolk. This supports the conclusion that the middle mid-gut cells are also derived from the pole cells.

Discussion

Types I and II involve disturbances of the mechanism of the distribution of the cleavage nuclei. All three types involve disturbances, in varying degrees, of cellular differentiation. Where the later morphogenetic movements occur at all, i.e. in type III, they are undisturbed. No hierarchical sequence of mechanical defects is found, except for the minor deformations of the body wall in type III, which are caused by the abnormalities of the muscular system. In addition to these three types it was suggested above that some "lethal" embryos develop normally and survive into the larval stage.

If it is the case that each of these types
/represents

represents a different manifestation of a single character, it is not easy to envisage what genic action may be basic to all four, or what determines which of the types will appear in a particular embryo. The same problem arises in the mutants X20 and S9 in this study.

The manifestation of the effects of lethal factors at specific periods in development has been termed by Hadorn "phase specificity" (Hadorn, 1948 and 1951). The majority of Drosophila lethals so far investigated have been shown to cause the death of the organism at a single phase of its life-history - in the embryo, the larva, or the pupa, and usually even more specifically within these periods (Hadorn and Chen, 1952; Rizki, 1952; Oster, 1952 and 1954). However, numerous exceptions to this rule do occur. For example, six 2nd-chromosome mutants have been found in which both early and late embryonic lethality occurs (Brody, 1940). The mutant translucida causes death either in an early or a late pupal stage (Hadorn, 1949); other mutants are lethal in both embryonic and late larval stages, and others in both embryonic and pupal stages (Hadorn and Chen, 1952). Similarly in the fowl, embryos which are homozygous for

/creeper

creeper usually die on the 3rd-4th day, but some survive this stage and die at the end of incubation (Landauer, 1932).

In some cases it has been shown that the time of effect is determined by environmental conditions. A factor was found in Drosophila pseudoobscura which was a complete lethal at 25½°C, semi-lethal at 21°C, and viable at 16½°C (Dobzhansky and Spassky, 1944). A maternal effect acting through the egg cytoplasm has been shown to determine the time of effect of three 2nd chromosome lethals; whether the organism dies in the late larval stage or in the pupal stage depends upon the age of the mother (Hadorn and Zeller, 1943).

The time of action of lethal factors is also affected by their genetic background. The mutant lethal giant larva (lgl) usually causes death in the larval stage, but a strain has been isolated in which it occurs in the embryo (Hadorn, 1940). Again, a number of stocks carrying the cryptocephal mutant have been isolated and selected for varying penetrance and expressivity; in "strong" stocks, all die as pupae, while "weak" stocks produce high

/percentage

percentage of adults (Hadorn and Gloor, 1943). Temperature also affects the expression of this mutant (Gloor, 1945).

Hadorn terms the individuals which escape the lethal effect in its earlier period of action "break-throughs" (Hadorn, 1948). The mechanism which produces such divergence must reside in the variability in expression and penetrance which is well-known to occur in viable mutants, in conjunction with developmental thresholds. It must be supposed that if the activity of the mutant gene is sufficiently great, a particular lethal disturbance is triggered off in development; on the other hand, if the gene activity falls below this particular threshold, the development proceeds normally until the next period in which its effect may be lethal.

This type of mechanism may well occur within the embryonic period. In the mutant X10 the first lethal period is at the time of migration of the cleavage nuclei, when the effect of the factor may stop further development completely (type I). Break-throughs occur in which, though migration is still disturbed, some differentiation, particularly

/into

into nervous tissue, occurs (type II). Other embryos escape this disturbance, and are break-throughs to the third period, in which the lethal effect of the gene is to slow down and modify cellular differentiation (type III). It is probable that some embryos escape this last embryonic disturbance, and become break-throughs to the larval stage. The environmental conditions in which embryonic development occurs vary very little, and which type of abnormality appears probably depends upon the uncontrolled genetic background. The changes which may have occurred in this could account for the discrepancy in the relative numbers in each of the different types between the earlier and the later observations.

Conclusions

X10 is a recessive lethal factor located on the first chromosome at about 0.0. It is usually lethal in the embryonic period, but it is variable in expression, and probably some embryos survive into the larval stage. Those which die as embryos are of three types: In type I an anterior cap of undifferentiated cells is formed. In type II there is no organization, but there is some cellular

/differentiation

differentiation, usually entirely into nervous tissue. In type III all the essential developmental events occur, the morphogenetic movements are undisturbed, and a highly organized embryo is produced. But cellular differentiation is retarded, and many of the final cell types are abnormal. The muscles of the body wall are particularly malformed, and cause a general disturbance of the hypoderm and its segmentation. No single primary effect is found which is basic to the terminal abnormalities; the effect of the gene must be upon the general metabolism of the developmental processes rather than upon the formation of any particular structure.

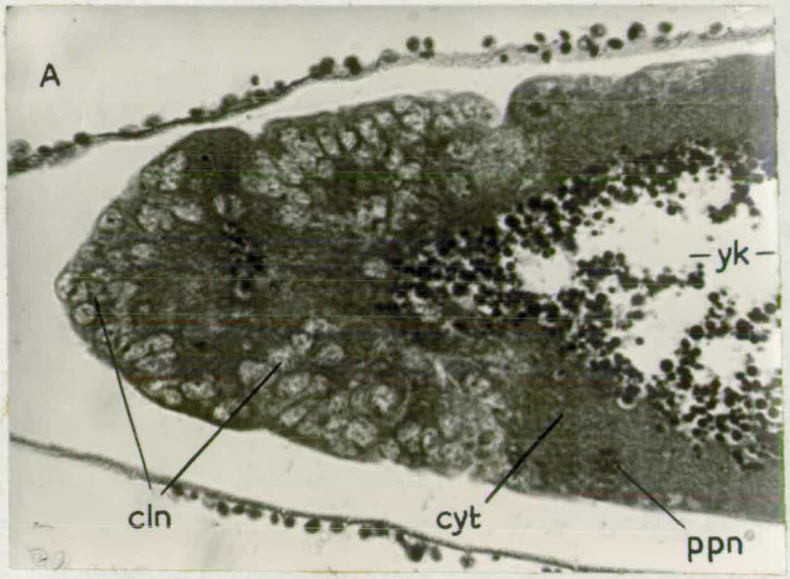
Fig.91. L.S. X10 type I, anterior region, 4-5 hours, x500.

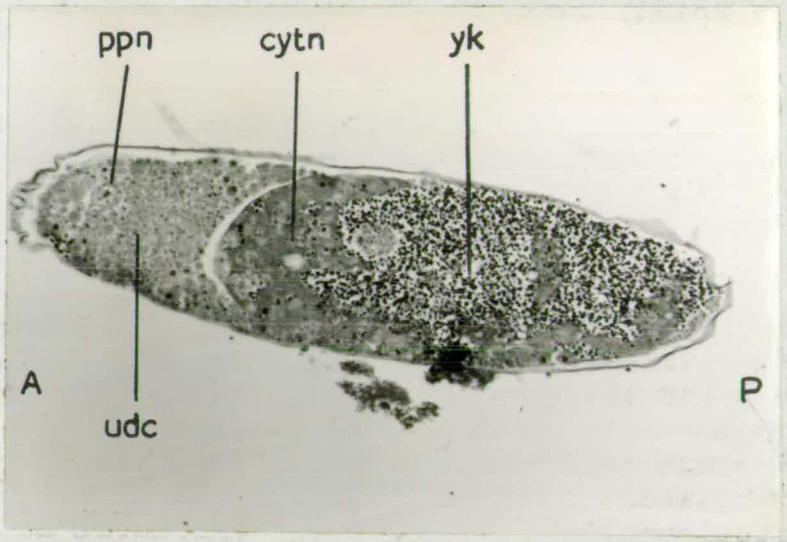
Fig.92. L.S. X10 type I, 15-16 hours, x200.

Fig.93. L.S. X10 type I, 12-13 hours, x400.

Key to figs. 91-102.

A, anterior.
amg, anterior mid-gut.
br, brain.
cln, cleavage nuclei.
cyt, cytoplasm.
cytn, cytoplasm with nuclei.
gnso, sub-oesophageal ganglion.
gt, gut.
hg, hind-gut.
hy, hypoderm.
mg, mid-gut.
mgc, mid-gut constriction.
mmgc, middle mid-gut cells.
mus, muscles.
musc, muscle cells.
nt, nervous tissue.
oes, oesophagus.
P, posterior.
pc, pole cells.
ph, pharynx.
pmg, posterior mid-gut rudiment.
ppn, polyploid nucleus.
pv, proventriculus.
sg, salivary gland.
st, stomodaeum.
tr, trachea.
udc, undifferentiated cells.
vem, ventral ectoderm and mesoderm.
vns, ventral nervous system.
yk, yolk.


A
yk
cln
cyt
ppn


ppn
cytn
yk
A
P
udc

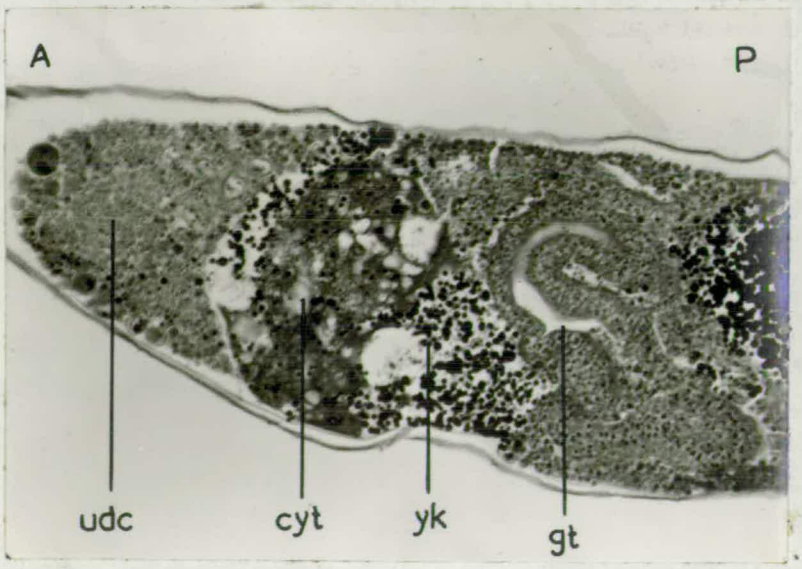
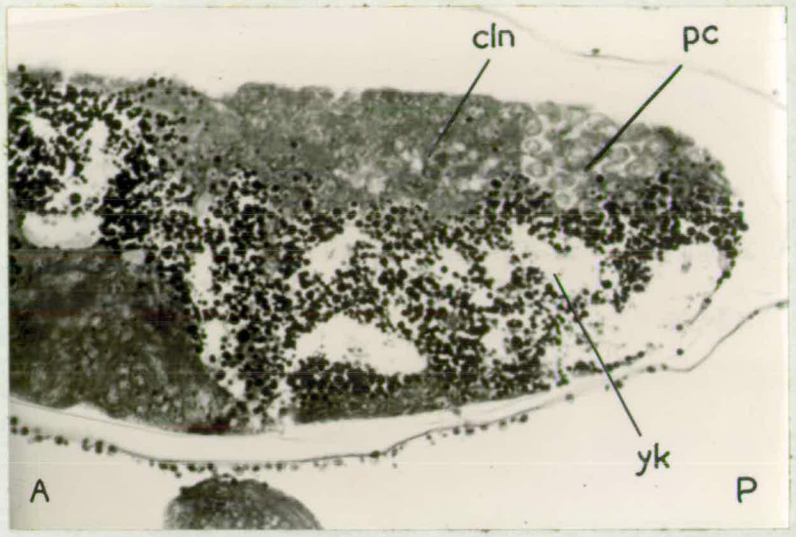
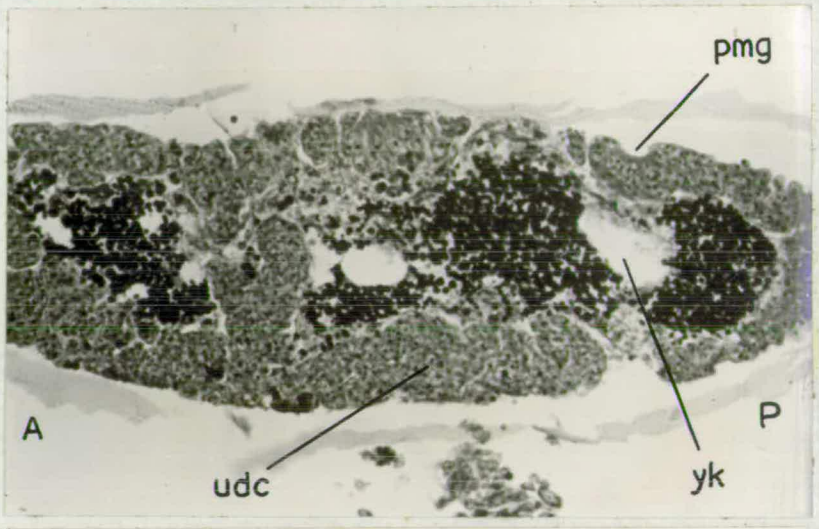

A
P
udc
cyt
yk
gt

Fig.94. L.S. X10 type II, 5-6 hours, x340.

Fig.95. L.S. X10 type II, 27-29 hours, x340.

Fig.96. L.S. X10 type II, 27-29 hours, x340.


cln
pc
yk
A
P


pmg
A
P
udc
yk

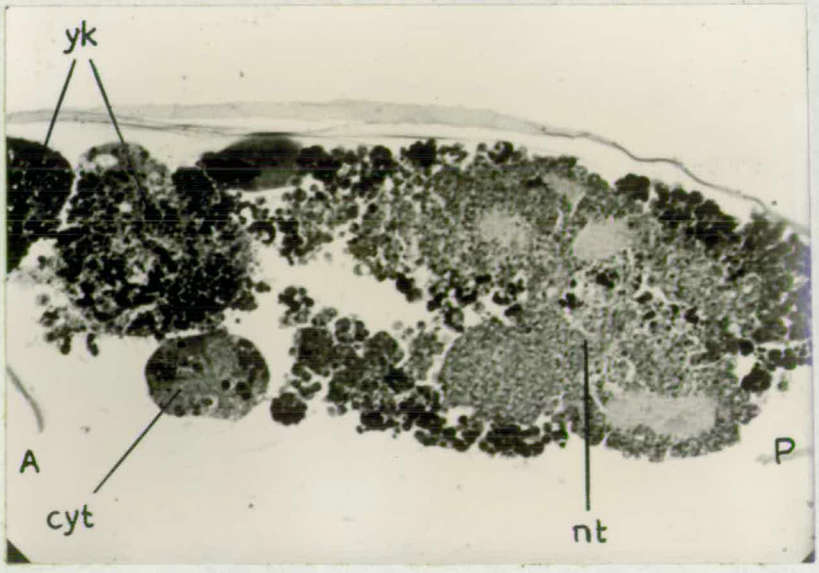
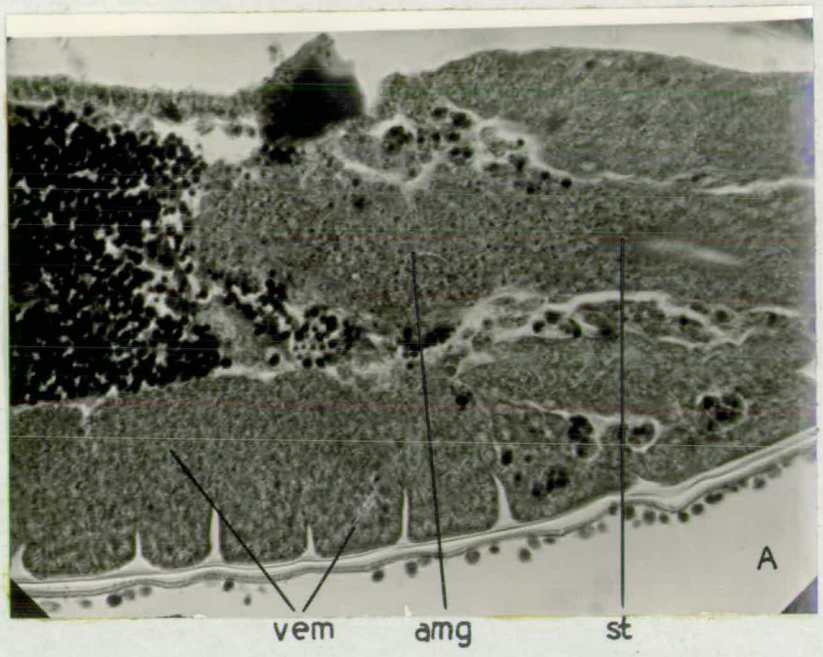

yk
A
P
cyt
nt

Fig.97. L.S. X10 type III,
10-11 hours, x500.

Fig.98. L.S. X10 type III,
13-14 hours, x500.


A
vem
amg
st

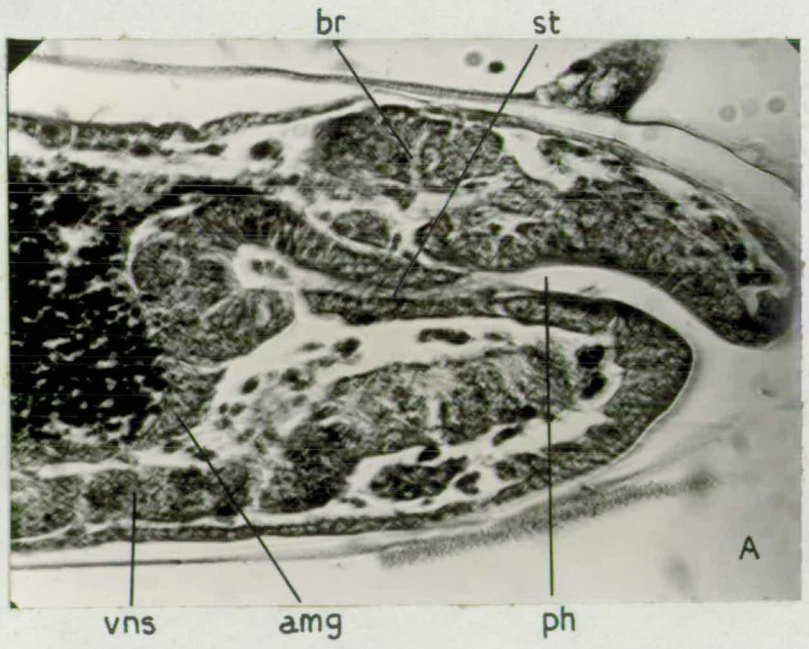
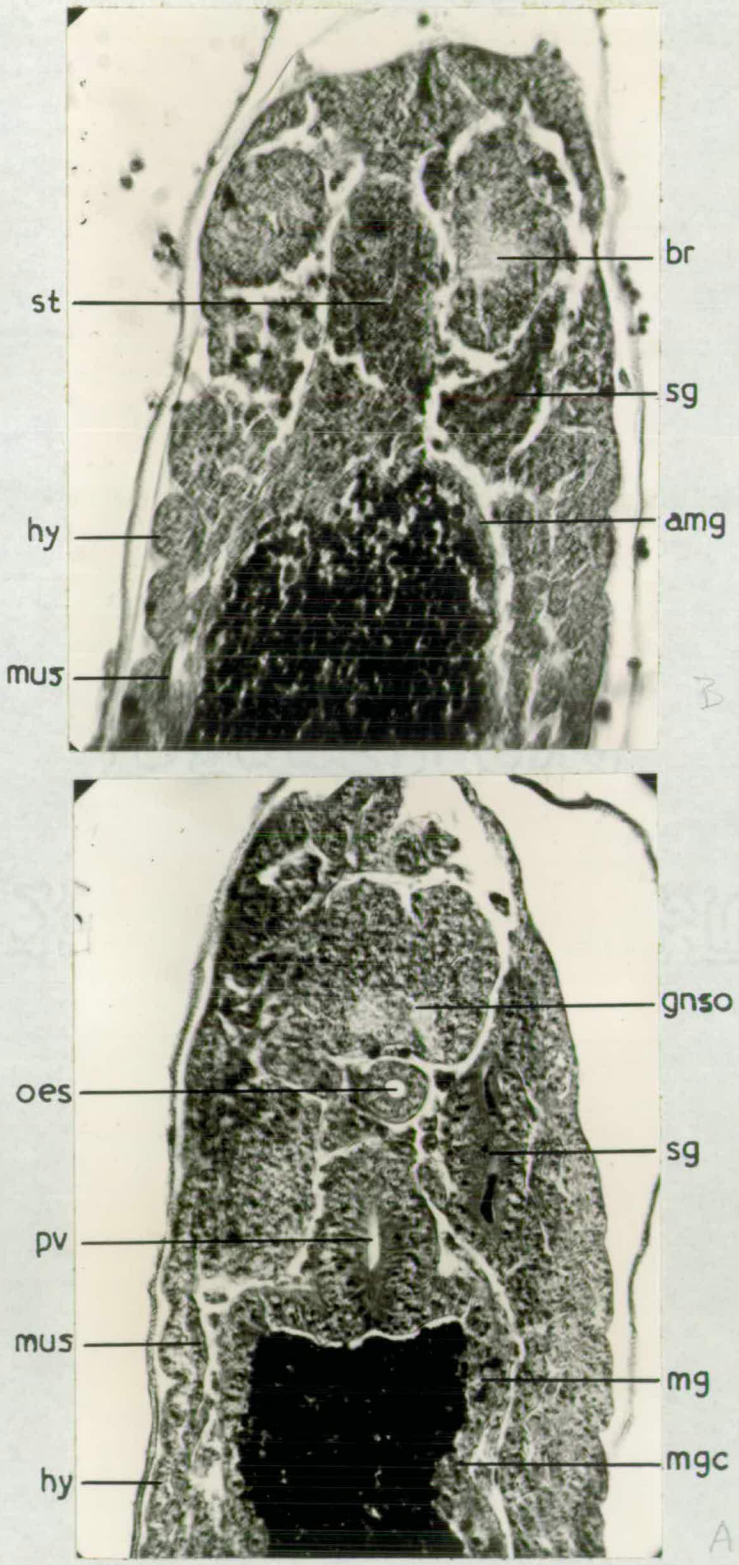

br
st
A
vns
amg
ph

Fig.99. H.S. X10 type III, anterior region, 12-13 hours, x500.

Fig.100. H.S. normal, anterior region, 12-13 hours, x500.


br
st
sg
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amg
mus
B
gnso
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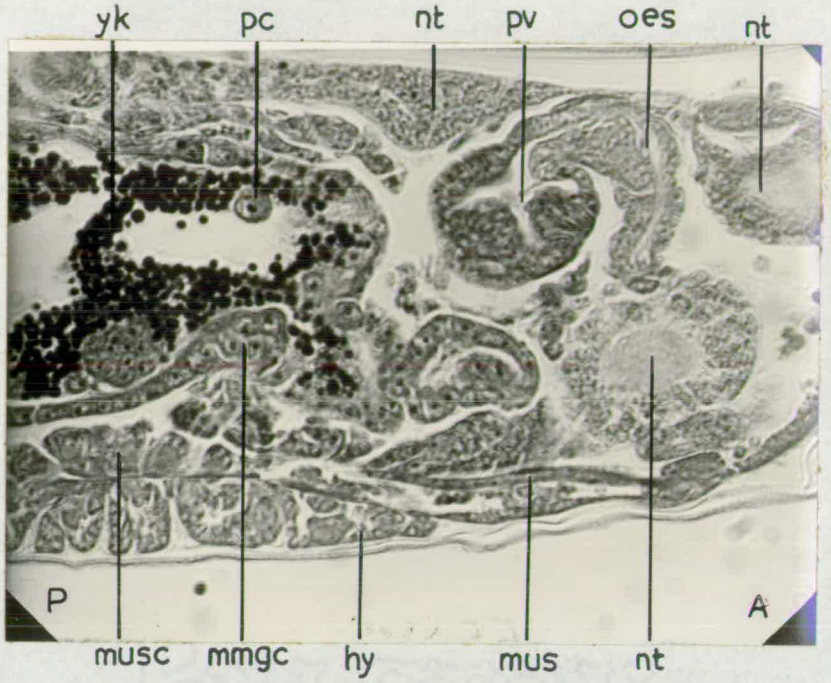
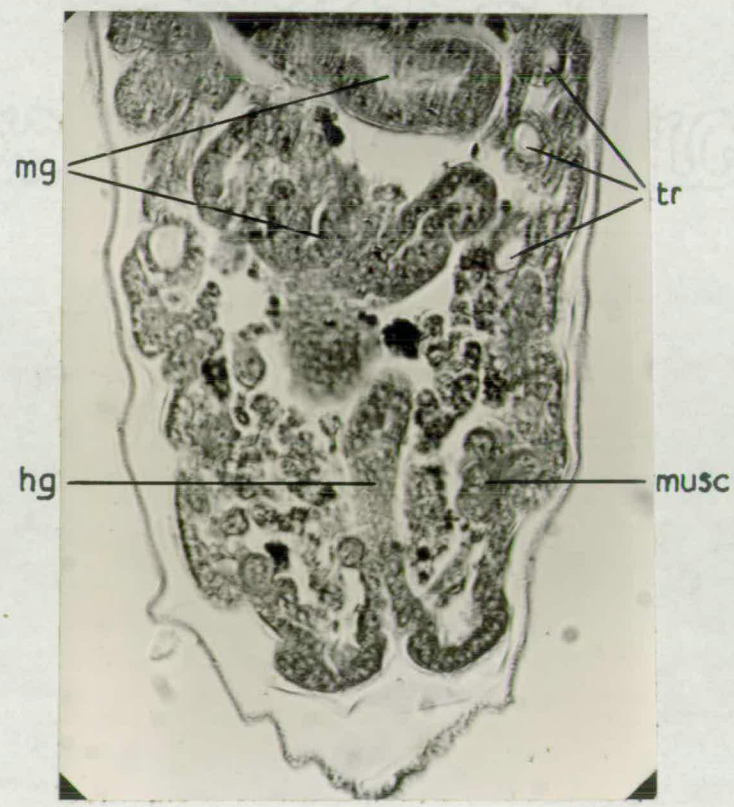
hy
mgc
A

Fig.101. L.S. X10 type III, anterior region, 19-20 hours, x500.

Fig.102. H.S. X10 type III, posterior region, 19-20 hours, x500.


yk
pc
nt
pv
oes
nt
P
A
musc
mmgc
hy
mus
nt


mg
tr
hg
musc

Mutant S9

Material:

Sections were made of embryos at all developmental stages after two hours, and living embryos were also examined.

Preliminary data:

1. This factor arose spontaneously, and has been approximately located, by genetic tests, close to the centromere, to the right of carnation (62.5) (H.Slizynska, unpublished).

2. When the stock was first tested, 60 out of 162 eggs (37%) failed to hatch, suggesting that it caused complete embryonic lethality. Subsequent tests and observations revealed a much more complex situation, which still requires further investigation.

Later hatchability tests showed a variable, but always low, proportion of eggs not hatching, and the stock showed a high pupal lethality. Some of the full-grown larvae would remain uncontracted, forming elongate pupae which died soon

/afterwards

afterwards. A number of single-pair matings were made, in order to determine whether the embryonic and pupal lethalities might arise from separate X-chromosome factors which had become mixed in the stock. However, with few exceptions, both embryonic and pupal lethality occurred in the progeny of each female; in the case of the exceptions, where one or other appeared to be absent, it occurred in the next generation. The duality of the lethal effect is not, therefore, simply due to a mixture of X-chromosome factors.

The summed figures from these matings gave a fraction of 72 out of 587 (12%) embryonic, and 60 out of 671 (9%) pupal lethality. This falls short of the expected 25% total lethality expected by ¼, suggesting that death may also occur in the larval stage.

3. Analysis of sectioned material:

The first series of sections was made from the original stock. The second series, constituting the majority of the sections, was made from a strain "f" originating from one of the single-pair matings. The progeny of the pair had shown a high embryonic lethality (14/54) and no pupal lethality (0/56); but these characteristics were not maintained in subsequent generations.

/Strain

Strain	Period	Unfert-ilized	Unident-ifiable	Normals	Abnormals
Original	8-20hrs.	30	4	90	9
Original	25-30	4	28	16	58
"f"	2-14	196	43	747	27
"f"	25-30	16	8	50	78
	Totals	246	83	903	172

The expression of the lethal factor:

The proportion of abnormal embryos in the sectioned material is extremely low: 9 out of 99 fertilized and identifiable eggs (9.1%) in the period 8-20 hours for the original stock, and 27 out of 774 (3.5%) in the period 2-14 for the "f" strain. It is possible that in the last case several mutants were misclassified as unfertilized, but it is clear that the embryonic lethal fraction is not stable, and that the expression of the lethal factor does not necessarily involve the embryonic stage. This question is taken up in the discussion. The abnormal embryos show a considerable range of variation, but they are not separable into distinct types, and conform to the same general developmental pattern.

Final Appearance of the Embryo

The degree to which organisation and cellular differ-

/entiation

entiation occurs varies greatly, and on this the final appearance of the embryo depends. But almost every one is characterized by the presence of a certain amount of undigested yolk between itself and the vitelline membrane, especially at the anterior end. The embryo is extremely deformed, particularly in this region (fig. 110).

The Development of the Embryo

Events up to gastrulation:

In this mutant the final abnormalities have their origin in disturbances of the earliest developmental events, i.e. in the distribution of the cleavage nuclei and in blastoderm formation. There is little to add to what has been said above about the cleavage nuclei in the normal embryo, but it is necessary in the present connection to describe more fully the normal formation of the blastoderm, and the changes which occur in it. A number of original observations are included in this account, derived from sectioned material and from the film.

The blastoderm in the normal embryo:

The cleavage nuclei arrive at the periphery of the egg at about 2 hours, and form a syncytial blastoderm (fig.3, 2.30-2.37; fig.4, 2.10; fig.9, 2.10). At about

Fig 1, 1.50

/2,30

2.30, furrows grow in from thesurface between the nuclei and into the cytoplasm beneath them, so that the blastoderm becomes a layer of columnar cells (fig.3, 2.50; fig.9, 2.35). The films shows something occurring shortly after this which is difficult to interpret. The inner border of each blastoderm nucleus appears to become detached and move rapidly towards the centre of the egg; the effect of this occurring in all the nuclei simultaneously is of a line of white dots moving inwards (fig.3, 2.53-3.09; fig.9, 3.15-3.20). The line does not advance quite to the inner border of the Keimhautblastem, and it appears to coincide with the inner cell boundaries. Whether this phenomenon is in fact connected with the formation of the latter, or whether it is an optical effect produced by layers of cells moving over each other during the general blastodermal rearrangements, is not certain.

When the cellular blastoderm is first formed, it is uniform inthickness; all the cells are columnar, and are at right angles to the surface of the egg (fig.9, 2.45). Towards the end of the events described above, changes occur in the formoof the blastoderm, and corresponding ones take place in the yolk. The latter contracts in the middle region, and the blastoderm becomes correspondingly thicker at this point (fig.4, 3.20-3.30; fig.9, 3.15).

/The

The non-cellular Keimhautblastem becomes heaped up here also. On the other hand, the blastoderm at either end becomes much thinner, especially at the anterior tip on the ventral side (fig.1, 2.40-3.13; fig.4, 3.30; fig.9, 3.30). The changes in thickness are accompanied by changes in the orientation of the cells. The withdrawal from the anterior and posterior ends ventrally is connected with the heaping up which occurs medially when the ventral furrow is formed; the film shows a strong contraction of the whole ventral area towards the centre during this process (fig.3, 3.18-3.22). This is quickly followed by a general dorsal and lateral contraction, with its focus on the point at which the cephalic furrow is formed, i.e. one third the length of the embryo from the anterior end. The cells of the lateral and dorsal regions become orientated towards this point; as remarked above (page 56), it appears likely that contractions of the yolk are responsible for these changes in the blastoderm (fig.4, 3.30). These are the first movements of gastrulation, whose more active later phases have already been described.

The blastoderm in the S9 embryo:

Disturbances appear in blastoderm formation; either the distribution of the cleavage nuclei is irregular, so that they do not arrive at the periphery simultaneously

/(fig.

(fig.103), or they do arrive together, but divide at different rates, so that an irregular blastoderm is formed, resembling that found occassionally in X2 (fig.~~104~~ 45). This variability is reflected in the blastoderm at a later stage, when cell walls have appeared. In those cases where the nuclear distribution was disturbed, the blastoderm is extremely irregular, with some areas devoid of nuclei, and many nuclei remaining in the yolk (fig.104). In the cases where the syncytial blastoderm was formed before disturbances appeared, the chief abnormalities are the abnormal thinness of the blastoderm in some regions, and the irregularity of the Keimhautblastem (fig.105). In both cases the blastoderm is most disturbed at the anterior end. As described above, it becomes thinner in this region, just before gastrulation, in normal embryos; in this mutant, the defective blastoderm apparently cannot take the strain during this movement, and the yolk breaks through it. As a result, a certain amount of yolk is unenclosed by the embryo, and usually remains in the anterior region throughout, where it distorts the development of the head (figs.106, 107, 108 and 110). Sometimes yolk breaks through at the posterior end, which also becomes thinner in the normal embryo.

Subsequent development:

/In

In addition to this initial disturbance, subsequent differentiation is affected to varying degrees, the extent of the abnormality probably being correlated with the extent to which the blastoderm is abnormal. The range of variability, together with the shortage of mutant embryos, made it impossible to obtain material for a stage by stage analysis of the development, and the following general remarks are given instead.

The general form of the body depends upon the extent to which the various embryonic movements have been disturbed. In some cases gastrulation is prevented, and the resulting embryo consists of a disorganised collection of partly differentiated tissues, in which nervous tissue predominates (fig. 106). Usually gastrulation does occur, but it is very often the last embryonic movement to do so; many embryos show a high degree of differentiation of the body wall, with hypoderm, cuticle, segmental divisions and musculature, but shortening does not take place, and the embryo remains doubled back upon itself, remaining in the configuration it is in at the end of gastrulation. In such embryos there is often extremely little development of the gut, so that the yolk remains in a mass in the middle of the embryo.

/In

In other cases the gut develops, but quite abnormally; fig. 109 shows an embryo in which the body wall and the ventral nervous system have differentiated relatively normally, but in the gastrulation configuration, while the gut has grown back from the proctodaeal invagination as a flask-shaped sac, with part of its wall protruding into itself.

In other cases shortening occurs, but in only a few cases is there complete dorsal closure; consequently the gut, when formed, is often exposed (fig. 110). Involution of the head never goes further than the invagination of some of the pharyngeal material, but even this, with the partly developed pharyngeal muscles attached to its inner surface, sometimes remains facing externally (fig. 108). The frontal sac is never formed.

Even in the embryos in which these general movements do occur to some extent, resulting in the formation of an embryo which is basically normal in the arrangement of its parts, the gut is always poorly developed. In some cases the wall of the middle region is almost absent (fig. 107), and it is always shapeless, containing much undigested yolk and undifferentiated

/cellular

cellular material (figs. 107 and 108). The proventriculus, and mid-gut caecae and malpighian tubules, are not formed.

The body wall is usually incomplete owing to the failure of the dorsal closure, and the absence of hypoderm over parts of the nervous system. Signs of segmentation sometimes occur, but it is always extremely irregular. The disturbances of the musculature varies in extent; sometimes it is relatively normally developed (fig.); in other cases the spindle-shaped muscles are only partly formed (figs. 107 and 108); while in some the muscles take the form of unattached spherical bodies without contractile fibrils, similar to those in X10 type III (fig. 111).

The nervous system is least disturbed in those embryos which remain in the gastrulation configuration (fig. 109). In others it is distorted, and not covered by hypoderm in many places (figs. 107 and 111). No embryos have been found in which a tracheal system is developed, or with gonads.

Discussion

The abnormalities described above are all the result of a primary disturbance in blastoderm formation, in conjunction with subsequent defects in the general embryonic movements and cellular differentiation. It is not

possible to say what the relation is between the blastoderm disturbances and the later defects. Certainly the extent to which the blastoderm is deformed plays a part in determining the degree of abnormality in the later embryo. But there may also be a general effect on cellular differentiation, similar to that in X1O type III.

Very similar disturbances of the cleavage nuclei and the blastoderm have been reported in the female sterile mutant deep-orange when homozygous females are mated to hemizygous males (Counce, 1953). In progeny from such matings, 65% of the embryos succumb to abnormalities in very early cleavage, but those which survive this period show the same sort of abnormalities in blastoderm formation, and proceed with a relatively normal gastrulation. However, the resemblance ends here, for there-after cellular degeneration sets in, and there is no further differentiation or development of any kind.

The instability of expression of the factor cannot be explained on the present evidence. It is possible that the situation is similar to that postulated for the X1O factor, in which it was suggested that the various types of abnormal embryo resulted from variations in its

/expression

expression, with some embryos escaping the first lethal phase, and going on to the next, or the next again, as "break-throughs". On a similar hypothesis in S9, some embryos would succumb in the embryonic phase of lethality, others would not die until the pupal phase, and possibly some would die as larvae. The genetic background determining the phase in which lethality occurs may have changed considerably since the stock was first picked out, thus accounting for the apparent reduction in the embryonic lethality.

On the other hand, it is possible that the pupal lethality and the embryonic lethality are not caused by the same factor. The resemblance between the early embryonic disturbances and those occurring in the deep-orange mutant suggests that the embryonic mortality may be caused by a female sterile gene, acting when homozygous in the female parent. But in this case the single-pair matings would have given an all or nothing embryonic lethality; since this was not the case lethality must have arisen from a factor acting when hemizygous in male embryos, as in the other mutants that have been discussed. The single-pair matings also established that the embryonic lethality and the pupal lethality are not caused by different X-chromosome factors, so that if they do arise

/from

from separate factors one of them must be autosomal.

Conclusions

S9 is a recessive lethal factor, probably located on the first chromosome close to the centromere. Its expression varies; pupal as well as embryonic lethality occurs, and probably larval also. The abnormal embryos all follow the same general pattern of development, but there is a considerable range of variation. Disturbances appear early, during the distribution of the cleavage nuclei or during blastoderm formation. Their most important consequence is the malformation of the blastoderm, and in particular its fragility at the anterior end. Here much of the yolk escapes and interferes with the subsequent development of the head region. Later development is disturbed both in its morphogenetic movements and in its cellular differentiation.

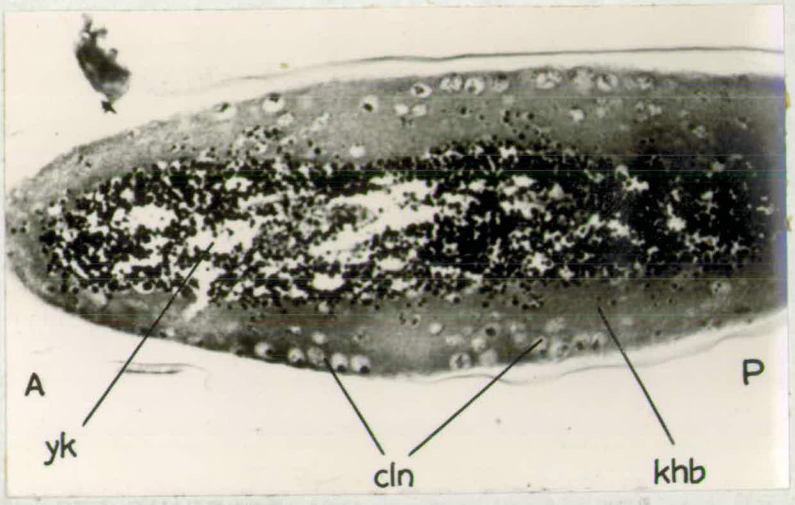
Fig.103. L.S. S9, 2-3 hours, x340.

Fig.104. L.S. S9, 3-4 hours, x150.

Fig.105. L.S. S9, anterior region, 3-4 hours, x340.

Key to figs. 103-111.

A, anterior.
bl, blastoderm.
br, brain.
cln, cleavage nuclei.
fgo, fore-gut opening.
gtc, gut cells.
hg, hind-gut.
hy, hypoderm.
icyt, irregular cytoplasm.
khb, Keimhautblastem.
mg, mid-gut.
msn, mesodermal mesenchyme.
mus, muscles.
musc, muscle cells.
nt, nervous tissue.
oes, oesophagus.
P, posterior.
phm, pharyngeal material.
pmus, pharyngeal muscles.
pmusc, pharyngeal muscle cells.
pv, proventriculus.
uyk, unenclosed yolk.
vns, ventral nervous system.
yk, yolk.
ykn, nuclei remaining in yolk.


A
P
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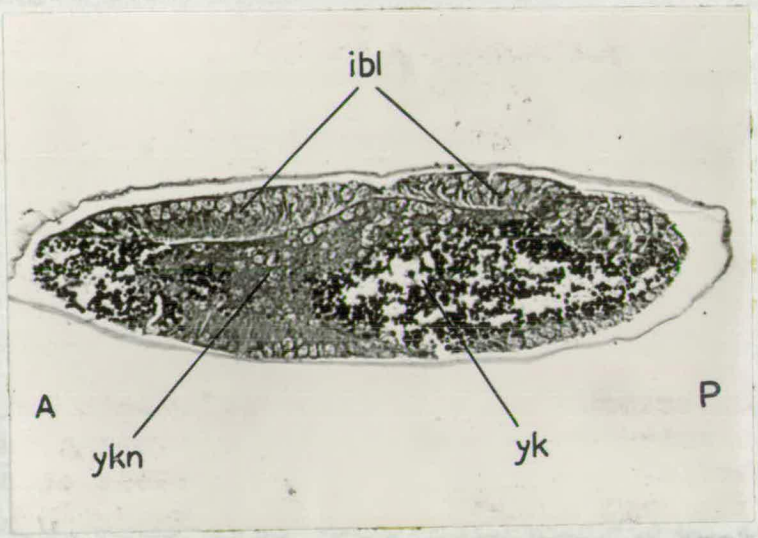
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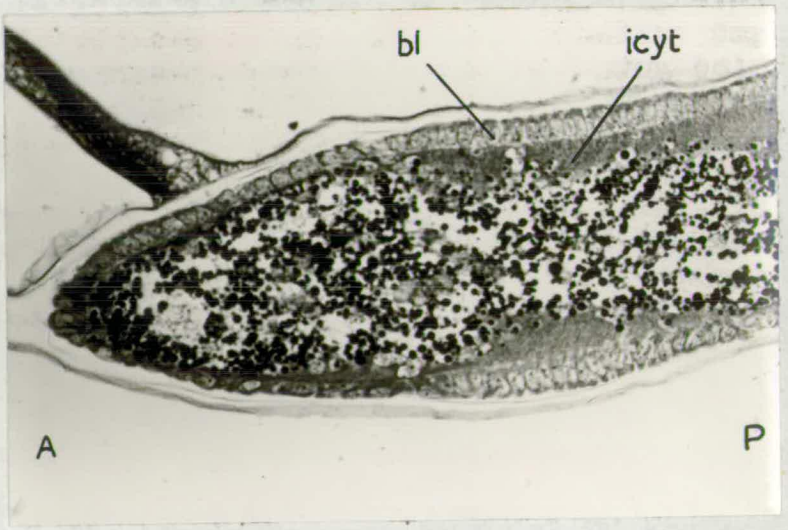
bl
icyt
A
P

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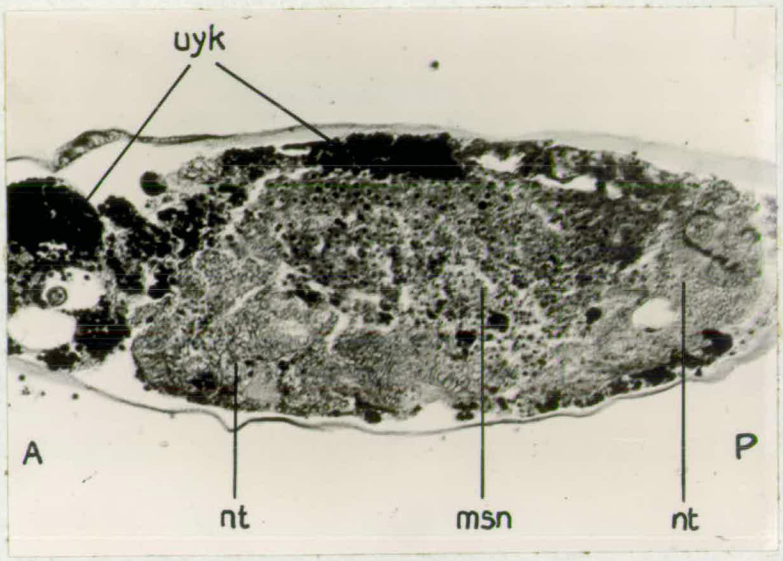
Fig.106. L.S. S9, 8-9 hours, x300.

Fig.107. L.S. S9, anterior region, 13-14 hours, x380.

Fig.108. L.S. S9, anterior region, 18-19 hours, x380.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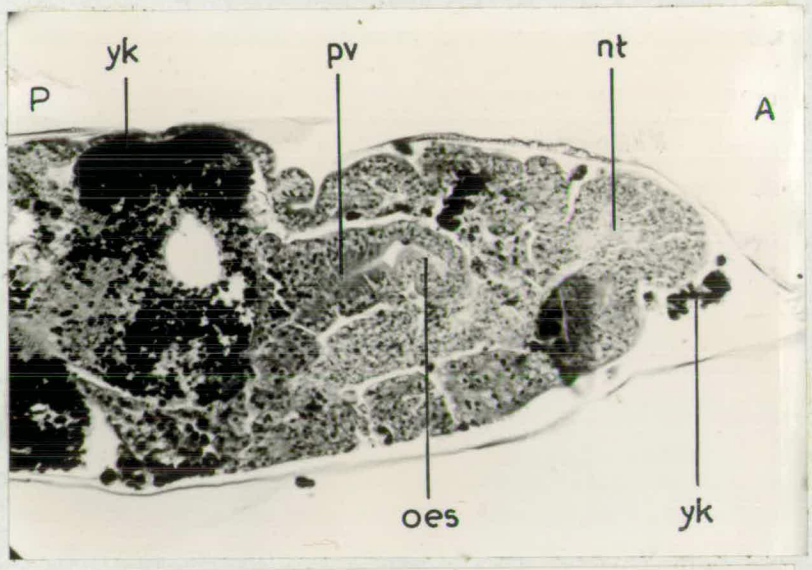
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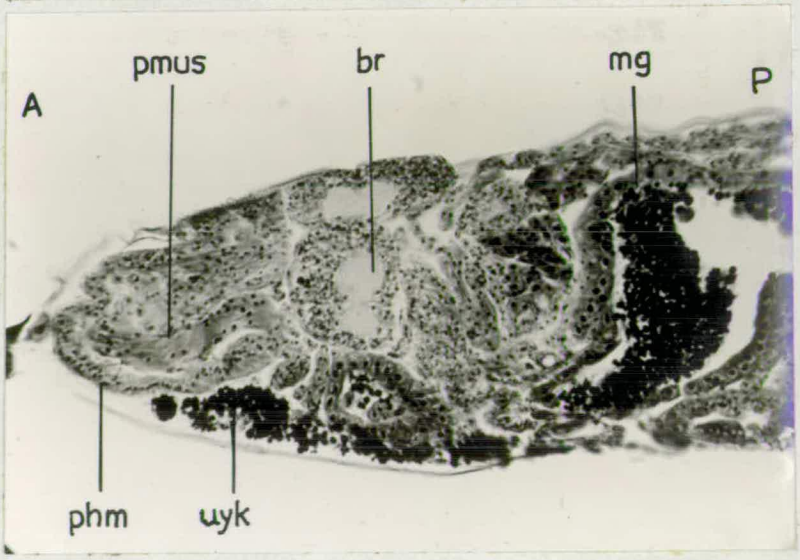
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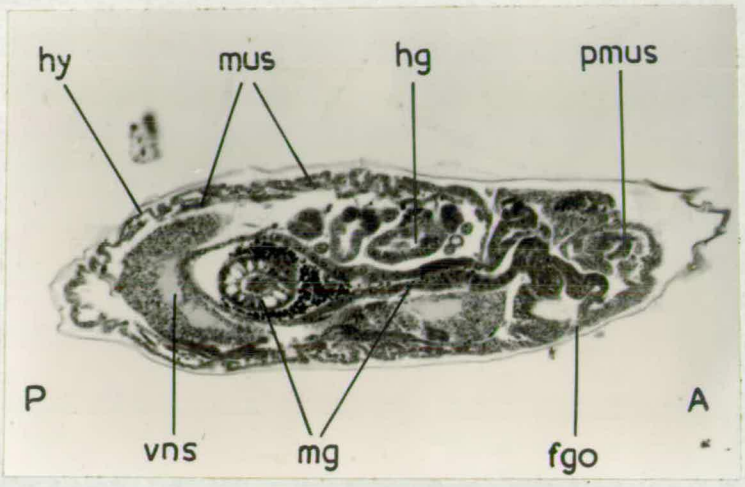
Fig.109. L.S. S9, 18-19 hours, x200.

Fig.110. L.S. S9, 25-29 hours, x200.

Fig.111. L.S. S9, anterior region, 25-29 hours, x460.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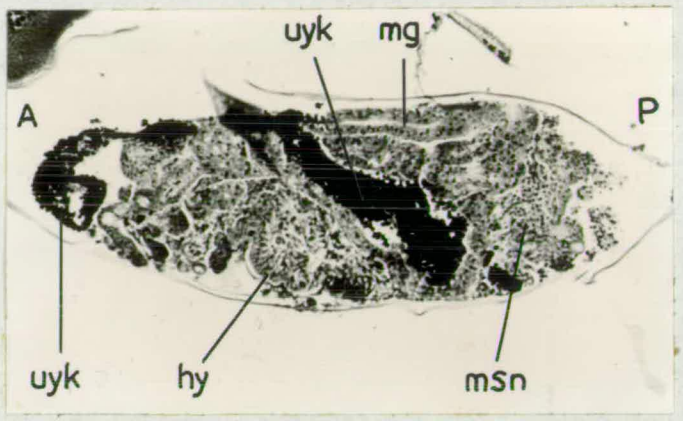
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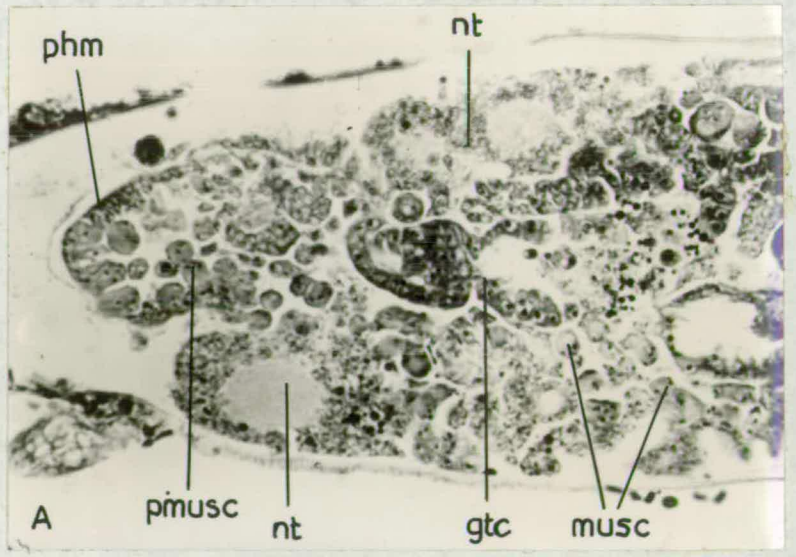
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General Discussion and Conclusions

The action of lethal factors:

Patterns of development in lethal embryos fall into three broad categories:

a) Early developmental disturbances, followed by little or no cellular differentiation or organisation: X20, types III and IV; X10, types I and II; nullo- and half-X (Poulson, 1940); the female sterile deep-orange (Counce, 1953).

b) Complete embryonic development, with the formation of a larva which does not hatch: six were found in the present study; other cases have been listed in the introduction.

c) Considerable cellular differentiation and organisation, but the development is disturbed in a variety of ways: Lff 11; X2; X27; X20, types I and II; X10, type II; S9; Notch and white deficiencies (Poulson, 1940 and 1945); Krüppel (Gloor, 1950); Minute-4 deficiency (Farnsworth, 1951); the female steriles fused and rudimentary (Counce, 1953).

/Demerec

Demerec has shown that, in the epidermis of the adult, many homozygous deficiencies have a direct lethal effect even on single cells containing them (Demerec, 1934 and 1936), and it is possible that such a direct gene-cell effect occurs in some embryonic lethals. It certainly does in a number of larval and pupal lethals, where it is possible to test the capacity of tissues to survive when transplanted to normal larvae (reviewed Hadorn, 1951). Unfortunately it is technically impossible to do such experiments with embryos, and the best that can be done is to observe whether or not cellular degeneration occurs in the course of development. It does in the case of all those in category "a", and is reported to occur in some tissues in fused and rudimentary. Probably it occurs to some extent in all those in which there is apparently a general retardation or disturbance of growth processes, such as has been reported in the last two mutants, and in Krüppel and Minute-4 deficiency. However, in others, while there are gross disturbances in their organisation, the cells and tissues appear to be quite viable; e.g. Lff 11 and X2. In other cases, such as Notch and white deficiencies, and X20, types I and II, cellular differentiation is abnormal in that certain types of cells appear at the expense of others, but in themselves they are viable. In many cases then, death occurs not because

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of a direct effect of the factors on the cells containing them, but because of developmental disturbances whose lethal effect is on the organism as a whole.

The localization of gene effects:

Where there are specific structural effects one of the aims of developmental studies is to distinguish those which are close to the original gene action from those which are remote. Grüneberg states the methodological approach as follows: "In developmental genetics, a generalized effect is more likely to be fundamental than a local one, and an early effect is likely to be closer to the primary gene action than a late one" (Grüneberg, 1953). An example from these embryonic lethals is the frequent failure of the involution of the head; since this is a local and a late effect, which may be caused by a great variety of antecedent disturbances in the embryonic organisation, it cannot be considered as a fundamental one.

The question arises as to how, if the primary effect is a general one, it can bring about specific localized structural effects. In principle the solution is clearly that the effects arise as a result, not simply of any effect of the factor under consideration, but of its interaction with the developmental pattern as a whole.

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As Sewall Wright has put it: "The apparent extreme localization of the effects of particular genes in certain cases turns out to be a matter of thresholds in the organism - not of preformed rudiments in the germ plasm. Genes and environment are alike modifiers of a pattern residing in the whole." (Sewall Wright, 1945). And the pattern in the whole is determined by the whole complement of genes, in conjunction with the pattern imposed by the cytoplasmic organisation of the egg, which is under genetic control in the previous generation.

The manner in which this interaction of mutant factors with the normal developmental pattern has been described, as far as possible, in the six lethals described in this work. It is clearest in those in which there is a disturbance of early morphogenetic movements, which bring about a number of subsidiary effects, e.g. Lff 11, X2, X27. The subsidiary effects are chiefly what would be expected from the early displacement of materials in mosaic eggs - the differentiation of tissues without relation to each other, resulting in the formation of teratological embryos which are incapable of further development.

The displacement of tissues relative to one another

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would lead to interesting results in organisms where organiser phenomena were important, and would provide evidence for such phenomena if they played a part in the development of Drosophila embryos. In an analogous way Raven has shown that although the main lines of development in Limnaea are determined by the mosaic development of differentiated regions of the egg cytoplasm, contact inductions do play a part in organogenesis (Raven, 1950). However, very little evidence of such inductive mechanisms has appeared. Nothing in the present work supports Poulson's suggestion that the nervous system has an inductive action upon the hypoderm (Poulson, 1940), but there are, on the other hand, strong indications that the degree to which the muscular sytem is developed is determined by the prior differentiation of the hypoderm (see the discussions of X20 and X27).

Phase specificity:

The localization of the expression of gene effects to particular structures implies a corresponding restriction with respect to the period of development at which these effects become apparent. This is related to the "phase specificity" which Hadorn has emphasized as a characteristic of lethal mutants, which refers to the time

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at which death occurs (Hadorn, 1948).

Many visible mutants show a general weakness and a lowered viability in ontogeny, and a similar sensitivity has been found in some larval and pupal lethals, which have a higher embryonic and larval mortality than normal controls (Hadorn, 1951). But all work on lethal mutants confirms that in general death occurs at a specific period for a particular mutant. There may be, as in X10, X20 and S9 in the present studies, several lethal periods in each, owing to "break-throughs" from one to the other, but they are still distinct; nowhere is there a general distribution of deaths through the whole ontogeny.

Hadorn points out that when large numbers of lethals are considered, the periods at which death occurs are clearly clustered at particular developmental stages (Hadorn, 1948). In the embryo these are the "first sensitive phase", which includes fertilization, cleavage, blastoderm formation and primary organogenesis, and the "second sensitive phase", which is the time of normal hatching, in which the important factors are the forces required for the hatching movement. Between them is an "insensitive interphase" in which death is rarely found to occur.

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This is confirmed by the present study; those mutants included in category "a" do not develop beyond the first sensitive phase, while those in categories "b" and "c" die at the second sensitive stage. But the classification is a very rough one; the first phase includes many different stages of development, and the second does not distinguish those embryos which produce larvae which are apparently normal except in not being able to hatch, and those in which there is a complete disorganisation brought about by a very early developmental disturbance. Where large-scale surveys of lethals are concerned it is scarcely possible to do more than classify embryos in this way; but detailed developmental studies such as the present one give much more information about the time of action of the factors, as opposed to the times at which death results.

Though Hadorn points out that these are not synonymous, his own scheme emphasizes the one which is least important for an understanding of developmental mechanisms. In particular, the clustering of so many lethals as resulting in death at the second sensitive phase shows little more than that the mechanical difficulties of hatching are decisive factors in preventing the further development of abnormal embryos; it reveals

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unfitness as organisms, but nothing about the reaction of the developmental pattern to genetic factors.

Epigenetic crises in development:

A more useful scheme for the present purpose is that developed by Waddington in connection with developmental studies on various genes affecting adult structures, such as the eyes and wings. In the following quotation the terms "epigenetic crisis" and "quiescent interphase" recall Hadorn's "sensitive phase" and "insensitive interphase", but they have a clearer relation to characteristics of the developmental mechanism: "..we can perhaps take it as a fairly general pattern...that there is an alternation of phases of epigenetic crisis, at which the predominant factor operating is often an evocator stimulus, with more quiescent periods during which a comparatively large number of minor epigenetic processes are governing a whole host of small adjustments, on whose overall effect the character of the next crisis will depend." (Waddington, 1948b).

One example given is the effect, in combination, of certain mutant genes upon the growth of the imaginal buds during the larval period. This results in a gross abnormality of the folding of the imaginal tissue into the buds at the time of pupation, and there are in consequence

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a number of disturbances in evocation of adult organs, though the genes themselves probably affected only the growth and thus the mechanical folding in the buds.

This is precisely comparable to some of the defects which in the present study have been found to occur in gastrulation, especially in Lff 11, X2 and X27, though the ensuing evocation phenomena are less clear, if present at all. In each of these cases the disturbances appearing in gastrulation are spectacular and clear-cut, but they have been preceded by a sequence of much more obscure minor abnormalities, which have only become drastic in their effects at the focal point represented by this epigenetic crisis.

Equilibrium in development:

Considering the complexity of movements involved in gastrulation, and the minuteness with which they must be integrated in the normal process, it is not surprising that it should be disturbed by many different mutant factors, which may act by altering slightly the speed of one component process, thus disrupting the co-ordination in time. Comparable disturbances have been found to occur in hybrid trout embryos (Rubaschev, 1937), and it is probable that processes involving the integration of

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complex cell movements are likely to be epigenetic crises.

This example illustrates the balance which normally exists between the factors involved in development, and their equilibrium until it is disturbed by the effects of a mutant factor. Waddington points out that this balance in developmental processes results in an important characteristic of differentiation - its "canalization", so that one or other type of tissue may be formed, but very rarely an intermediate type: "..there is a strong tendency for the end-result to be a typical representative of a repertoire of definite and distinct organs or tissues." (Waddington, 1948a).

The example given is the range of potentialities which exist in the early vertebrate ectoderm, which, having been started on one path or another according to the evocator stimulus it receives, continues to differentiate inexorably into one particular type of tissue. And it is pointed out in another connection that "genes act in a way formally like that of evocators, in that they control the choice of alternative" (Waddington, 1940b).

This principle is well illustrated by the present studies, especially with respect to the differentiation

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of the ectoderm. In this the delicate balance which exists between the tendency to give rise to hypoderm, and the tendency to give rise to nervous tissue, has frequently been referred to. The case of X20 is particularly striking, where in type I the ventral nervous sytem develops normally, but with no hypoderm over it, while in type II there is a complete hypoderm, but no nervous tissue is developed. Here the normal action of the gene controlling the choice of alternatives has been disturbed, and differentiation of the ventral hypoderm is canalized into one or other of these contrary directions. The self-reinforcing nature of the differentiation process is well shown in X10, type II, where frequently the entire mass of differentiating cells gives rise to nervous tissue, somewhat analogous to evocation without individuation as it sometimes occurs in amphibians.

Variation and buffering:

Biometrical studies have shown that small variations in development occur even when the environmental and genetic variables are reduced to a minimum; such variation has been provisionally ascribed, in these studies, to "'intangible' accidents of development" (Robertson and Reeve, 1952). But such accidents are usually controlled by the equilibrium and balance mentioned above, as Waddington has pointed out in his explanation of the way in which normal development is "buffered" (Waddington,

1942#). In mutants however the buffering mechanism is upset, and the variation found even in visible mutants is much more extensive than in normals; slight initial deviations will tend to lead to greater divergence, instead of being adjusted. In the mutants described here the variation between individual embryos is very large, and in fact is one of the chief difficulties in such work. It is most marked where the disturbances are of general differentiation processes, as in X10, type III. Where there is a specific structural effect, as in Lff 11 and X2, the variation is less. S9 is interesting in this connection, for there is an initial structural defect of the blastoderm, which is subject to relatively little variation, and subsequent general effects on cellular differentiation, in which there is much more.

Slight initial deviations will therefore often operate as "switch" mechanisms, being followed by canalization of the development into a particular path. Some such mechanism must account for the large proportion of the mutants which show a number of distinct types of expression in embryonic development (X20 and X10), or in which "break-throughs" occur to another stage of development (S9).

/Evolution

Evolution:

The relevance of these studies to two related larger problems may be briefly noted. These are those of the comparative embryology of insects, and of the evolution of higher categories than species.

In the first place, the comparative embryology of insects lacks any unifying principle relating the very different types of development in the various orders (Johannsen and Butt, 1941, passim). In the second, a number of biologists suggest that the current neo-Darwinian explanations of the mechanism of evolution fail with respect to the emergence of supra-specific categories (Dalcq, 1949; Goldschmidt, 1940 and 1952). Goldschmidt in particular has suggested that their evolution has come about, not by the accumulation of small mutations, but by large macro-mutations. These would cause early disturbances, but the organism would be viable, giving, as he puts it, a "hopeful monster", which might survive as a progenitor of a group of organisms of a new type of organisation.

The present studies show that early disturbances arising from mutations can cause drastic modifications of developmental pattern, while the organism as a whole

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may survive until its inadequacy as a functional organism leads to its death at abou the time of hatching. Moreover, those which are most striking in this respect - the lethals which cause disturbances in gastrulation - bring about modifications in the relative length of the germ band, extent of the embryonic membranes, alterations in movements, etc., of the type which is involved in the differences between the embryonic organisation of one group of insects and another. And it has been stressed that these changes are probably brought about by alterations in the rates of normal processes, which is well known to be a common mechanism in evolutionary change (de Beer, 1940). In Drosophila there is no regulation in embryonic development following these disturbances, and the resulting monsters would be hopeful indeed if they expected to contribute to evolutionary advance. But in ancestral insects, as in many existing orders, a greater capacity for regulation might have made promising material for natural selection out of such mutants.

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ABSTRACT OF THESIS

Name of Candidate Donald Albert EDE

Degree Ph.D. *Date* May 14th, 1954.

Title of Thesis Some problems on physiological genetics in Drosophila: effects of genetic lethal factors on embryonic development.

The studies of embryology and genetics grew up separately, but in recent years great interest has arisen in the study of physiological genetics, which aims at discovering the relations between them, and in particular the way in which genes act in controlling development. One difficulty in its study is that genetic mutants which affect fundamental developmental processes usually cause the death of the organism, and are therefore impossible to keep in most animals. However, in Drosophila techniques exist for maintaining stocks which carry genetic lethal factors; more-over, large numbers of them are produced in the course of research into the induction of mutations, so that there is an abundant supply of material. It is known that many of these factors produce their lethal effect by disturbing embryonic development, but few detailed studies have been done on the exact way in which this occurs. An obstacle in the way of such studies is that whereas there is an abundance of genetical knowledge relating to Drosophila, comparatively little is known about its normal developmental mechanisms. Therefore the present study aimed at contributing to knowledge of normal embryonic development, both by studying normal embryos and by noting the reaction of the developmental processes to disturbances caused by lethal factors, in addition to investigating the mechanism of gene action in development.

Twelve factors were selected which caused death in the embryonic stage. Six of them had no apparent structural effects, but produced fully-developed embryos which were unable to hatch. The other six caused disturbances in development which led to the production of extremely abnormal embryos. These were studied in detail by means of

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serial sections, prepared at all stages, and in some cases by time-lapse ciné-studies. In generalthe complicated final array of abnormalities characterizing each mutant could be traced back to the effects of simpler earlier structural effects, which were considered to be the primary effects of the genetic factors. The primary effects occurred at specific times in development, and in three cases involved the gastrulation process, which they were believed to affect by disturbing the time relations of its component events. One mutant caused an earlier disturbance, in affecting the distribution of the cleavage nuclei. In some cases there also occurred apparently unrelated disturbances in cellular differentiation, and in one case these occurred in the absence of any primary structural abnormality. Effects upon the differentiation of the ectoderm were particularly striking, and showed that a delicate balance exists between the tendency to become hypoderm, and the tendency to become nervous tissue, which is under genetic control. Little evidence for the existence of induction mechanisms was found, but the degree to which the hypoderm is developed does appear to affect the development of the somatic mesoderm. There is much individual variation in the expression of the factors, and in some mutants several distinct types of embryo were found. These findings are discussed in relation to general embryological concepts, in particular to those of equilibrium and canalization.