CYTOCHEMICAL STUDIES ON
DROSOPHILA DEVELOPMENT

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BY

T. YAO

B. Sc., NATIONAL UNIVERSITY OF CHEKIANG, CHINA.

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I. INTRODUCTION

The importance of a combined genetical and embryological approach in the causal analysis of development has been stressed by Waddington (1940b). *Drosophila*, in view of our detailed knowledge of its genetics, should be the best material for such analysis. With these points in mind, the present work has been undertaken with the hope of acquiring more information about the development of the fly.

Experimental embryology, like other branches of the experimental biological sciences, seems to have reached a stage at which further advancement is limited by the lack of chemical knowledge at the cellular level. It is for this reason that the current development of cytochemistry and the application of cytochemical methods to embryological studies have received wide attention. Danielli (1947) has put it clearly that, in the realm of cytochemistry, the distribution of various chemical species should first be known before further approach at the physicochemical level can be made possible. The present study is, therefore, mainly concerned with the localization of certain particular chemical substances during the development of *Drosophila*. These substances include/-

include chiefly the protein sulphhydryl groups (or fixed -SH groups), ribonucleic acid and phosphomonoesterases. The reason for which the first two substances have been chosen for study is, among others, their significance in connection with the determination of the embryo axis in vertebrates (Brachet, 1947). The distribution of phosphatases is studied because of their suggested relation to the chemistry of differentiation (Moog, 1944).

On the other hand, the Drosophila egg, as that of a holometabolous insect, is necessarily endowed with a double organization, involving both the larval and imaginal developmental patterns. This has actually been borne out by Geigy's (1931b), irradiation experiments. Since the processes through which these two patterns are realised are fundamentally the same, cytochemical observations have been extended to post-embryonic stages of Drosophila, in an attempt to find some general similarities between embryonic development and metamorphosis.

II. MATERIAL AND METHODS

Wild-type Oregon S stock of Drosophila melanogaster was used and cultured by the usual technique at 25$\pm$0.2°C. Oocytes in various developmental phases were obtained from one day old females which contained practically all successive stages. For the collection of fertilized eggs, virgin females were kept separate from the males for two days. They were then mated and the males removed after one hour. Egg collection was made from such females on a yeasted simple medium containing 2% ethyl alcohol and 1% acetic acid in 2% agar. After discarding the first two or three lots (which may contain some eggs with advanced embryos), eggs were collected at ten minute intervals, timed and incubated. The embryos for study were first dechorionated free-hand as described by Poulson (1937) and then carefully punctured in the fixative with a very fine tungsten needle.

In order to collect larvae of known age, 30-50 rapidly laying females were allowed to lay eggs in a fresh food bottle for one hour. Larval age was computed from the time of egg laying, the total duration of embryonic life being 18-19 hours at/-

at 25±0.2°C. The limit of age difference within such a group of larvae is approximately ± 1 hour. To obtain the requisite stages of prepupae and pupae, mature larvae prior to puparium formation were transfered to agar plates in Petri dishes. Following Robertson (1936), the moment when the anterior spiracles ceased to move was carefully recorded for a group of individuals and taken as zero hour. Due to the impermeability of cuticle, pricking with tungsten needles in the fixative is also necessary for the larvae, prepupae and pupae.

Formal-alcohol-acetic acid mixture (Huettner, 1923) was used as fixative for the following cytochemical tests: fixed -SH groups, ribo- and desoxyribo-nucleic acids. 80% alcohol was used for alkaline phosphatase, and chilled acetone for acid phosphatase. The materials were embedded in the usual way and sectioned at 10 µ.

For the detection of fixed -SH groups the method devised by Chèvremont and Frédéric (1943) was employed. Control sections were treated with saturated mercuric chloride for one hour to block the -SH groups. Ribonucleic acid distribution was studied by the method of Brachet (1942): crude ribonuclease was isolated from calf pancreas: sections were incubated in enzyme solution (in distilled/-

distilled water) for 1-2 hours at 37.5°C.: controls for the same period in pure distilled water. Desoxyribonucleic acid was demonstrated by the standard Feulgen technique (Stowell, 1945).

For the demonstration of alkaline phosphatase, Danielli's (1946) schedule was generally adhered to. Certain minor modifications in timing were, however, found to be necessary for the present material, because of its relatively low enzyme content. The duration of the steps between fixation and incubation was therefore deliberately shortened (especially that in the paraffin bath (m.p. 52°C., 1-1½ hr. at 56°C.)) so that the enzyme loss can be reduced to a minimum. Sodium glycerophosphate (B.D.H.), consisting of almost pure beta-salt, was used as substrate. Magnesium chloride was added to the incubation mixture at a final concentration of 0.01 M. The pH of the mixture was 9.40. The duration of incubation was 4 hours at 37.5°C. For post-embryonic stages, this was extended to 12 hours in order to get the maximum possible reaction in tissues of low phosphatase content. In several cases, negative results were checked by a 24- or

even/-

even 48-hour incubation.

In post-embryonic development, alkaline phosphatase activity shows a considerable difference in degree between different organs and between different morphological stages. In order to express such differences as truly as possible, enzyme activity has been classified into three grades: these are by no means satisfactory but they are sufficient to describe some important changes of enzyme activity. A "strong" reaction refers to a tissue or a part of a tissue which appears so black that its internal structure is often difficult to identify. A "moderate" reaction describes material showing dark brown precipitates in the nucleus and diffuse, brownish to grayish colours in the cytoplasm. Histological details can easily be made out in these. A tissue is referred to as "weakly" reactive when the nuclei only contained brownish precipitates.

Gomori's technique for the demonstration of acid phosphatase has recently been questioned on certain grounds (Bartelmez and Bensley, 1947). The problem of non-specific absorption was therefore studied, using Drosophila ovaries, by superimposing active sections on inactivated sections and incubating/-

incubating them together for various lengths of time (8, 12, 24, 48 and 72 hours). It was found that after short incubation (8, 12 hours) the inactivated sections were practically unstained. Non-specific, diffuse yellowish staining in these sections became evident in 24-hour series and more so after 48 and 72 hour's incubation. From this result, it appears that incubation requires very critical timing. Moreover, I was unable to confirm the instability of lead phosphate as claimed by Bartelmez and Bensley. Further, the distribution of acid phosphatase in the Drosophila ovary has been confirmed by incubating fresh unfixed material: this will be described later. Granting that acid phosphatase technique is not quite so satisfactory as the method for alkaline phosphatase, information about its general activity can, nevertheless, be obtained, if the conditions of experiment are carefully controlled.

In this work, Moog's (1944) modified Gomori's technique for betaglycerophosphate was used. The pH of the incubation mixture was 4.70. The duration of incubation was 16 hours at 37.5°C. Control sections were incubated either without substrate or in the presence of 0.01 M. fluoride.

In order to get some information about the mature sperm, most cytochemical tests were repeated on

Drosophila testes from one day old males. These results will be briefly dealt with under the section on oocyte development.

III. RESULTS

(1) Oocyte development

A. Morphological observations on oocyte development:

Since the development of the Drosophila oocyte has not been adequately described, it must first be summarized here. The whole ovarian history can be divided arbitrarily into eight stages, based on the size of the oocyte and other morphological characters of the nurse cells and follicular epithelial cells. These stages are shown in the figure 1 and a brief description of them is given below: (the median cross sectional area (A) of the oocyte was measured by a planimeter under a linear magnification of 160; the diameter (d) of the largest nurse cell nucleus was measured by an ocular micrometer)

1. $A=25\ mm^2$. $d=15\mu$. The oocyte can be distinguished from the nurse cells by its smaller-sized nucleus and weaker cytoplasmic basophily.
2. $A=160\ mm^2$. $d=20\mu$. The whole follicle elongates and the follicular cells become flattened out at the nurse cell end. Nurse cell cytoplasm develops strong basophily.

3. /-

3. A=310 mm^2. d=27μ. Deposition of yolk granules starts at the peripheral region of the oocyte. Follicular cells concentrate around the egg: a very thin envelope consisting of a few squamous cells is all that encircles the whole set of nurse cells. Nurse cells and follicular cells show strong cytoplasmic basophily. Intracellular vacuoles appear in the nurse cells.

4. A=560 mm^2. d=40μ. Secretory activities of the nurse cells and follicular cells are in full swing. The nurse cell cytoplasm is highly vacuolated and its contents begin to pour into the egg. Not infrequently, whole nurse cell nuclei can be found inside ooplasm.

5. A=1080 mm^2. d=40-42μ. The general features of this stage are similar to those of the preceding stage. The physiological activity of the nurse cells has probably passed its peak as evidenced by the decline of cytoplasmic basophily. The follicular cells show no signs of degeneration.

6. A=2030 mm^2. The nurse cells show a steady decrease of cytoplasmic volume and basophily. Their nuclei become pycnotic, those adjoining the oocyte often being the first affected. A great number of vacuoles can be seen in the oocyte/-

oocyte cytoplasm. They are derived from the nurse cells and are observable under living conditions.

7. A=2600 mm^2. The cytoplasmic contehts of the fifteen nurse cells are completely absorbed The follicular cells become flattened, but their cytoplasm is still moderately basophilic. The whole egg cortex is covered by a thin, homogenous, yellowish and refractile structure ——— the vitelline membrane.

8. This stage corresponds to a mature oocyte and is not shown in the figure. Follicular cells degenerate and the chorion with filaments is well formed. No pycnotic nurse cell nuclei remain visible. The volume of the oocyte increases slightly and a further synthesis of yolk granules occurs.

B. Fixed -SH groups:

Fixed -SH groups can be demonstrated in both nucleus and cytoplasm of the oocyte, nurse cells and follicular cells. In the nucleus, they are mainly concentrated in the chromatin substances and in the nucleolus. The nuclear sap is negative. In the cytoplasm, certain discrete granules always

stain/-

stain more intensely.

In the oocyte the intensity of the -SH reaction increases after stage 3, especially in the cortical ooplasm which stains deep blue as compared with the greenish-blue of the central ooplasm. As maturation proceeds, the distribution of fixed -SH groups becomes more even, but a uniform and higher concentration is still evident in the egg cortex.

In the nurse cells fixed -SH groups are very abundant in the cytoplasm in stages 1-5. Particularly reactive are those areas near the nuclear and vacuolar membranes.

Like the nurse cells, the follicular cells are very rich in fixed -SH groups. In certain stages (3 and 4) an intracellular gradient of -SH reaction can be seen, with deep blue granules accumulated mostly in the proximal half of the cell facing the egg surface. The follicular cells still give a strong -SH reaction in stage 7.

Control sections gave no reaction in most cases. Occasionally, the structures containing a high concentration of fixed -SH groups such as the egg cortex or chromatin materials in nuclei remain faintly positive after mercuric chloride treatment.

The adult testes are equally rich in fixed -SH groups. Sperm heads are extremely reactive.

C. /-

C. Ribonucleic acid

In stage 1, nurse cells contain more ribonucleic acid than either the oocyte or follicular cells. In the next three stages (2-4), an enormous increase of cytoplasmic ribonucleic acid occurs in the nurse cells. They now stain deep red with pyronin. The acid content starts to decrease in stage 6.

The follicular epithelial cells give as intense a staining as do the nurse cells in stages 2-4. They retain most of their ribonucleic acid in stages 6 and 7, a fact which is possibly related to the formation of the chorion and filaments of the egg.

In general the oocyte cytoplasm takes less pyronin than the nurse cells or follicular cells in most developmental stages. Comparing oocytes of different stages, the ooplasm of younger oocytes (before stage 2) often contains relatively more ribonucleic acid than that of older oocytes (stages 3 and 4). Nevertheless, in these latter stages, cytoplasmic materials at the end of the oocyte adjacent to the nurse cells take pyronin as strongly as do the nurse cell's cytoplasm. This indicates the beginning of the nurse cell absorption. In stages 5-7, the ribonucleic acid content of the egg shows a definite increase as a result of the direct incorporation/-

incorporation of all nurse cells. The acid content of mature oocytes decreases very sharply again. This is probably connected with further synthesis of yolk materials before maturation (Pasteels (1948) recently found that in Ascaris ovogenesis the decrease of cytoplasmic basophily is correlated with yolk formation). Brachet (1942) has mentioned that ribonucleic acid decreases during the oogenesis of Drosophila. In the light of present evidence, his statement is true only when the comparison is made between certain definite developmental stages.

Ribonucleic acid is not demonstrable in the oocyte nucleus at any developmental stage.

Treatment with ribonuclease completely abolishes the cytoplasmic affinity towards pyronin. Pycnotic nuclei of the nurse cells (in stages 6 and 7) show various shades of colour (green, purple, red etc.) both in the control and enzyme treated sections. This probably indicates that depolymerization of desoxyribonucleic acid occurs during the nuclear degeneration, since pyronin will stain desoxyribonucleic acid only in the depolymerized form (Kurnick, 1947).

The testes from freshly emerged or adult males contain a negligible amount of ribonucleic acid in comparison/-

comparison with the ovaries. Mature sperm take up practically no pyronin.

D. Desoxyribonucleic acid

Using the Feulgen reaction, Painter and Reindorp (1939) have made a detailed study of nuclear phenomenan in the nurse cells of Drosophila ovaries. Analysis of the very early follicles of our preparations has confirmed their findings of the general phenomenon of endomitotic growth in the nurse cells. A few points which are related to the development of the oocyte as a whole will be mentioned here. In the first place, there seems to be some relation between the physiological activity of the nurse cells and their degree of polyteny. Morphological evidence and ribonucleic acid content both indicate that the nurse cells are physiologically most active in stage 4. The nuclear diameter of the largest nurse cell at this stage has already reached 40μ which is at least 512-ploid according to Painter and Reindorp. Secondly, the reactivity of the nurse cell nucleus as a whole towards the Schiff's reagent and the number of the Feulgen-positive granules around the nuclear membrane/-

membrane decrease sharply in the stage 5 follicles. This is then followed by pycnosis and reassumption of Feulgen positivity. Lastly it may be mentioned that there is no demonstrable change in the Feulgen reaction of nuclei of either the oocytes or follicular cells throughout the ovarian history.

E. Alkaline phosphatase

Alkaline phosphatase is not demonstrable in <u>Drosophila</u> ovaries taken from one day old females. Even when the incubation was extended to 24 or 48 hours, only a very feeble reaction could be noticed in the nucleolar materials of the nurse cells and follicular cells. Both experimental and control sections appear grayish due possibly to the presence of preformed phosphates. The epithelium of the oviducts gives, however, a moderate alkaline phosphatase reaction (fig. 2).

Testes from one day old males also show no histochemical trace of the enzyme.

F. Acid phosphatase

Acid phosphatase has a wide distribution in the <u>Drosophila</u> ovary. It is present in both the nuclei and cytoplasm of the oocytes, nurse cells and follicular cells (fig. 3). The nuclear reaction is generally stronger than the cytoplasmic one, which, especially in the nurse cells, varies between different developmental stages.

In the case of follicular cells there seems to be very little change of acid phosphatase activity as the oocyte grows. Their nuclei stain as heavily as those of the nurse cells and oocytes, but their cytoplasmic activity is slightly weaker.

In the very young follicle, nurse cells are undergoing endomitosis and the resulting mutiple chromatids show a strong tendency to remain together in definite groups (Painter and Reindorp, 1939). These polytene chromosomes give a very intense acid phosphatase reaction. Cytoplasmic activity of the nurse cells in these young follicles is comparatively low. It increases substantially in the older follicles (stages 4 and 5), especially around the the intracellular vacuoles and the cell border. The activity falls off at stage 6, but the pycnotic nuclei remain very reactive.

As is evident from figure 5, the oocyte is very rich in acid phosphatase, both in the nucleus and cytoplasm. In the latter, the concentration of enzyme near the peripheral part of the egg is a distinct feature in follicles of stages 4-6. The

yolk/-

yolk granules as they are formed in this peripheral region are likewise endowed with high acid phosphatase activity. A similar concentration of enzyme is also noticeable around the vacuoles present in the ooplasm in stage 6. In mature oocytes cortical localization of acid phosphatase becomes less evident, but instead the vitelline membrane appears to be positive.

The distribution of acid phosphatase in Drosophila ovaries has also been studied by incubating directly fresh material in lead-glycerophosphate reagent. After half an hour of incubation, the nuclear reaction is already evident in the peripheral follicles of the ovaries, chiefly in the follicular cells and nurse cells. The slower reaction in the oocyte is perhaps due to the limitation set by the rate of penetration of the reagents. The whole reaction becomes more intense and definite as the duration of incubation increases. Figures 4 and 5 present the differences found between a group of experimental and control follicles after two hour's incubation. If one compares figure 4 with figure 3, it is obvious that relative distribution of acid phosphatase is precisely the same in both cases.

Acid phosphatase is also demonstrable in the oviduct epithelium.

Like the ovaries, Drosophila testes are rich in acid phosphatase. Sperm heads and the whole matrix in which sperm are embedded seem to contain the enzyme.

G. Summary

It is evident from the above account that the egg of Drosophila, as a type of so called mosaic egg, does not show any differential distribution of the chemical substances (so far studied) in different parts of the egg whose spatial axes are probably determined very early during its development.

Mature oocytes are very rich in fixed -SH groups. A definite, but general, concentration of these groups in cortical ooplasm has been observed. Sperm heads are equally rich in fixed -SH groups.

Mature oocytes (and in particular immature ones) contain a considerable amount of ribonucleic acid which is diffusely distributed.

Acid phosphatase is demonstrable in both mature oocytes and sperms. The concentration of this enzyme in the egg cortex in the early stages of/-

of oogenesis seems to be connected with yolk synthesis, since such localization is no longer distinguishable after maturation.

Both adult ovaries and testes, and consequently the mature germ cells, contain no histochemical trace of alkaline phosphatase.

(2) <u>Embryonic development</u>

A. Fixed -SH groups:

A cortical concentration of fixed -SH groups is clearly visible in the early cleavage and blastoderm stages which agrees with the distribution already found in mature oocytes. Mitosis is always synchronic in these stages. If the nuclei of an early embryo happened to be in the course of mitosis at the time of fixation, the cytoplasmic -SH reaction is far more intense then that of a corresponding embryo whose nuclei were not in division. This fact is in accordance with the general view concerning the relation between fixed -SH groups and mitosis (cf. Needham, 1942). Like the chromatin substances in a non-dividing nucleus, mitotic chromosomes invariably show a vivid blue reaction.

As/-

As gastrulation and germ layer formation proceed the ectoderm which forms the future hypodermis obtains a greater share of fixed -SH groups than the endoderm and mesoderm. This ectodermal localization is traceable in all parts of the ectoderm of the embryo. It is still noticeable before the contraction of the germ band which takes place at about the 9th hour after laying at 25±0.2°C.

Apart from this differential distribution, no difference in the intensity of -SH reaction has been noticed between the mesoderm and nervous tissue rudiment or endoderm.

In later embryos (12 hours or older), an increase of -SH reaction has been observed in certain organs, such as gut epithelia, salivary glands, gonads and cuticle. With the exception of cuticle, the increase in fixed -SH groups seems to be correlated with the increase of ribonucleic acid.

B. Ribonucleic acid

In the newly laid eggs the distribution of ribonucleic acid is diffuse. As soon as the cleavage nuclei begin their migration to the egg periphery, there occurs a simultaneous segregation of/-

of ribonucleic acid. Thus, in the incipient or single blastoderm stage, the whole surface blastoderm is so heavily stained by pyronin that it gives one the impression that the ribonucleic acid content is increased. Especially noticeable is the higher concentration of the acid in the so called "innere blastema" which is later to be partially incorporated with the blastoderm cells. No ribonucleic acid is now left in the yolk except in the thin protoplasmic areas surrounding the yolk nuclei.

Between gastrulation and the contraction of the germ band (3-9 hours embryos), most principal organ rudiments have been laid down. During this interval no conspicuous difference in ribonucleic acid content between different germ layers and their derivatives has been observed. Within the ectoderm itself, however, a differential distribution exists. The half of the cell facing the yolk always contains more ribonucleic acid than the other half. Since this condition was found in the ventral lateral and dorsal ectoderm as well as in the invaginating stomodaeum and proctodaeum, it is possibly a mere consequence of the concentration of ribonucleic acid in the "innere blastema" in the blastoderm stage.

In/-

In older embryos (12-15 hours) a differential distribution of ribonucleic acid due to differential acquisition of synthetic activity arises. For example the mid-gut seems to acquire this ability soon after the coiling of the intestine has taken place (between 11th and 12th hour). Other organs whose ribonucleic acid content is comparable to that of the mid-gut are the proventriculus, gastric caeca, salivary glands, trachea buds and the hind-gut. Organs such as stomodaeum, Malpighian tubes, muscles, fat cells and hypodermis contain less ribonucleic acid. Still, the cytoplasmic basophily of these latter organs is not very much different from that of the cells of a gastrula. Nerve cells possess the least amount of ribonucleic acid at most stages: the nerve fibre region is devoid of it.

Comparing the pyronin stainability between embryos of various developmental stages (from cleavage to about 15th hour after egg laying), it was found that there seems to be very little, if any, decrease of ribonucleic acid throughout the most part of Drosophila embryonic development. Between the 15th and 18-19th (hatching of the larva) hours, ribonucleic acid content is maintained in the gut epithelia and salivary glands. In other organs a decrease of the acid content is noticeable.

Treatment/-

Treatment with ribonuclease removed the pyronin stainability of the embryonic cells. There is, however, a tendency for the yolk to take some pyronin in both control and treated sections.

C. Desoxyribonucleic acid:

The Feulgen reaction of Drosophila embryos has been studied with a view to getting some information about the time of the synthesis of desoxyribonucleic acid. It was found that mitosis occurs most frequently between the 5th and 8th hour after egg laying at 25±0.2°C. After the contraction of the germ band (9th hour) cell division can be seen only in the brain, mesenchyme cells (?) and occasionally in the ventral ganglion; but not in other tissues. This result is in entire agreement with the findings of Poulson (1945).

Since the growth of the larval tissues in Drosophila takes place almost exclusively by the process of endomitosis (Cooper, 1938), it would be interesting to know whether or not endomitotic growth starts early in later embryonic development. The nuclear size of the cells of mid-gut and salivary glands has thus been compared between the stages/-

stages immediately after the contraction of the germ band and just prior to hatching. Although the nuclear size in the latter stage is definitely larger than that in the former, the total increase in nuclear volume amounts to less than 50% (from about $28\mu^3$ to $40\mu^3$ in the case of the salivary gland cells). If the relation between the nuclear division cycle and doubling of nuclear volume holds true, this would suggest that endomitosis does not occur during embryonic life.

D. Alkaline phosphatase

Following on the negative reaction of mature oocyte and sperm, alkaline phosphatase is not detectable, even in the nuclei, in fertilized eggs nor in embryos up to a stage just prior to the contraction of the germ band. The enzyme arises suddenly in the embryo during, or more likely immediately after, that morphogenetic stage, and makes its first appearance in the ventral part of the embryo near the future thorax. The exact centre of origin is rather difficult to locate. It lies probably in the latero-ventral ectoderm (opposite the ventral nerve cord) of the metathoracic/-

metathoracic and first two abdominal segments. Topographically, these segments occupy almost a central position along the antero-posterior axis of the embryo and they always show a stronger alkaline phosphatase reaction than do other segments in the period soon after the appearance of enzyme activity (fig. 6). In most cases, only one such centre is found on the one side of the median embryo axis. In a few cases, two centres, one on either side of the median axis, are found.

From the centre (or centres) the enzyme activity spreads to the neighbouring ectoderm and to the interior organs in a quite characteristic manner, for definite gradients of alkaline phosphatase reaction are always observable in embryos fixed between the 9th and 12th hour after laying. At least two such gradients are noticeable:

(a) Extero-interior gradient ---- This is indicated by (i) the surface ectoderm always shows more enzyme activity than internal organs; (ii) the ventral nerve cord is active only in its ventral part at first, later to be followed by a distinct gradient showing a strong reaction in the ventral part but a moderate (to weak) one in the dorsal portion (a ventrodorsal gradient might be involved in this case); and/-

and (iii) the mesodermal components of the intestine invariably respond to the wave of enzyme spreading earlier than do the intestinal cells themselves.

(b) Antero-posterior gradient ---- The posterior part of the embryo is more reactive than the anterior part, a characteristic which can be very well seen in 10-11 hour embryos. This gradation also manifests itself within the surface ectoderm. In the case of the proctodaeum, the highest enzyme activity is found near the anal opening, gradually fading away towards the mid-gut. These two gradients of alkaline phosphatase reaction can be traced in the figure 7.

The possibility that gradient activities might be artifacts of some kind has been ruled out by the following experiment: a part of the enzyme activity of a number of fixed 11-12 hour embryos was destroyed individually by the local application of heat from a micro-cautery needle before embedding: the inactive areas in such embryos always gave a negative reaction irrespective of the positive reaction of the uninjured regions and of the duration of incubation. The results indicate that diffusion which might otherwise be the distrubing factor does not take place within the sections under experimental conditions. Consequently, the observed gradients must be attributed/-

attributed to differential rates of spreading of enzyme activity.

Further spreading takes place between the 12th and 14th hour, mainly in the anterior and central parts of the embryo. At this time the gradients of alkaline phosphatase reaction are no longer very distinct. In fact, it was often found that, in 12-13 hour embryos, the anterior part is more reactive than the posterior part. This is apparently due to the fact that organs in the anterior region (such as the brain, stomodaeum, cephalopharyngeal apparatus and head mesoderm) have reached their maximal enzyme activity, whereas the activity in the posterior part of the embryo has possibly already passed its peak. Because the highest enzyme activity of a particular organ seems to be maintained for only a short period, it is difficult to say at which stage the Drosophila embryo, as a whole, has its maximal alkaline phosphatase content. It may be stated that all embryonic tissues show a positive reaction in 12-15 hour embryos (fig. 8).

Alkaline phosphatase is more concentrated in nuclei, presumably in the chromatin material. In the cytoplasm, a diffuse and moderate reaction is demonstrable in every organ between the 12th and 15th hour/-

hour. When a cell begins to acquire phosphatase activity, the nucleus seems to be the first region to become active. Activity in the cytoplasm comes later.

At about the 15th hour enzyme activity starts to decrease in most tissues, especially in the cytoplasm. However, the cells of the tracheal tubes, hypodermis and cephalopharyngeal apparatus still retain their full activity, with perhaps even more activity in their cytoplasm. It is noteworthy that these three organs have a common physiological function i. e. the secretion of cuticle.

By the time of hatching, alkaline phosphatase remains active in the salivary glands, mid- and hind-guts and the posterior branches of Malpighian tubes (fig. 9). In other organs such as the nervous system, fore-gut, proventriculus, gastric caeca, muscles, fat bodies, tracheae, cephalopharyngeal apparatus and hypodermis, only some residual nuclear activity can be demonstrated after 24-hour incubation.

No alkaline phosphatase activity has been found in the yolk during most of the embryonic period. In older embryos (16-18 hour) the disintegrating yolk granules in certain sections of mid-gut show a positive reaction. Since in these cases the gut epithelia/-

epithelia and their striated borders have a very high phosphatase content, the enzyme activity found in the yolk is probably not of intrinsic origin. Yolk nuclei, on the other hand, show moderate activity in most stages.

E. Acid phosphatase:

Acid phosphatase can be demonstrated in the developing Drosophila embryo from cleavage up to the hatching of the larva. No apparent change in enzyme activity occurs during the whole embryonic development, although irregular results have often been encountered due to the destructive effects of histological proceedures on the enzyme (Stafford and Atkinson, 1948).

Generally speaking, the most active site of acid phosphatase is the yolk which stains dark brown to black. Nuclei of the embryo cells are the next most active structures, giving a golden brown appearance. Cytoplasm is the site of the weakest activity, showing a light yellow to yellowish brown colour. Alternate sections as controls are always colourless.

In cleavage stages acid phosphatase is mostly concentrated in the subcortical plasm and in the yolk/-

yolk; whereas the egg cortex appears to be free from the enzyme. In dividing cells, chromosomes and spindle area are found to be moderately positive.

In the single blastoderm stage, acid phosphatase is most active in the nuclei of blastoderm cells and in the yolk (fig. 10). In this same figure a stronger reaction in the "innere blastema" which more or less corresponds to the subcortical plasma in the cleavage stage can be also seen. Throughout the gastrulation stage, the situation remains the same: a stronger reaction in the yolk and cell nuclei (fig. 11). No difference has been found between the cells of the three germ layers, nor is there any differential intensity of reaction in different parts of an embryo at this or in any other later stages.

Figure 12 is taken from a 12½ hour embryo. The nuclear acid phosphatase reaction in the nervous system, proventriculus, cephalopharyngeal apparatus and hind-gut (as are shown in the figure) is still comparable to that of the early embryonic cells. The mid-gut with its enclosed yolk forms the most active centre of activity. Comparing this distribution with the alkaline phosphatase reaction of/-

of an embryo of the same age, a most noticeable difference is that the yolk and nerve fibres give a strong to moderate acid phosphatase reaction, but give no trace of (yolk) or very weak (nerve fibres) alkaline phosphatase activity.

Further differences between the activity of the two kinds of phosphatase during Drosophila embryogenesis are found in embryos prior to hatching. Unlike alkaline phosphatase, no decline in acid phosphatase activity has been noticed (fig. 13). Both nuclear and cytoplasmic reactions are still demonstrable in every tissue. In the case of gut epithelia (including proventriculus and gastric caeca) and salivary glands, it is even possible that there is some increase in cytoplasmic activity.

Due to the extreme impermeability of the vitelline membrane, it was not possible to check the acid phosphatase distribution in Drosophila embryos by using fresh unfixed material.

F. Summary:

As a consequence of the cortical localization of fixed -SH groups during oogenesis, a concentration of these groups in the ectoderm of the developing Drosophila embryo has been observed. Similarly, a higher concentration of ribonucleic acid in the "innere blastema" in the blastoderm stage has led to the/-

the differential distribution of the acid within the ectoderm in older embryos. These two differential properties are, however, of general occurrence in the sense that they are restricted to the ectodermal layer but not to a particular region of an embryo.

The ribonucleic acid content of the Drosophila embryo does not show a conspicuous decrease during the first three-quarters of embryonic life. The increase of ribonucleic acid content through synthetic activity is very pronounced in the case of salivary glands and gut epithelia.

Judging from the frequency of mitosis, synthesis of desoxyribonucleic acid during Drosophila embryogenesis takes place mostly before the stage of the contraction of the germ band.

Endomitotic growth probably does not occur in the later half of embryonic life.

A histochemical study of the distribution of phosphatases reveals two different patterns of enzyme activity: acid phosphatase is active throughout the whole embryonic development with no detectable changes in its activity; alkaline phosphatase/-

phosphatase only arises after the stage of the contraction of the germ band, increases its activity and recedes (in most tissues).

The evidence that alkaline phosphatase originates in the ventral ectoderm near the future thorax demonstrates again the importance of the ventral region of an embryo in insect development.

The coincidence in time between the cessation of mitosis and the appearance of alkaline phosphatase activity seems to suggest that the latter enzyme is related to histo-differentiation.

The high acid phosphatase activity of the yolk in developing embryos, together with a similar activity in the cytoplasm of the nurse cells and oocyte during oogensis, indicates that this enzyme plays some role both in the synthesis and in the degradation of the yolk.

(3) Larval development

A. Alkaline phosphatase:

The localization of alkaline phosphatase has been studied in a series of Drosophila larvae from hatching up to puparium formation with an age interval of about 8 hours. The following general results/-

results are noteworthy:

(i) during most of the 1st instar larval period, enzyme activity is very weak and is similar to that of a larva before hatching; it increases in the later part of 1st instar life (between the 16th and 24th hour after hatching):

(ii) in 2nd instar larva (approximately between the 24th and 48th hour), alkaline phosphatase activity is more easily demonstrable and

(iii) the activity in early 3rd instar larvae (from the 48th to 64th hour) is still not very different from that of 2nd instar larva, but larvae which had ceased to feed and which were crawling about on the sides of the culture bottle (generally between 72th and 80th hour) give quite a different picture of enzyme distribution.

In general, nuclear alkaline phosphatase is demonstrable in every tissue, but cytoplasmic phosphatase shows a strict organ specificity. The differential high enzyme activity in the gut, salivary glands and Malpighian tubes, as has been found in the larva before hatching, is maintained during the larval life.

The following account of enzyme distribution is based on the observations made on larvae aged between/-

between 16 and 64 hours.

NERVOUS SYSTEM ---- Nerve cells are moderately reactive. Mitoses were found during the larval period, especially among the giant ganglion cells. The phosphatase reaction of the nerve fibres is weak.

DIGESTIVE SYSTEM ---- The phosphatase reaction of the pharynx, proventriculus, gastric caeca and hind-gut is weak. So is the reaction of the cephalopharyngeal apparatus. In the mid-gut, the nuclei are moderately positive and the striated border strongly positive (fig. 14,mg). In the cytoplasm of mid-gut cells, the enzyme is more concentrated between the nucleus and the striated border as if it is secreting into the intestinal lumen. Salivary glands are the most active organs (fig. 15,sl). The enzyme is present in both the nucleus and cytoplasm as well as in the contents of the lumen. The activity is often so high after 12 hours of incubation that its neighbouring tissues are affected by a contamination reaction. The presence of alkaline phosphatase in salivary glands has been previously reported (Danielli and Catcheside, 1945; Kruglis, 1945, 1946).

MALPIGHIAN TUBES ---- Moderate activity is found in the nucleus and some intracellular granules. The brush/-

brush border and sometimes the whole lumen are strongly positive (figs. 14,21,Ma). The lumen of the anterior branches of the Malpighian tubes are much wider than that of the corresponding posterior branches and its width increases as the larva grows. The contents in such dilated lumens stain in both experimental and control sections. Evidently, this is due to the presence of preformed calcium salts (Eastham, 1925).

HYPODERMIS ---- The phosphatase reaction varies from weak to moderate (figs. 15,16,h). It is possible that those hypodermal cells which are engaged in cuticle secretion show more phosphatase activity than those which are not so engaged. Oenocytes lying beneath the hypodermis give a moderate nuclear reaction only.

TRACHEAE ---- The epithelium as well as the cuticular intima are strongly reactive at the time of moulting (fig. 16,tr,ti). The strong positive reaction of the shed cuticle of the previous instar is clearly shown in the figure. Otherwise, the phosphatase reaction of the trachea is moderate.

MUSCLES ---- In a fully developed muscle fibre, nuclei and myofibrils show a moderate reaction, whereas the sarcoplasm is almost negative. The anisotropic/-

anisotropic discs of the myofibils are far more reactive than the isotropic discs, giving thus a typical banded structure (fig. 17).

FAT BODIES ---- Fat cells give a weak to moderate nuclear reaction, but their cytoplasm is negative (figs. 15,16,27a). The stronger reaction of those fat cells situated near to a very active organ (in fig. 15) is due to contamination.

GONADS ---- Both ovaries and testes are weakly positive throughout the whole larval life.

IMAGINAL DISCS ---- All imaginal discs give a moderate alkaline phosphatase reaction which is constant throughout the first and second larval instars and the early phase of the third instar. In general, the phosphatase reaction of imaginal discs is comparable to that of the nerve cells and is stronger than that of the hypodermis from which they are mostly derived. This higher activity is evidently linked to the proliferative growth of the discs. Some of these discs are shown in the figures 15a and b (Fr,W). It should be pointed out that the difference in reaction intensity between the salivary glands and imaginal discs, while distinct in actual preparations, is not evident from the photographs. The alkaline phosphatase reaction of imaginal discs declines almost to zero in larvae preparing for puparium/-

puparium formation (fig. 18).

RING GLAND ---- From the embryological study of Poulson (1945), it is certain that the ring gland of Drosophila constitutes a fusion between paired corpora cardiaca and a single corpus allatum. The alkaline phosphatase reaction of this organ is very weak in the 1st instar larvae. It becomes moderately active in 2nd instar larvae (fig. 15b, Rg), the enzyme activity being mostly confined to the nucleus: nuclei of the corpora cardiaca are much more active than the smaller nuclei of the corpus allatum. Parallel to the phenomenon observed in the case of imaginal discs, the enzyme activity of the ring gland is again very weak in the 3rd instar larvae (60, 72 and 80 hours old).

HEART AND RELATED STRUCTURES ---- A moderate reaction is visible in the nuclei of the heart cells. Their cross-striated contractile fibrils react only weakly. Paired lymph glands are moderately positive.

There are two groups of pericardial cells in the larva of Drosophila. About 16 pairs of large pericardial cells are situated on either side of the heart. The small pericardial cells, about 32 in number are binucleate and are located between the brain and proventriculus. Although these latter bear no direct relationship to the heart, their phosphatase/-

phosphatase activity, intracellular ribonucleic acid distribution and their behaviour during metamorphosis point to their similarity to the large pericardial cells. Both types of pericardial cells give a moderate alkaline phosphatase reaction during the larval period: a concentration of the enzyme around the cell membrane can often be noticed (figs. 15c, Sp, 29, Lp).

It was stated at the beginning of this section that the distribution of alkaline phosphatase in the late 3rd instar larvae (72 - 80 hours after hatching at 25±0.2°C.) differs from that in young larvae. The most significant differences are the general decrease of alkaline phosphatase activity in the internal organs and the simultaneous increase in the hypodermis (fig. 18, Pm+h) and possibly also in the muscles below. For example, nervous system, imaginal discs and ring gland which show moderate activity in the 2nd and early 3rd instar life are now only weakly positive in their nuclei. Some decrease of enzyme activity probably also occurs in the salivary glands, mid-gut, Malpighian tubes and pericardial cells, although they are still the most active organs at this stage. The cause of this change of alkaline phosphatase activity is not known/-

known. But since the larvae are preparing for pupation, it is only natural to connect the high alkaline phosphatase activity of the hypodermis with the formation of the puparium. How close such a connection may be can only be answered by transplantation of the ring gland.

B. Acid phosphatase

Acid phosphatase is very difficult to demonstrate in the first and early second instar larvae: organs such as the salivary glands, gonads, gut epithelia, Malpighian tubes and nervous system show moderate reaction, while the rest appears negative. Since it has been shown that only a small fraction of the original acid phosphatase activity is preserved in the embedded tissue sections (Stafford and Atkinson, 1948), the negative reaction of an organ does not necessarily mean the intrinsic absence of the enzyme in that organ. However, the acid phosphatase reaction becomes more intense and more easy to demonstrate in late second (38-50 hours after hatching) and third instar larvae, probably due to an increase of the enzyme content. The following summarized account refers to these older larvae.

Like alkaline phosphatase, acid phosphatase is present in the nuclei of every tissue. The whole nucleus often stains dark brown or black without any internal differentiation. Occasionally, if the reaction is not so intense, a distinct active nucleolus and active polytene chromosome structures can be seen within the nucleus of such larval tissue as gut epithelia and fat bodies. On the other hand, cytoplasmic acid phosphatase shows a much wider distribution than does the cytoplasmic alkaline phosphatase. The strongest cytoplasmic reaction is found in the following organs, arranged in order of decreasing grade of activity: salivary glands, gonads Malpighian tubes, mid-gut, proventriculus and gastric caeca. Moderate cytoplasmic reaction is present in the ring gland, oenocytes and nerve fibre region. The cytoplasmic activity of all imaginal discs, nerve cells, hind-gut, muscles and fat cells is rather weak; and that of the tracheal epithelium, pericardial cells and hypodermal cells is almost negligible.

Comparing the cytoplasmic distribution of alkaline and acid phosphatases in the larval organs of Drosophila, it seems that the latter can be classified into three groups:

(i) two enzymes of similar activity, either very active or very weak: salivary glands, Malpighian tubes, mid-gut, nerve cells, imaginal discs, muscles and fat bodies:

(ii) alkaline phosphatase predominates: pericardial cells, tracheae and hypodermis:

(iii) acid phosphatase predominates: gonads, proventriculus, gastric caeca, ring gland and oenocytes.

Figure 19 is taken from two 72-hour larvae, showing the strong acid phosphatase reaction of the salivary glands, proventriculus, gastric caeca, ring gland, nerve fibres, mid-gut and testes.

Apart from the difference in distribution among different organs, acid phosphatase differs from alkaline phosphatase in another important respect: no decline of acid phosphatase activity in internal organs prior to puparium formation, nor any increase of activity in the hypodermis.

C. Ribonucleic acid:

The distribution of ribonucleic acid is more or less the same in the larvae of different instars. The differentials of the distribution found in the larva before hatching are still maintained. Thus, the/-

the following organs are the most rich in ribonucleic acid and stain deep red with pyronin: salivary glands, proventriculus, gastric caeca, mid-gut, all imaginal discs, lymph glands, some nerve cells and gonads. The other organs, including Malpighian tubes, tracheae, fore- and hind-guts, ring gland, most nerve cells, pericardial cells and fat bodies, are comparatively poor in ribonucleic acid and stain pink to red. The staining of the hypodermal cells varies from deep red to pink among different individuals of certain definite age, e.g. 20-30 hour larvae. It is possible that this variation is related to the growth cycle of the hypodermal cells accompanying each moulting process. Fully developed muscle fibres of the 2nd and 3rd instar larvae seem to contain very little ribonucleic acid.

The imaginal buds of the intestine are very easy to pick out from the larval gut cells by virtue of their higher content of ribonucleic acid. The difference becomes extremely striking in late 3rd instar larvae due to a reduction of cytoplasmic basophily of the larval gut cells themselves. The imaginal buds now stain purplish red, whereas the larval gut cells appear pink. Similar results

havē/-

have previously been noted by using absorption (Caspersson and Schultz, 1939) and staining (Brachet, 1942) techniques.

In the nervous system, high ribonucleic acid content is confined to some four patches of nerve cells in the brain (Bauer's (1904) outer and inner "Bildungsherde" which later organize into optic ganglia) and many solitary giant ganglion cells ("Riesenzelle" of Hertweck, 1931), both in the brain and ventral ganglion.

In the ring gland, a difference in ribonucleic acid content also exists between the corpora cardiaca and corpus allatum, the former being the richer. Moreover, the degree of basophily of the corpora cardiaca changes with the age of the larvae: those of 80- hour larvae definitely stain more weakly than those of 55-60 hour larvae.

Fat cells are characterized by their red nucleolus and pink cytoplasmic meshworks separated by empty spaces previously occupied by fat substances. Just prior to puparium formation, changes take place within these fat cells which lead to the breakdown of the fat vacuoles and the formation of globules of unknown nature, especially around the nucleus. These globules are stainable by pyronin in both control and enzyme treated sections (Brachet (1942) had/-

had the same experience). Furthermore, there is evidence that such intracellular changes start at the anterior end of the larva and advance in an antero-posterior direction.

Another point worthy of mention is the intracellular distribution of ribonucleic acid in the two groups of pericardial cells which seems to be quite unique. Ribonucleic acid is concentrated in one or two irregular blocks around the nucleus or nuclei (in the small pericardial cells), while the rest of the cytoplasm is practically free from the acid.

D. Summary:

The distribution of alkaline and acid phosphatases and ribonucleic acid in the larvae of Drosophila has been studied.

Both alkaline and acid phosphatases are demonstrable in the nuclei of every larval tissue. Cytoplasmic phosphatase, on the other hand, shows a definite organ specificity: in some organs such as the salivary glands, Malpighian tubes and mid-gut, both phosphatases are extremely active; in fat cells their activity is very weak; alkaline phosphatase activity predominates in the pericardial cells, tracheae and hypodermis; while only an acid phosphatase/-

phatase reaction is detectable in the gonads, proventriculus, gastric caeca, ring gland and oenocytes.

Imaginal discs possess both alkaline and acid phosphatases and are very rich in ribonucleic acid. The moderate alkaline phosphatase activity decreases in the late 3rd instar larvae to an extant which is almost beyond the limit of histochemical detection. Their acid phosphatase activity remains unchanged during the same period.

The formation of the puparium case is preceeded by a tremendous increase of alkaline phosphatase in the hypodermis.

With the exception of the imaginal discs and the nerve fibre region, there exists a positive correlation between the acid phosphatase activity and the ribonucleic acid content of an organ.

(4) Prepupal and pupal development (metamorphosis)

Since the processes of metamorphosis which involve histolysis and histogenesis start in mid-prepupal life, it seems to be more appropriate to treat together the results obtained from the cytochemical studies made on the prepupae and pupae.

A. Alkaline phosphatase:

Within the first few hours after the anterior spiracles cease to move, the alkaline phosphatase reaction of a prepupa is rather weak, just like that found in a larva prior to puparium formation (fig. 20, compare with fig. 18). Salivary glands, hypodermis, muscles, mid-gut, Malpighian tubes, pericardial cells and lymph glands are among the relatively active organs. The reaction of the nervous system, ring gland and all imaginal discs is still exceedingly weak. However, from the 5th hour onwards, there begins a definite increase in reaction intensity. This is especially noticeable in the organs having weak enzyme activity such as the nerve cells, ring gland and imaginal tissues.

In prepupae prior to the head eversion (fig. 21), the relative distribution of alkaline phosphatase activity is this: salivary glands, yellow body, larval hypodermis, anterior portions of the tracheae, pericardial cells, lymph glands and histolyzing structures (detached head and thoracic muscles and detached hypodermal cells) all show a moderate to strong reaction; fat bodies, abdominal muscles, hind-gut, posterior parts of the larval tracheae and gonads react/-

react very weakly. Because of this differential activity, the anterior part of the prepupa is definitely more reactive than the posterior part. This also is clearly indicated in the same figure.

When the larval and imaginal components of an organ are lying side by side as for example in the case of the hypodermis, mid-gut or salivary glands, it is generally true that the larval cells are much richer in alkaline phosphatase content than do the imaginal cells (see fig. 26).

If the increased alkaline phosphatase activity due to histolysis in the late prepupae is not taken into account, the enzyme activity of the prepupae is similar to that of the larvae rather than to that of the pupae.

Pupation occurs between the 11th and 12th hour after the larva has become quiescent at 25±0.2°C. The visible morphological changes which mark its beginning are the sudden eversion of the whole cephalic complex, the breakdown of a part of the fat bodies into individual cells and the pouring of the latter into the newly formed head. A comparative study of the alkaline phosphatase reaction of the prepupae just before the head eversion and of that of the pupae immediately after/-

after it has revealed a definite and sudden increase of enzyme activity accompanying pupation. Since a moderate nuclear reaction is present in every tissue before pupation, the most noticeable increase is found in the cytoplasm.

Just after the head eversion, the phosphatase reaction in the head and thorax is very strong (fig. 22), but comparatively weak in the abdomen except in those parts adjoining the thorax. A few hours later, the reaction in the abdomen increases, due to advancing histolysis of the abdominal muscles, posterior larval tracheae and hypodermis, as well as fat bodies. Such high phosphatase activity is maintained for the first 24 hours or so after the head eversion (fig. 24), during which period histogenesis and histolysis are progressing actively. Then the enzyme activity begins to decline, first in the head and thorax. In pupae older than 48 hours and in newly emerged flies, phosphatase reaction is very weak in the head and thorax, with nerve cells as an exception. The reaction in the abdomen is, however, still moderate to strong (fig. 25). This relatively high enzyme activity in the abdomen of a late pupa is probably connected with:

(i)/-

(i) the late histogenesis of the abdominal muscles

(ii) the descending of phosphatase-active mid-gut and yellow body into the abdomen;

(iii) the delayed decline of enzyme activity of the gonads and possibly

(iv) the slow histolysis of the abdominal fat bodies.

The following is an account of the change of the alkaline phosphatase activity in different organ systems during metamorphosis:

(a) NERVOUS SYSTEM ----- The phosphatase reaction is very weak in the early, and moderate in the late prepupal periods. It is greatly enhanced after pupation (compare figs. 21 and 22; figs. 23a and 23b). The nerve fibre region now also becomes moderately positive. Such moderate activity remains unchanged until about the 48th hour after puparium formation. The reaction is again very weak in pupae aged between 52 and 68 hours. After the 72nd hour, a secondary increase of alkaline phosphatase activity occurs in the nerve system. The nerve cells of the brain proper and ventral ganglion give a moderate reaction; the middle and inner optic ganglia a weak to moderate one; and the outer optic ganglia a very weak, or even negative one. This condition persists in the pupae prior to emergence and in the 24-hour old adult flies.

(b)/-

(b) ANTENNAE AND COMPOUND EYE ---- Both antennal and eye discs are almost negative in the young prepupae (fig. 20,Fr); but they are moderately active in 5 to 11½ hour prepupae. After pupation, the phosphatase reaction is intensified just as the rest of the head ectoderm (fig. 22,Od). At this time antennal discs are in the form of two thickened ectodermal plates. In a few hours, the two major antennal joints appear by processes of folding and extension. Antennae are well defined in a 26-hour pupa. During this period, alkaline phosphatase reaction is moderate to strong. It declines afterwards and becomes almost negative in 44-hour pupae. Johnston's organs are recognizable in 72-hour pupa, but they show only a weak nuclear reaction.

Similarly, eye discs are also represented by two thickened ectodermal plates, each consisting of several rows of cells. Unlike the antennae, no very striking histological changes occur during the first 16 to 20 hours after the head eversion, except an obvious increase of the width and a concomitant decrease of the thickness of the eye rudiments. Between the 30th and 52nd hour, histological differentiation of the compound eyes is in full swing, and individual ommatidium and optic lobes are very well formed. The compound eye of a 72-hour pupa resembles/-

resembles in every respect the structure of an imaginal eye. Moderate to strong phosphatase reaction of the eye rudiments is a constant feature in pupae aged between 12 and 42 hours. The enzyme activity then drops and only a weak nuclear reaction is demonstrable in 60-, 72- and 80-hour pupae and in the freshly emerged flies.

(c) WINGS AND LEGS ---- These organ discs show very weak phosphatase reaction in early prepupal period (fig. 20,W,L). They become moderately reactive after their eversion in mid-prepupal stage. Accompanying with pupation, there is an increase of both nuclear and cytoplasmic phosphatase in these organs (compare figs. 21 and 22; 23a and 23b). This high phosphatase activity prevails in both organs up to about 36-hour pupae (fig. 24,L). Inactivation or rather destruction of the enzyme then begins, leading first to a moderate (48-hour pupa) and finally to a weak reaction (72 hour pupa, fig. 25). Waddington (1940a) has made an extensive study of the development of the normal and mutant wings of <u>Drosophila</u>. From his description, it is evident that the most important morphogenetic events in normal wing development occur during the "definite wing stage" (stage P2 (18-45 hour) in his paper). The observed strong alkaline phosphatase activity/-

activity of the wings in 12-36 hour pupae and a moderate activity in 36-48 hour pupae therefore strongly suggest that the enzyme is particularly concerned with histo-differentiation.

During the transition period (36-72 hour), it has been noticed that the distal parts of these organs often lose their enzyme activity earlier than the corresponding proximal parts; and that the wings lose their enzyme activity earlier than the legs.

Residual nuclear phosphatase activity is still present in the wings and legs of the newly emerged flies.

Trichogenic cells are very difficult to recognize in phosphatase reaction slides.

The development of leg muscles follows the same temporal course as the development of most thoracic muscles and will be discussed in the section on muscles.

(d) HYPODERMIS ---- Following the strong phosphatase reaction before the puparium formation, hypodermis is always an active site of phosphatase activity in prepupae (figs. 20,pm+h;21,h). During the 12 hours of prepupal life, all larval thoracic hypodermis is replaced by an imaginal one, whereas in the abdomen hypodermis remains mostly larval, with only a few scattered groups of imaginal cells. The phosphatase/-

phosphatase reaction is very strong in the larval cells, but only moderate in the imaginal cells.

Soon after pupation, the phosphatase reaction of the head and thoracic hypodermis becomes very strong and remains so until about the 38th hour (figs. 22,23b,24). The reaction gradually fades away and becomes negative in 60-hour pupae.

On the other hand, no change in the enzyme activity has been observed in the abdominal hypodermis shortly after pupation. The contrast between the activity of the larval and imaginal cells can still be seen (fig. 26). Six hours later, this differential reaction becomes less and less striking due to the increasing enzyme activity in the imaginal cells. When the abdominal hypodermis is completely renewed, its phosphatase reaction is very strong (fig. 24). Folding of the hypodermis to form tergites and sternites happens between 36- and 48-hour pupae. The phosphatase reaction is strong during this period. It becomes moderate after the 48th hour and weak after the 60th hour.

Trichogenic cells or the histoblasts of the hairs and bristles in the head and thorax can be easily distinguished from the rest of the hypodermal cells by their larger size and stronger nuclear phosphatase reaction. In the abdomen, they are smaller and hence are hard to discriminate from the ordinary/-

ordinary hypodermal cells.

(e) MUSCLES ---- Larval muscles are completely destroyed during metamorphosis. They give a weak to moderate phosphatase reaction in the early prepupae. As soon as they are detached and undergo histolysis, the sarcolytes show moderate to strong reaction. Those sarcolytes derived from the head and thoracic muscles can be seen in the anterior Part of an old prepupa (figs. 21,23a). The abdominal muscles, however, undergo histolysis a few hours after pupation. Thus, strongly reactive sarcolytes can be found in the abdomen of 15-24 hour pupae. A similar increase of alkaline phosphatase activity occurs in the muscular coats of the gut when they are undergoing histolysis. Myocytes in the thorax of a late prepupa are moderately active (fig. 21,My). After the head eversion, intense proliferation goes on and the phosphatase reaction becomes very strong (fig. 23b, My). The resulting mass of myocytes is more or less in the form of a syncytium. In 20-24 hour pupa, the first sign of muscle differentiation -- the formation of myofibrils and the arrangement of the nuclei into parallel rows -- becomes visible. Further differentiation between 24- and 48-hour pupae involves the growth of the muscle fibre as a whole/-

whole and the continuous formation of myofibrils. As a rule, the phosphatase reaction of a developing muscle is very strong in 12-36 hour pupae, but moderate in 38-52 hour pupae. Furthermore, the development of the dorsal thoracic muscles proceeds several hours ahead of that of the other thoracic, head and leg muscles. In 54-56 hour pupae, the dorsal thoracic muscles almost reach their final length and their phosphatase reaction is very weak.

Myofibrils give a positive phosphatase reaction. When the isotropic and anisotropic discs become differentiated, a slightly stronger reaction is found in the anisotropic discs. The cross striation appears in the dorsal thoracic muscles in 48-52 hour pupae; in other muscles it develops somewhat later.

The development of the abdominal muscles occurs much later. Definite myocytes, each regularly spaced, can be readily seen in 48-hour pupae. The myofibrils first appear in 54-56 hour pupae. When the phosphatase activity of the thoracic, head and leg muscles is already in decline, abdominal muscles show very strong reaction in 48-72 hour pupae. They are still moderately active in 80-hour pupae.

In the newly emerged flies, residual phosphatase activity can still be demonstrated in the

nuclei/-

nuclei and myofibrils of all muscles.

(f) DIGESTIVE SYSTEM ---- The phosphatase reaction of the fore-gut is rather weak in the prepupal stages. Unlike the hypodermis, the larval and imaginal components show no difference in enzyme activity. The fore-gut gives a strong reaction in the first day after pupation (fig. 24,Ph). The reaction is weak to moderate in the second and third days of pupation. The crop which is entirely an imaginal organ is strongly reactive before the 38th hour and moderately so after that.

Because of the high phosphatase activity of the salivary glands in prepupae, it is not sure whether their enzyme activity is also enhanced after pupation. Nevertheless, the fragments of the glands after their disintegration in 15-hour pupae show a very strong reaction (fig. 23a,sl), until finally they disappear. The imaginal salivary glands are very difficult to trace in young pupae in view of the strong phosphatase reaction of the thorax as a whole. They are found to be strongly positive in 28-48 hour pupae. Moderate reaction is retained in late pupae and in the adult flies.

The imaginal mid-gut formed in the early phase of prepupal life gives a weak alkaline phosphatase reaction in comparison with the strong reaction of the/-

the yellow body (fig. 21, Mg and y). In the latter structure, the higher enzyme activity in the striated border of the larval gut cells is still recognizable. Pupation brings about a marked increase of phosphatase activity in both the imaginal mid-gut and yellow body (fig. 22). Being different from most other organs, the mid-gut does not show any appreciable decrease of enzyme activity in the second and third days after the head eversion.

The moderate reaction of the larval hind-gut in prepupae changes into a stronger one in 16-32 hour pupae, as histogenesis and histolysis set in. Moderate phosphatase activity is found in the hind-gut and rectal papillae at the time of emergence.

(g) MALPIGHIAN TUBES ----- According to Robertson (1936), the larval organ transforms directly into the adult one without any visible morphological changes. In correspondance with this, it was found that the phosphatase reaction of the Malpighian tubes of prepupae and pupae is the same as that of the larvae. The nuclear reaction is moderate, whereas the brush border and many cytoplasmic granules react very strongly. However, variations have also been found as, for example, segments of the tubes showing no reaction in the brush/-

brush border or segments free from the positive cytoplasmic granules.

The contents of the dilated anterior branches show positive reaction in both experimental and control sections.

(h) FAT BODIES ---- Since fat is the main energy source required for the morphogenetic processes during metamorphosis, fat bodies are mostly broken down after pupation. In the larval and prepupal periods, fat cells are arranged into definite sheets and their phosphatase reaction is moderate in the nuclei, but almost nil in the cytoplasm (fig. 27a). Accompanying the head eversion, there is a sudden dissolution of the fat bodies and a concomitant increase of alkaline phosphatase activity in the isolated fat cells (fig. 27b). The increase is mostly confined to the cytoplasm, where the enzyme is localized in the protoplasmic mesh-work separating the numerous globules. These individual fat cells soon undergo histolysis and dissolve away.

On the whole, the histolysis of the fat bodies is a graded process, for the thoracic fat bodies are always first attacked and the most posterior abdominal fat bodies the last. Thus, the abdominal fat bodies of a pupa shortly after pupation maintain their layered structure and show no increase of/-

of phosphatase activity. A similar gradient of the histolysis of the fat bodies has been observed during metamorphosis of Calliphora (Perez, 1910).

At the end of the first day after the head eversion, the layered structure of the fat bodies in the abdomen has mainly vanished. However, not all larval fat cells are destroyed during the pupal stage. Those partially changed fat cells left behind in the abdomen still give a moderate phosphatase reaction at the time of emergence. Further histolysis of these cells takes place in the adult life.

The imaginal fat bodies can readily be recognized in 60-hour pupae. They appear as groups of small cells situated beneath the hypodermis and give a moderate nuclear reaction.

(i) TRACHEAE ----- The imaginal buds of the tracheae can be traced to the early second instar larvae. They are located near the anterior spiracles and show a moderate nuclear phosphatase reaction. During the prepupal stages, proliferation of the imaginal tracheal cells and histolysis of the larval tracheae are the main features. In 5 hour or older prepupae, the anterior portions of the tracheae give a very strong phosphatase reaction (figs. 21,23a;tr,Tr), but the posterior trunks

(approximately/-

(approximately one third of the total length) show invariably a very weak reaction.

Just after pupation the prothoracic spiracles, main trunks in the thorax and the principal branches to the head are all composed of imaginal cells. The anterior commissure and the main trunks in the anterior part of the abdomen are still larval. Both the imaginal and larval tracheae are strongly reactive (fig. 22, Tr). The posterior larval trunks in the abdomen first become solid cords and then undergo histolysis in 14-24 hour pupae, with an accompanying rise in phosphatase activity.

The lateral spiracles as seen in the prepupae give a weak to moderate nuclear reaction. They become strongly positive after pupation.

The phosphatase activity of the tracheae and tracheoles, very active the first day after the head eversion, decreases in the second day. In 72-hour or older pupae the tracheae of the head and thorax are only weakly reactive, whereas those in the abdomen, in keeping with the activity of other abdominal organs, are moderately active.

(j) RING GLAND ---- As in the imaginal discs and nervous system, the phosphatase reaction of the ring gland is almost negative in 0-5 hour prepupae. After/-

After the 5th hour enzyme activity is increased. The corpora cardiaca now give a moderate reaction, but the corpus allatum is only weakly positive (fig. 28). This is exactly the same situation as was met in the second instar larvae.

Within the first few hours after the head eversion, there is no change of phosphatase activity of the ring gland. However, the whole gland becomes very reactive in 18-38 hour pupae, no longer showing a difference in activity between the corpora cardiaca and corpus allatum. The ring glands of 46, 54, 62, 66 and 72 hour pupae show moderate activity. At the time of emergence, the nuclear phosphatase reaction is still demonstrable in the gland.

In 30-hour or older pupae, the cells of the larval corpora cardiaca show a reduction of the nuclear size and cytoplasmic content. In some cases the beginning of nuclear pycnosis has been observed. These are probably the preludes to their actual histolysis which takes place after the emergence (Vogt, 1941, 1942a).

(k) HEART AND RELATED STRUCTURES ---- The larval heart transforms directly into that of the adult with a change of form (Robertson, 1936). The phosphatase reaction of the heart in prepupal stages is/-

is the same as that of the larvae. The enzyme activity, as in other organs, is increased after pupation. Both the nuclei of the cardiac cells and the muscle fibres are positive (fig. 29, Dv).

The lymph glands or the "blood forming organ" of Stark and Marshall (1930) give a moderate phosphatase reaction in the prepupal period. In pupae shortly after the head eversion, two pairs (one pair according to Robertson) of such glands are found (fig. 29, lg). They do not show any increase of phosphatase activity and disappear before the 18th hour.

The two groups of pericardial cells persist throughout metamorphosis and are present in the adult flies. During the prepupal period, their phosphatase reaction is moderate. Accompanying pupation, the increase of cytoplasmic phosphatase in these pericardial cells is very marked (fig. 29Lp). In the case of the large pericardial cells, this increase is especially clear in 18-32 hour pupae, for they appear as solid black bodies (fig. 24, Lp). Both the large and small pericardial cells show a moderate phosphatase activity in the mid-pupal period. In later pupal stages, the phosphatase reaction becomes rather weak and remains so in the emerged flies.

(1)/-

(1) GONADS ---- During metamorphosis, the ovary and testis show similar behaviour in phosphatase activity. Like those in the larvae, they react very weakly in the prepupal stages and exhibit a delayed response to the general increase of phosphatase activity after pupation. Together with the abdominal fat bodies, they thus constitute those structures which give a relatively weaker reaction in the early pupae. A very definite increase of phosphatase activity is, however, noticeable in the 32-hour pupae, when the gonads are found to be already joined by the genital ducts. From this stage onward, the ovary and testis always give a moderate to strong phosphatase reaction until the time of emergence.

Figure 30 is taken from a 32-hour male pupa showing the enzyme distribution in the testis and vas efferens. It is evident from the figure that alkaline phosphatase is more concentrated in the nucleus than in the cytoplasm of the spermatocytes. Consequently, the sperm heads are strongly positive.

In the ovary, the enzyme is present in the ovarian cords and the surrounding somatic tissues in the early developmental stage (fig. 31). When the egg follicles begin to differentiate after the 72nd hour, phosphatase is also found to be mostly localized/-

localized in the nuclei of the follicular cells, nurse cells and oocytes (fig. 32).

The development of the external genitalia and other accessory organs has not been followed in detail. The phosphatase reaction of the abdominal disc which gives rise to these structures is very weak in the prepupal period (fig. 21,ad). It increases at about six hours after the head eversion, parallel to the general increase of enzyme activity in the imaginal hypodermis of the abdomin. In 32 hour pupae the well formed genital ducts are as reactive as the gonads.

B. Acid phosphatase

During metamorphosis, acid phosphatase differs from alkaline phosphatase in the following respects:

(i) it is active in the early as well as late prepupae, with a relative distribution among various organs the same as in the 3rd instar larvae;

(ii) it does not show any increased activity after the head eversion, possibly even a slight decrease;

(iii) it becomes very active in 32-68 hour pupae, without showing any difference in activity between the head, thorax and abdomen;

(iv) its activity also decreases after the 72nd hour but to a much lesser extent than does the alkaline phosphatase/-

phosphatase activity. Perhaps, in both cases, the disappearence of the enzymatically active histolyzing fragments is a factor which, with others, determines the relatively low phosphatase activity of the old pupae.

Acid phosphatase reaction is very weak in the freshly emerged flies. It increases slightly in the 24-hour old adults and is demonstrable in the nervous system, proventriculus, mid- and hind-guts, Malpighian tubes, gonads, salivary glands, muscles and fat bodies.

The following is a brief account of the acid phosphatase activity in various organ systems during metamorphosis:

(a) NERVOUS SYSTEM ---- Both the brain and ventral ganglion give a moderate reaction in the prepupal and early pupal (12-28 hour) periods. They become strongly reactive in 32-70 hour pupae (fig. 33,G). Comparing figure 33 with figure 25, it is evident that the nerve fibre region shows as strong an acid phosphatase reaction as do the nerve cells. Moreover, the outer optic ganglia contain a high content of acid phosphatase, but apparently no alkaline phosphatase. In pupae older than 72 hours and in adult flies, the nerve system as a whole still gives moderate acid phosphatase reaction.

(b)/-

(b) EYES AND ANTENNAE ---- Following their moderate activity in the larvae prior to puparium formation, eye and antennal discs show a similar activity throughout the prepupal period, with perhaps some increase of activity in late prepupae. In young pupae their acid phosphatase activity appears very low (their exposed position might render them more vulnerable to enzyme inactivation). Nevertheless, they are strongly reactive after the 32nd hour after the quiescence of the larva and remain moderately so at the time of emergence. In the case of the compound eye, retinal cells show a very strong nuclear and cytoplasmic acid phosphatase reaction (fig. 33,od; compare with fig. 25).

(c) WINGS AND LEGS ---- Their acid phosphatase reaction is comparable to that of the eyes and antennae, except that they appear negative in pupae older than 72 hours.

(d) HYPODERMIS ---- In prepupae and early pupae, only nuclear activity is demonstrable in the hypodermal cells. Sometimes they give an entirely negative reaction. Difference of activity between the imaginal and larval hypodermal cells (which has been observed in the alkaline phosphatase reaction) was not found for acid phosphatase. Although the enzyme/-

enzyme activity is more easy to demonstrate in 32-68 hour pupae, it is still mostly confined to the nuclei. The hypodermal cells are again weakly reactive or negative in the old pupae. The oenocytes situated beneath the hypodermis show, however, a fairly strong acid phosphatase reaction, both in the nucleus and in the cytoplasm.

(e) MUSCLES ---- The larval muscles give a rather weak acid phosphatase reaction. When they become detached and are undergoing histolysis, the reaction is intensified.

Myocytes usually show only a nuclear reaction in the prepupal and early pupal stages. When they begin to differentiate in 24-hour pupae, acid phosphatase starts to increase. The enzyme reaction of the developing muscles is extremely strong in 32-56 hour pupae. Unlike alkaline phosphatase, the acid enzyme of the imaginal muscles is still moderately active in pupae older than 72 hours. Muscles of the adult flies give a weak acid phosphatase reaction.

(f) DIGESTIVE SYSTEM ---- The larval proventriculus, gastric caeca and mid-gut are all very rich in acid phosphatase. They later form the so called yellow body, which retains a correspondingly high enzyme activity. However, the imaginal mid-gut epithelium which/-

which shows very weak alkaline phosphatase reaction is moderately active in acid phosphatase. The imaginal fore-gut and larval hind-gut give a similar moderate reaction in later prepupal period.

Shortly after the head eversion, the acid Phosphatase activity of the digestive system does not show any appreciable increase, except that of the yellow body. The activity is definitely increased after the 32nd hour, and the fore-gut, proventriculus, crop and mid- and hind-guts are all strongly reactive. These organs still possess a good deal of acid phosphatase in the late pupae (fig. 33) and even in the emerged flies.

The larval salivary glands are highly active during the prepupal period. When they are broken down into fragments soon after pupation and are undergoing histolysis, acid phosphatase activity remains very high. The imaginal salivary glands also give a strong positive reaction (fig. 33, 31).

(g) MALPIGHIAN TUBES ---- Very strong nuclear and cytoplasmic acid phosphatase reactions have been observed in the Malpighian tubes throughout the period of metamorphosis. Their brush border and the contents of the lumen are also reactive, but, as in the alkaline phosphatase reaction, the contents in the anterior branches give a positive reaction in both/-

both the experimental and control sections.

(h) FAT BODIES ---- In prepupae only a nuclear reaction is demonstrable in the fat cells. This is still true for the pupae shortly after the head eversion. Gradually the cytoplasmic activity becomes visible in pupae older than 24 hours. In 32-hour pupae, all larval fat cells show very strong nuclear and moderate cytoplasmic acid phosphatase reactions. The latter appears more intenser around the nucleus. This condition is maintained up to the time of emergence, although the fat cells are found only in the abdomen in the pupae older than 72 hours.

Imaginal fat cells give a moderate nuclear reaction.

(i) TRACHEAE ---- A moderate reaction is the general feature in later prepupal stages when the histolysis of the larval tracheal epithelium and the proliferation of the imaginal cells are in progress. After pupation, the reaction is rather weak in 12-28 hour pupae. It becomes moderate to strong in 32-68 hour pupae (fig. 33,Tr). Prior to emergence, only a weak nuclear reaction is demonstrable.

(j) RING GLAND ---- Differing from alkaline phosphatase, acid phosphatase is moderately active in young/-

young and old prepupae and in pupae aged between 12 and 24 hours. No difference in enzyme activity has been found between the corpora cardiaca and corpus allatum. Acid phosphatase is very active in the ring gland in 32-56 hour pupae. Its activity decreases after the 72nd hour.

(k) LYMPH GLANDS AND PERICARDIAL CELLS ---- Lymph glands show strong acid phosphatase reaction throughout prepupal life. On the other hand, only nuclear acid phosphatase reaction is detectable in the large and small pericardial cells in most prepupal and pupal stages. Some cytoplasmic activity has been observed in the small pericardial cells in 32-56 hour pupae, but it is very weak.

(l) GONADS ---- Ovary and testis give an unbroken record of high acid phosphatase activity throughout metamorphosis. The whole organ always stains black without showing any internal differentiation (fig. 33,0). In the developing testis, it was found that spermatogonia contain less acid phosphatase than the spermatocytes which are, in turn, less reactive than the spermatids and sperm~~t~~.

C./-

C. Ribonucleic acid:

(a) NERVOUS SYSTEM ---- The differential distribution of ribonucleic acid in the prepupae is essentially the same as that found in the larvae. Soon after pupation, the "Bildungsherde" start to organize into optic ganglia, with a concomitant decrease of ribonucleic acid content in these cells. Apart from this, there seems to be very little change during metamorphosis. The solitary giant ganglion cells are still very rich in ribonucleic acid at the time of emergence.

(b) ANTENNAE AND COMPOUND EYES ---- Eye and antennal discs stain deep red with pyronin in the prepupal stages. After the head eversion, these organ rudiments are still very basophilic. Decrease in staining capacity occurs in later pupal life, as the organs become well formed. However, the hypodermis, muscles and Johnston's organs of the antennae retain some ribonucleic acid even in newly emerged flies.

In the compound eyes of pupae older than 60 hours, the localization of ribonucleic acid in the retinal and corneal cells is very characteristic, indicating a close correlation between the acid phosphatase activity and ribonucleic acid content.

(c) WINGS AND LEGS ---- The high ribonucleic acid content of the wing and leg discs shows no perceptible change after their eversion during prepupal life; nor is there any decrease in the early pupal stages. Decrease of basophily is noticeable after the first day after the head eversion. Thus, the wings of 48-hour pupae contain very little ribonucleic acid, except in those cells near the blood lacunae. Both the wings and the legs lose all their ribonucleic acid before emergence.

Trichogenic cells, being themselves rich in ribonucleic acid, can be readily picked out among the other hypodermal cells of the wings and legs which are relatively poor in the acid content. They have been found in 24, 32 and 48 hour pupae. Even in 60-hour pupae, many pairs of trichogenic cells can be seen in close proximity to certain leg bristles.

(d) HYPODERMIS ---- In prepupae and young pupae, larval and imaginal hypodermal cells are both rich in ribonucleic acids (like other imaginal structures), a fact which is quite in contrast to their differential activity in alkaline phosphatase. When all imaginal hypodermis is completed (32-36 hour) a slight decrease of basophily takes place.

Nevertheless/-

Nevertheless, the basophily of the trichogenic cells appears to be increased, which is possibly due to their own capacity to synthesize ribonucleic acid.

(e) MUSCLES ---- The larval muscles contain very little ribonucleic acid. During histolysis, the sarcolytes often take some pyronin, both in the control and ribonuclease treated sections.

In the prepupal and early pupal periods, myocytes have a ribonucleic acid content similar to that of the other imaginal organs. When the imaginal differentiation of these myocytes begins in 24 hour pupae, a concomitant increase of ribonucleic acid has been observed in them. Furthermore, there seems to be a direct correlation between the ribonucleic acid content of a developing muscle and its state of differentiation. For instance, the dorsal thoracic muscles whose development is several hours ahead of the other muscles always stain more heavily with pyronin than the other muscles in the second day after the head eversion. This difference becomes, however, less distinct in the third day when most head and thoracic muscles have gained their maximal ribonucleic acid content.

Most/-

Most ribonucleic acid is localized in the sarcoplasm around the nuclei which is especially clear in the cross sections. The peripheral region of a muscle fibre, where myofibrils are being formed, stains very lightly with pyronin. As differentiation goes further, the myofibril containing portion grows at the expense of the inner sarcoplasm which becomes greatly reduced in its volume and loses most of its ribonucleic acid content at the time of emergence.

A similar increase of ribonucleic acid has been observed during the development of the leg muscles.

During the development of the abdominal muscles, the anisotropic discs of the myofibrils stain far more intensely than the isotropic discs, giving a banded appearance which is also very distinct in alkaline and acid phosphatase preparations. This feature can readily be seen in 60-72 hour pupae.

(f) DIGESTIVE SYSTEM ---- In prepupae the imaginal fore- and mid-guts are as basophilic as the other imaginal structures. The hind-gut, which remains larval, stains lightly with pyronin.

The crop contains an amount of ribonucleic acid similar to that of the fore-gut as it arises from the latter/-

latter. However, it loses most of its acid content in a 48-hour pupa and it becomes practically unstained in a 72-hour pupa.

About twenty-four hours after the head eversion, there begins an accumulation of ribonucleic acid in the mid-gut (including the proventriculus) and salivary glands. Their strong basophilic character is very well seen in 72-hour pupae. There is no increase whatsoever in the fore- and hind-guts, possibly even some decrease in late pupal life. High ribonucleic acid content in the salivary glands and mid-gut persists in the adult flies.

The yellow body is composed mainly of larval gut cells; it stains at first pink in the cytoplasmic areas and green in the nuclei. In pupae older than 20 hours, the degenerating nuclei stain, besides green, various shades of blue, violet and reddish violet in both control and ribonuclease treated sections. As in the nuclear pycnosis of the nurse Cells during oocyte development, this suggests the depolymerization of desoxyribonucleic acid in cell degeneration. In 80 hour pupae, the yellow body no longer takes any stain, either pyronin or methyl green.

(g)/-

(g) MALPIGHIAN TUBES ---- The ribonucleic acid content is relatively low and shows no changes throughout metamorphosis. The lumen of the swollen anterior branches appears empty in pyronin-methyl green slides.

(h) FAT BODIES ---- It has been mentioned before that at the time of puparium formation, globules of unknown nature appear in the fat cells. The accumulation of such globules continues in the prepupal stages and advances in the antero-posterior direction until all the fat cells have been so transformed (a few hours after the head eversion). Although these globules have a special affinity towards pyronin, it has nothing to do with ribonucleic acid and probably indicates the presence of phospholipides. These globules gradually disappear in the course of histolysis, but few of them are still present in the abdominal fat cells at the time of emergence.

Comparable to the mid-gut, the imaginal fat bodies are very rich in ribonucleic acid.

(i) TRACHEAE ---- The imaginal tracheal epithelium is very rich in ribonucleic acid. This strong cytoplasmic basophily maintains for the first day after the head eversion, but it decreases steadily afterwards.

(j) RING GLAND ---- In prepupae and early pupae the corpora cardiaca contain more cytoplasmic ribonucleic acid than the corpus allatum. In pupae older than twenty hours, the difference is no longer very distinct, due probably to the increase of ribonucleic acid in the corpus allatum. Secretion vacuoles have been found in the cells of corpora cardiaca in prepupal and most pupal stages, but not in the corpus allatum.

(k) HEART AND RELATED STRUCTURES ---- The ribonucleic acid content of the heart is negligible. The lymph glands are rich in the acid owing to their proliferative function in producing a large number of leucocytes (phagocytes) engaging in histolysis. The intracellular distribution of ribonucleic acid in the two groups of pericardial cells does not show any change throughout metamorphosis. Like those in the larvae, the acid ~~are~~ is concentrated in one or two blocks around the nucleus.

(l) GONADS ---- The basophily of the larval and prepupal ovaries is similar to that of the other imaginal organs. In the first two days after the head eversion, the condition remains unchanged. By the third day, the differentiation of the egg follicles begins and a sudden increase of ribonucleic acid

has/-

has been noticed in those cells which are destined to become the oocytes and nurse cells. The pyronin-stained preparation at this stage is comparable to the figure 34 of Strasburger's (1935) paper. At the time of emergence, stages 2 follicles can be found in the pupal ovary. It is in these more advanced follicles that a similar increase of ribonucleic acid has been observed in the follicular cells.

The development of the testis proceeds at a much quicker pace than does that of the ovary. Spermatogenesis occurs early in prepupal life and fully matured sperm can be found in the young pupae (about 30 hour old). It has been noticed that only the young spermatogonium cells located at the anterior end of the testis retain their high ribonucleic acid content. The spermatocytes, spermatids and mature sperms lose their ribonucleic acid in successive degrees until the mature sperm are practically colourless to pyronin. Since ribonucleic acid is responsible for the cytoplasmic basophily, the pyronin-methyl green stained testis sections resemble in every respect the pictures given by Strasburger (1935, figs. 32a,33). A similar phenomenon of the progressive decrease of basophily during spermatogenesis has been reported in amphibians (Brachet, 1942).

D./-

D. Summary:

The distribution of alkaline and acid phosphatases and ribonucleic acid during Drosophila metamorphosis has been described. So far as these three cytochemical reactions are concerned, the prepupa bears a general resemblance to the larva rather than to the pupa. This fact therefore supports the conclusion derived from purely morphological evidence that the prepupa is actually an intrapuparial larval instar (Snodgrass, 1924; Robertson, 1936).

Pupation is accompanied by a general and sudden increase of alkaline phosphatase, notably in the cytoplasm. This high alkaline phosphatase activity is maintained almost for the first day and a half after the head eversion. Then it declines, first in the head and thorax, but much later in the abdomen. Weak to moderate enzyme activity is present in the nervous system, mid-gut, salivary glands and Malpighian tubes of the adult flies.

Analysis of the change of alkaline phosphatase activity in various organs during metamorphosis reveals again the importance of this enzyme to histo-differentiation. This is especially true during the development of imaginal organs such as eyes, antennae, wings, legs, muscles, digestive organs/-

organs, hypodermis and tracheae. In the case of gonads, the relation is not so clear, for spermatogenesis takes place in the prepupal and early pupal periods when the alkaline phosphatase reaction is very weak and oogenesis proceeds mainly after emergence when the enzyme activity is incapable of demonstration by the histochemical method.

Acid phosphatase does not show any increase after pupation. On the contrary, there is probably a slight decrease. However, the enzyme is very active in 32-36 hour pupae. Since during this period histogenesis is still going on, it seems that acid phosphatase may, as well, has something to do with differentiation. Although acid phosphatase activity shows a decrease in the later pupal period similar to that of alkaline phosphatase, the extent of this decrease is much lesser for acid phosphatase, especially among the head and thoracic organs.

A strong acid phosphatase reaction seems to be a characteristic of Drosophila gonads. The maintenance of this high enzyme activity throughout post-embryonic development explains the particular richness of this enzyme in the mature germ cells.

Both acid and alkaline phosphatase participate in the processes of histolysis, for the histolysing fragments always give strong phosphatase reaction.

The/-

The imaginal organ rudiments in the prepupal and early pupal stages are characterized by their high ribonucleic acid content. As histo-differentiation goes on, there is generally a decrease of acid content. In certain particular organs, an accumulation of ribonucleic acid, however, occurs in the course of differentiation. In the developing muscles, this phenomenon is evidently connected with the formation of myofibrils. In the case of salivary glands, mid-gut and ovary, this is possibly related to the normal physiological function of these organs and may well be the result of histo-differentiation. For these latter organs and for the muscles, the increase of cytoplasmic basophily is always associated with an increase of acid phosphatase activity. Nevertheless, it should be pointed out that high acid phosphatase activity does not necessitate the presence of ribonucleic acid. This is particularly true for the nerve fibres and the advanced stages in spermatogenesis.

IV. GENERAL DISCUSSION

(1)

The importance of fixed -SH groups and ribonucleic acid in the determination of the embryo axis in vertebrate development has been particularly emphasized by Brachet (1947). From the results of the present study, there is no reason to suppose that such substances also play a prominent role in Drosophila embryogenesis. Some concentration of fixed -SH groups and a differential distribution of ribonucleic acid have been found in the ectoderm of Drosophila embryos. However, owing to the general occurrence of these properties, it is difficult to attach any morphogenetic implications to them. Raven (1946) found a similar situation in Limnaea.

Histochemical evidence indicates that there is very little decrease of the basophily of the embryo cells during most of embryonic development (from 0 to about 15th hour). This maintenance of a relatively high content of ribonucleic acid must be due to some simultaneous synthesis of the acid, for otherwise one would expect a gradual decrease of basophily parallel to the increase in cell number and the scale of organization of the embryo as in the case of the sea-urchin (Brachet, 1947). In fact, an increase of ribonucleic acid through synthetic activity in organs like/-

like gut epithelia and salivary glands is beyond doubt. However, since the technique used is not sensitive enough to detect a small change of ribonucleic acid content, the possibility of the conversion of ribonucleic acid into desoxyribonucleic acid in the early embryonic period is not excluded by the present observation. Therefore, the resemblance between the nuclein metabolism of the Drosophila embryo and that of the chick embryo, as seems to be the case, must await direct confirmation.

The very fact that mitosis is of rare occurrence after the contraction of the germ band and the lack of evidence for endomitotic growth during the later half of embryonic development leads me to suggest that the stage of "germ band contraction" marks the transition from a growth phase of embryonic development into a differentiation phase. The term differentiation used here refers more to histo-differentiation rather than to the invisible or chemo-differentiation. The latter probably occurs well before that stage, as indicated by Geigy's (1931a,b) works.

(2)/-

(2)

During Drosophila embryogenesis, acid phosphatase shows no change of activity, at least cytochemically; whereas alkaline phosphatase arises only after the contraction of the germ band, increases in activity and then decreases again. The demonstration of the existence of these two different patterns of enzyme activity is itself quite suggestive. Perhaps the contraction of the germ band represents a very important morphogenetic stage and further investigation could reveal a good deal of biochemical differences between embryos before and after that stage. Quite recently, some preliminary data concerning the phosphatase activity in the early development of Arbacia has become available (Mazia et al, 1948). They found that in the unfertilized eggs, acid phosphatase predominates and its activity remains constant during early development. On the other hand, alkaline phosphatase keeps constant until before gastrulation, but increases very sharply after that. Obviously, the general pattern of phosphatase activity in the embryonic development of Drosophila and of Arbacia is very much the same. In chick embryogenesis, the situation is slightly different because the concentration of alkaline phosphatase is much greater/-

greater than that of acid phosphatase from the very beginning (Moog, 1944, 1946). Nevertheless, the trend of the change of acid phosphatase activity between 2 and 12 days old chick embryos is far less marked than that of alkaline phosphatase in the same period (Moog, 1946). From the above facts, it seems quite safe to say that acid and alkaline phosphatases are two different enzymes and play different physiological roles, even though they may be present simultaneously within the same cell.

The factors which determine the origin (or activation?) of alkaline phosphatase in the ventral ectoderm after the contraction of the germ band as well as the differential rates of spreading after its first appearance are not clear. The fact that the spreading of enzyme activity is a gradual process seems to suggest that the primary process involved in such spreading is actually a direct contact between the active and inactive cells. If this is true, the observed extero-interior gradient of alkaline phosphatase reaction can, probably, be partly accounted for. Thus, the closer contact between ectodermal cells themselves than between an ectodermal cell and for example a nerve cell would explain a quicker spreading within the surface ectoderm than spreading from the surface to the interior parts of/-

of an embryo. In this connection, it is interesting to mention that the salivary glands which are still directly connected with the ventro-lateral ectoderm (after the germ band contraction stage) show a strong alkaline phosphatase reaction earlier than other internal organs do.

That the contact is not the sole factor is indicated by the presence of an antero-posterior gradient which is also observable within the surface ectoderm itself. Evidently, there must exist within the embryo some physiological differences between the anterior and posterior parts and possibly such difference controls the differential rate of spreading along the antero-posterior axis. The presence of such a hypothetical physiological gradient is also shown by Geigy's (1931b) irradiation experiments, in which he discovered that the sensitization and de-sensitization of ventral ectoderm towards ultraviolet light exhibit a quite distinct "thoraco-abdominal" gradation.

Since Seidel's classical work on Platycnemis, it is generally admitted in experimental embryology that the organized development of insects involves the operation of two centres of organization, namely the activation centre and the differentiation centre. The presence of these centres in Drosophila has not hitherto been demonstrated, although Geigy's work just/-

just referred to has already hinted at their existence. Whether or not the centre of the origin of alkaline phosphatase in the ventral ectoderm near the future thorax represents actually the differentiation centre in Drosophila can not yet be fully ascertained; but that it is so appears very likely.

(3)

The interesting correlation between the phosphatase activity and the growth and differentiation is first demonstrated in chick embryogenesis by Moog (1944). Since then similar evidence has been briefly presented in the early development of amphibia (Brachet, 1946) and sea-urchin (Mazia et al, 1948). The present observation on Drosophila supports and strengthens this general correlation. However, the different behaviour of alkaline and acid phosphatases merits their separate discussion.

During embryogenesis alkaline phosphatase appears immediately after the contraction of the germ band and disappears in most tissues before the hatching of the larva. This fact tends to indicate that the enzyme is primarily concerned with histodifferentiation. In post-embryonic development, the/-

the alkaline phosphatase activity of the imaginal discs illustrates the same principle. In the larval and prepupal periods, their moderate enzyme activity is obviously linked to their proliferative growth. Then the activity suddenly increases at the time of pupation and maintains itself at this high level for the next day and a half. After that, alkaline phosphatase decreases in most organs, but remains at a high level in certain others. Considering now the developmental status of the imaginal organs at the time of pupation, the results of transplantation work on the organ discs (Bodenstein, 1943; Vogt, 1943) certainly indicates that they have already passed their stage of invisible or chemo-differentiation. Consequently, the increased alkaline phosphatase activity must be concerned with histo-differentiation. In this connection, the observed fact that the increased fraction is mainly cytoplasmic phosphatase is important, since differentiation is primarily a cytoplasmic process.

The similar behaviour of alkaline phosphatase during embryogenesis and metamorphosis not only suggests the basic identity between the processes underlying the realization of the larval and imaginal/-

imaginal developmental patterns, but also lends support to the general belief that the larval period is a period of growth and that of metamorphosis a period of differentiation (see Bodenstein, 1942).

On the other hand, the relation of acid phosphatase to histo-differentiation is not so obvious in view of its constant activity during embryogenesis and its low activity during the first day after pupation. Nevertheless, since acid phosphatase activity increases in the mid-pupal period (32-68 hour pupae, during which time histogenic differentiation of the imaginal organs is not yet completed), the enzyme may also serve a similar function. Apart from this, acid phosphatase activity in Drosophila development shows a rather general correlation to the ribonucleic acid metabolism. This is chiefly indicated by the following facts:

(a) high enzyme activity in the nurse cells and oocytes during the synthesis of ribonucleic acid and yolk during oogenesis;

(b) a constant enzyme activity during embryonic development and the maintenance of a relatively high ribonucleic acid content in the embryo cells;

(c)/-

(c) a moderate activity in all imaginal discs throughout the whole larval and prepupal periods and (d) the accumulation of ribonucleic acid during the development of the imaginal muscles, salivary glands, mid-gut and ovary being paralleled by a similar increase of acid phosphatase activity.

Furthermore, there seems to be some complementarity between the activity of the two kinds of phosphatases. At the cellular level, this is shown by the relative distribution of the two enzymes in various larval and imaginal organs (page 43). For the body as a whole, it is evident in the decrease of alkaline phosphatase but the maintenance of acid phosphatase in late 3rd instar larvae and early prepupae; and by the high alkaline phosphatase activity in early pupae and a weak one in late pupae, with a reverse situation for acid phosphatase activity.

(4)

The relation between the phosphatase activity and cellular degeneration has so far not been fully recognized. The nearest example in the existing literature (to my knowledge) is perhaps the facts discovered by Bodian and Mellors (1944) in nerve degeneration/-

degeneration. Even then, the increased acid phosphatase activity is attributed to the resynthesis of Nissl bodies rather than to their destruction (Bodian, 1947). In the present study it was found that the increase of phosphatase activity is almost concomitant with the actual progress of histolysis. This happens in every larval organ that is going to be destroyed, but it is especially clear in the case of fat bodies, muscles, hypodermis and tracheae.

As histolysis of fat cells sets in after pupation, the increase of cytoplasmic alkaline phosphatase is so sudden and so marked that one is inclined to think that the increased portion is due to the activation of the pre-existing enzyme molecules. Whether this represents the general mechanism by which the increase of alkaline phosphatase after pupation can be accounted for, and whether there occurs at the same time some "de novo" synthesis of the enzyme or not, are questions very hard to answer at the present stage.

(5)

Another aspect of phosphatase activity in Drosophila development which I would like to discuss is/-

is their normal physiological function. Thus, the concentration of alkaline phosphatase in the striated border of the mid-gut and in the brush border of the Malpighian tubes is of some comparative interest, since analogous localization in the mammalian intestinal epithelium and kidney tubules is well known. Whether or not this means that a similar transport function is played by this enzyme in Drosophila is difficult to say in view of our limited knowledge about insect digestion and excretion. Nevertheless the similar enzyme localization does indicate such a possibility.

One component of the insect cuticle is a nitrogenous polysaccharide called chitin. As a corollary to the current conception of biosynthesis (Lipmann, 1941), the synthesis and breakdown of chitin may well involve phosphorylative processes as in the case of glycogen. The strong alkaline phosphatase reaction of the tracheal epithelium and its cuticular intima is therefore what one would expect in considering the complementary function of phosphatase in the complex phosphorylative processes. Similarly, the high alkaline phosphatase activity of the hypodermis during the formation of the puparium in the larvae and that of exoskeleton in the pupae can be interpreted on the same basis.

The high alkaline phosphatase activity of the pericardial cells during prepupal and early pupal periods and the persistence of these cells throughout metamorphosis suggest that they are probably the incretory organs. This contention is supported by the following facts:

(a) they show definite cycles of change of cell size in the course of metamorphosis;

(b) secretion vacuoles have been observed in them in the larvae prior to puparium formation, and in prepupae and pupae

(c) a distinct sexual dimorphism has been found in the large pericardial cells, both during metamorphosis and in the adult flies. In this connection, it is interesting to note that Bodenstein (1943) (in the course of his extensive study of the physiology of the <u>Drosophila</u> ring gland) came to the conclusion that ring gland hormone does not act directly but, rather, indirectly through the intervention of some factors in the host. Therefore, it occurs to me that the pericardial cells are the most likely organs which cause such interaction complication. In fact, an interaction among different endocrine glands in Orthoptera has been reported (Pflugfelder, 1939). However, it is not possible/-

possible at present to compare these pericardial cells in *Drosophila* with the pericardial gland in *Dixippus* (Pflugfelder, 1938). It can only be stated that the pericardial cells are similar to the pericardial gland in showing sexual dimorphism, but differ from the latter by their persistence in the adult flies.

From the transplantation work of Hadorn (1937), Nyst (1941), Bodenstein (1943,1944) and Vogt (1942b,1943), it is certain that the ring gland in *Drosophila* produces both moulting and metamorphosis hormones. The individual roles played respectively by the corpora cardiaca and corpus allatum are, however, not clear. Based upon morphological (1942b) and experimental (1943) evidences, Vogt is of the opinion that the corpora cardiaca ("grosse Ringdrussenzelle" in her paper) is more concerned with metamorphosis than the corpus allatum. The data presented in this paper seem to support her conclusion, if the higher alkaline phosphatase activity and ribonucleic acid content of the corpora cardiaca in the larval and prepupal periods can be considered as indices of a higher level of physiological function. On the other hand, the present data also indicate that the corpus allatum becomes more active

six/-

six hours after the head eversion, if the above assumption is correct. However, the presence of acid phosphatase activity in the ring gland makes the situation even more complicated. The enzyme is moderately active during the larval, prepupal and early pupal periods, but strongly active in mid-pupal stage. But differing from the alkaline phosphatase reaction, no differential activity between the corpora cardiac and corpus allatum has been observed. Obviously, further study is needed before these facts can be correlated with the physiological activity of the ring gland.

V. GENERAL SUMMARY

(1) The Drosophila egg does not show any regional localization of the chemical substances so far studied: a definite, but general, cortical concentration of fixed -SH groups has been observed; ribonucleic acid is diffusely distributed; alkaline phosphatase is histochemically not demonstrable; but acid phosphatase is very active.

(2) Mature Drosophila sperm is also rich in fixed -SH groups and acid phosphatase, but contains no ribonucleic acid and alkaline phosphatase.

(3) Fixed -SH groups and ribonucleic acid do not seem to play an important morphogenetic role during Drosophila embryogenesis.

(4) Cytochemical evidence suggests that the nuclein metabolism of Drosophila is probably similar to that of chick embryo, in the sense that both desoxyribo- and ribo-nucleic acids are synthesized during early embryonic development.

(5) It is suggested that the stage of the contraction of the germ band marks the division between the growth phase of Drosophila embryonic development and its differentiation phase.

(6) Acid phosphatase shows constant activity during embryogenesis; whereas alkaline phosphatase appears only after the contraction of the germ band,

increases its activity and then decreases in most tissues before hatching.

(7) The centre of the origin of alkaline phosphatase in the ventral ectoderm near the future thorax is considered as the "differentiation centre" of the Drosophila embryo. The enzyme activity spreads from this centre to all parts of the embryo in characteristic patterns. The possible mechanism of spreading is discussed.

(8) Nuclear alkaline and acid phosphatase is demonstrable in all larval tissue of Drosophila. Cytoplasmic phosphatases, however, show a strict organ specificity.

(9) All imaginal discs possess a moderate amount of alkaline and acid phosphatases and they are very rich in ribonucleic acid.

(10) Larvae prior to puparium formation are characterized by an enormous increase of alkaline phosphatase in the hypodermis and simultaneous decrease of it in the internal organs. No such change has been found in the case of acid phosphatase.

(11) The result of cytochemical study confirms the contention that the prepupa is actually an intrapuparial larval instar.

(12) Pupation is accompanied by a general and sudden increase of alkaline phosphatase activity which is mainly confined to the cytoplasm. This high enzyme activity is maintained for the first day and a half after the head eversion. It declines afterwards until finally it disappears from most organs at the time of emergence, but is retained in certain others.

(13) The behaviour of alkaline phosphatase during metamorphosis is exactly similar to its behaviour during embryogenesis. In both cases, the possible bearing of alkaline phosphatase on histo-differentiation is suggested.

(14) Acid phosphatase does not show any increased activity shortly after pupation, but its activity increases in the mid pupal period (32-68 hour pupae). In the later pupal period, a decline of enzyme activity has also been found, although to a much lesser degree as alkaline phosphatase activity does.

(15) A general correlation between acid phosphatase activity and ribonucleic acid metabolism has been found. Otherwise, the function of acid phosphatase in *Drosophila* development is less readily explicable.

(16) Alkaline and acid phosphatases actively participates in the processes of histolysis or cellular degeneration.

(17) Histochemical evidence suggests that alkaline Phosphatase in Drosophila is probably playing a part in the carriage of organic substances across the membrane barrier.

(18) Alkaline phosphatase activity of the pericardial cells, together with other morphological evidences, indicates that these cells are endocrine organs and play important role in Drosophila metamorphosis.

VI. ACKNOWLEDGEMENT

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VIII. ABBREVIATIONS

ad,	abdominal disc
An,	antennal disc
c,	cuticle
Ca,	corpus allatum
cc,	corpora cardiaca
Dv,	heart or dorsal vessel
f,	larval fat body
fc,	histolysing fat cell
Fr,	Frontal sac
G,	cerebral ganglion or brain
gc,	gastric caecum
h,	larval hypodermis
H,	imaginal hypodermis
Ha,	imaginal bud of haltere
hg,	larval hind gut
L,	section of leg
L1,	imaginal disc of prothoracic leg
L2,	imaginal disc of mesothoracic leg
L3,	imaginal disc of metathoracic leg
lg,	lymph gland
Lp,	large pericardial cell
lu,	leucocyte or phagocyte
m,	larval muscle
M,	Imaginal muscle
Ma,	Malpighian tube

Mc,/-

Mc,	mesenchyme cell
mg,	larval mid gut
Mg,	imaginal mid gut
My,	myocyte
O,	ovary
Od,	optic disc or compound eye
Oes,	oesophagus
Ov,	oviduct
P,	proventriculus
Ph,	pharynx
pm,	puparium
Rg,	ring gland
Rp,	rectal papillae
S,	mature sperm
sc,	sarcolyte
sl.,,	larval salivary gland or its histolysing fragment
Sl,	imaginal salivary gland
Sp,	small pericardial cell
Te,	testis
ti,	chitinous intima of trachea
tr,	larval trachea
Tr,	imaginal trachea
Ve,	vas efferens
Vg,	ventral ganglion or ventral nerve cord
W,	wing or its imaginal disc
y,	yellow body

IX. FIGURE EXPLANATION

1. Camera lucida sketches of the different stages of the oocyte development of Drosophila melanogaster, from iron hematoxylin preparations. f. e. c., follicular epithelial cell; n. c., nurse cell; oc., oocyte. Descriptions in the text.

   2, 6-9, 14-18, 20-32 ---- alkaline phosphatase

   3-5, 10-13, 19, 33 ------ acid phosphatase

2. Section of ovary taken from a 24-hour female. Note the positive reaction of the oviduct. X 70.

3. Section of ovary showing the intense nuclear and cytoplasmic acid phosphatase reaction of the follicular cells, nurse cells and oocytes. X 95.

4. Acid phosphatase reaction of the fresh ovarian follicles incubated for two hours in lead-glycerophosphate reagent. X 82.

5. Same as figure 4, but incubated in the presence of 0.01 M fluoride. X 82.

6. An oblique sagittal section of a 9 hour embryo. The arrow head indicates approximately the centre of the origin of alkaline phosphatase activity. X 120.

7. Sagittal section of a 10-10½ hour embryo showing the spreading of enzyme activity towards the interior and posterior parts of the embryo. X 120.
8. Longitudinal section of a 14 hour embryo. X 120.
9. Sagittal section of an embryo just prior to hatching. Note the positive reaction of the salivary gland and gut epithelia. X 120.
10. Longitudinal section of an early embryo in single blastoderm stage. X 120.
11. Sagittal section of a 4½ hour embryo in advanced stage of gastrulation. X 120.
12. Sagittal section of a 12½ hour embryo. X 120;
13. Sagittal section of a 17½ hour embryo. X 120.
14. Portion of the sections of the mid-gut and Malpighian tube taken from a 24-hour larva. X 340.
15. Cross sections of the head region of a 32-hour larva: X 130.
    (a) Taken at the level of the frontal sac.
    (b) Taken at the level of the ring gland.
    (c) Taken at the level of the small pericardial cells.
16. Longitudinal section of the posterior part of a 24-hour larva (just after the first moulting) showing the high enzyme activity of both the new/-

new and the shed tracheae. The arrow indicates the point of puncture during fixation. X 120.

17. Thoracic muscle fibres taken from a 32-hour larva showing the concentration of enzyme in the anisotropic discs of the myofibrils. X 340.

18. Cross section through the head region of a larva prior to puparium formation showing the contrast between the strong phosphatase reaction of the hypodermis and salivary glands and the negative reaction of the imaginal discs. X 95.

19. Lateral sagittal section of a 72-hour larva showing the high acid phosphatase activity of the salivary gland, proventriculus, mid-gut, ring gland and eye and antenna discs. The arrow indicates the point of puncture during fixation. The high enzyme activity of testes can be seen in another larva of the same age but in longitudinal section. X 30.

20. Cross section of a 2-hour prepupa at a corresponding body level as that of the figure 18. X 95.

21. Sagittal section of an old prepupa just before the head eversion. The head region is slightly everted through manipulation during fixation. X 32.

22. Longitudinal section of a 12-hour pupa. X 40.

23. Cross sections at the level of the wings and legs showing the general increase of alkaline phosphatase after pupation: X 70.

    (a) 10-hour prepupa (b) 15-hour pupa

24. Sagittal section of a 32-hour pupa. X 40.

25. Longitudinal section of a 72-hour pupa. The actual intensity of the phosphatase reaction of the nerve cells and proventriculus is much weaker than that can be judged from the photograph. X 40.

26. Portion of the abdominal hypodermis taken from a pupa just after the head eversion showing the relative phosphatase activity of the larval and imaginal hypodermal cells. X 340.

27. Alkaline phosphatase reaction of fat body: X 340.

    (a) a group of fat cells (in sheet structure) taken from a 10-hour prepupa representing the characteric reaction of the larval and prepupal fat body.

    (b) a group of detached individual fat cells taken from a 12½ hour pupa showing the tremendous increase of cytoplasmic phosphatase accompanying histolysis.

28. Section of the ring gland of a 7-hour prepupa showing the difference in phosphatase activity between the corpora cardiaca and the corpus allatum. This condition is entirely comparable to that found in the 2nd instar larva (fig. 15b). X 340.

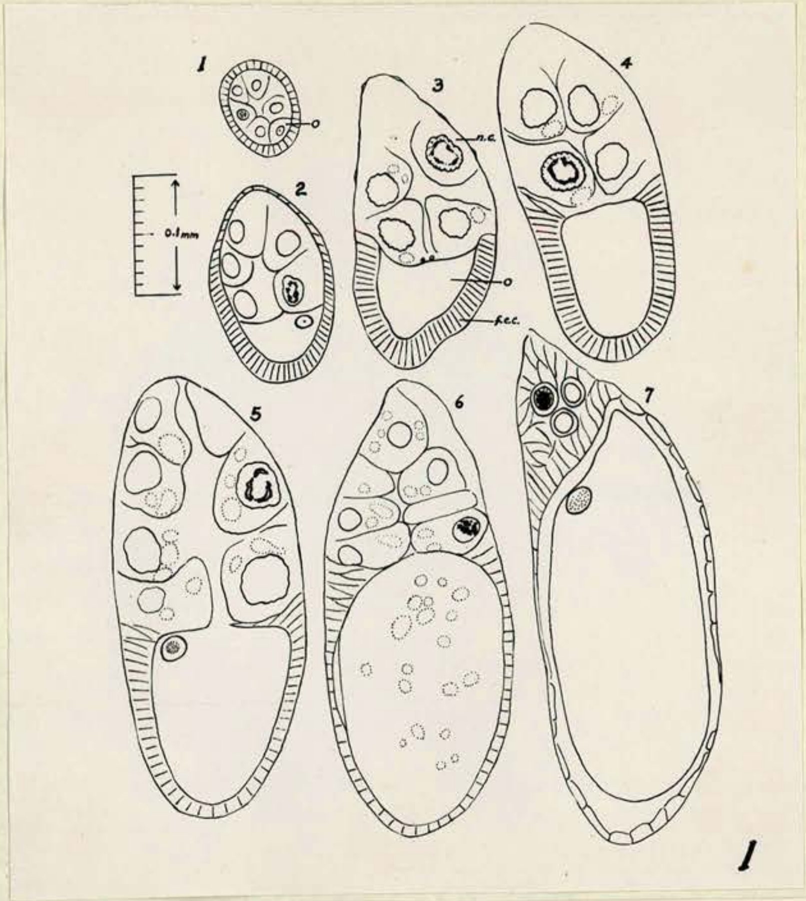
29. Section through the large pericardial cells, heart and lymph glands of a pupa just after the head eversion. X 100.

30. Portion of a testis from a 32-hour male pupa. Note the strong enzyme reaction of the sperm heads and the nuclei of the cells of vas efferens. X 280.

31. Section through the ovaries and oviduct of a 48-hour pupa. X 100.

32. Section through the ovary of a female pupa shortly before the emergence. The enzyme is mostly localized in the nuclei of the follicular cells and of the undifferentiated nurse cells and oocytes. X 280.

33. Acid phosphatase reaction of a 68-hour pupa in longitudinal section. Note the positive reaction of the nerve fibre region, compound eyes and tracheae as compared with those structures shown in the figure 25. X 30.



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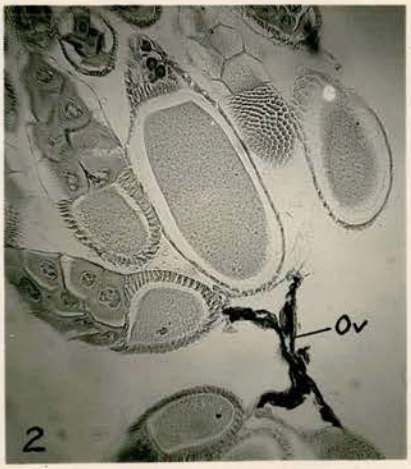

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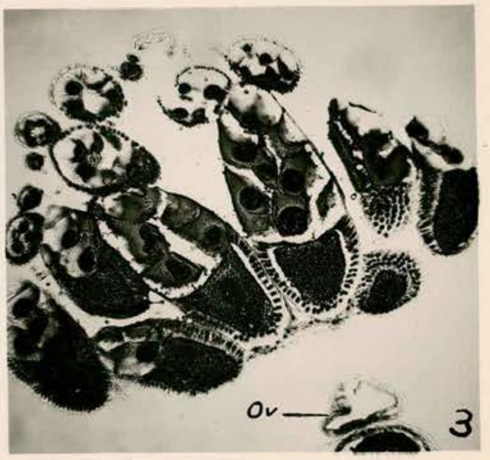

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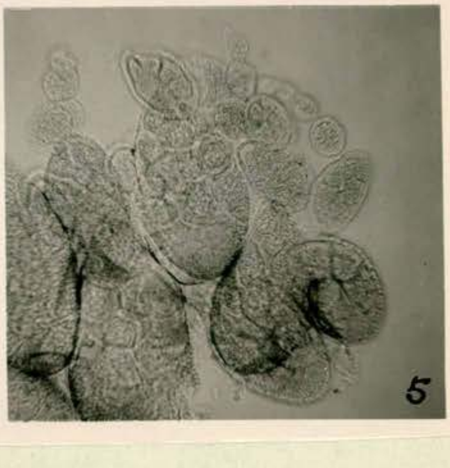

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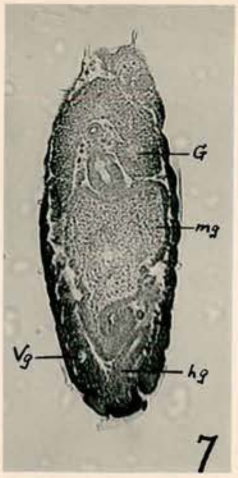

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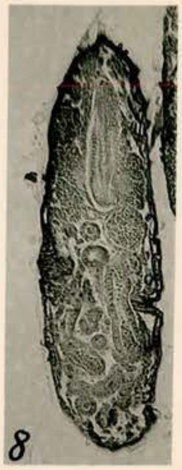

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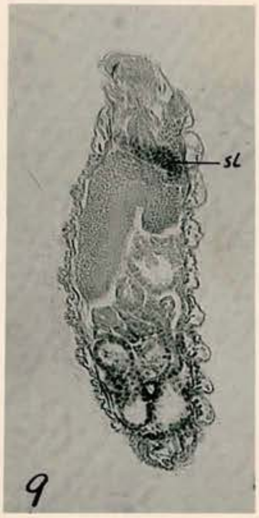

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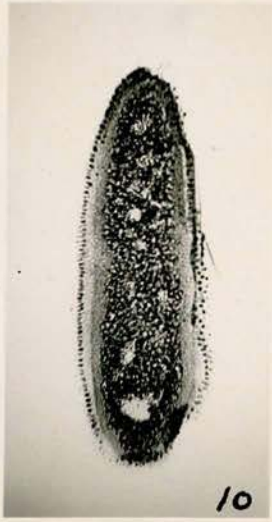

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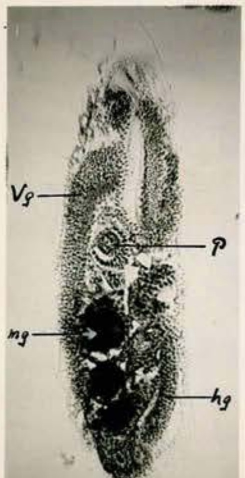

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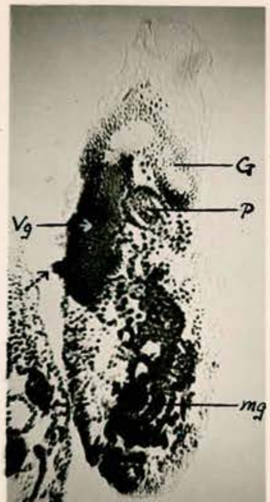

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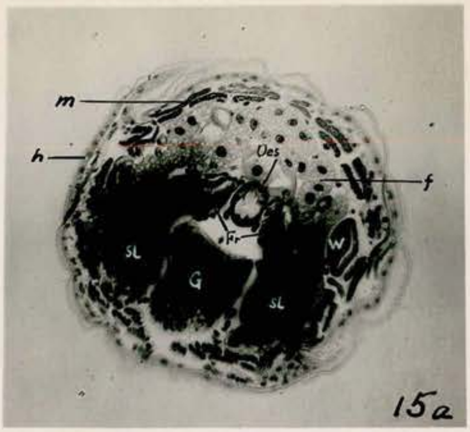

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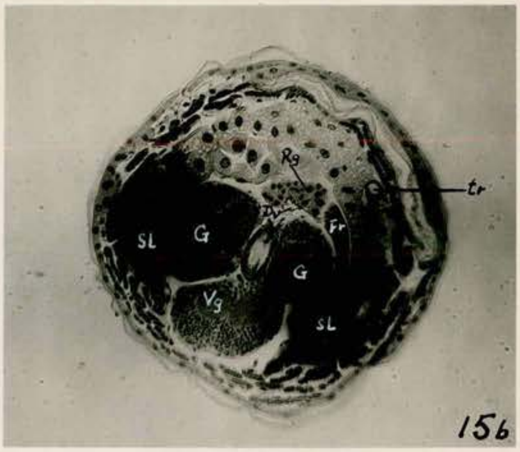

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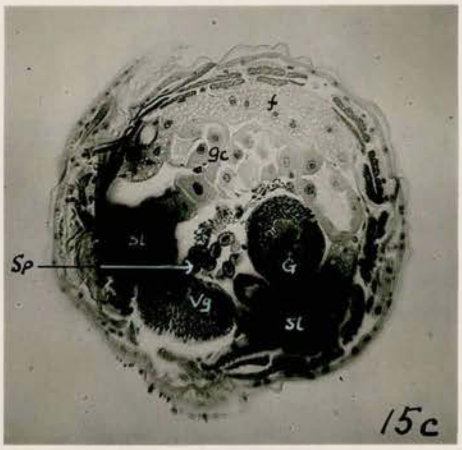

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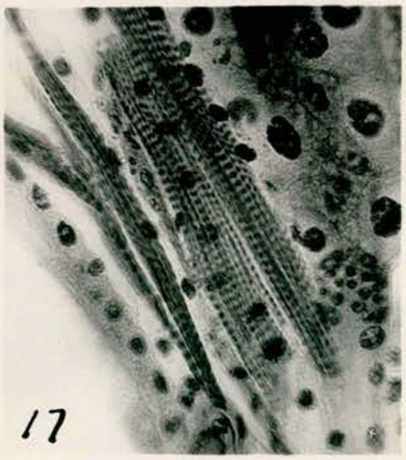

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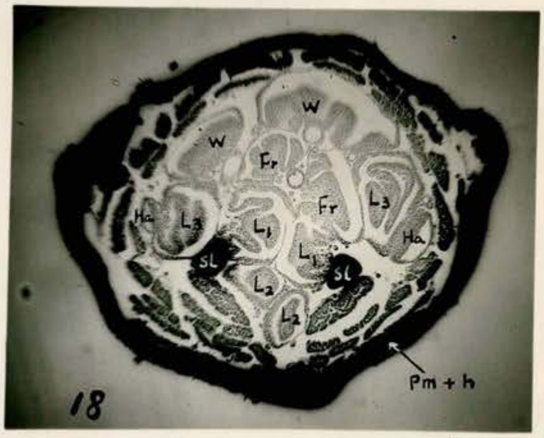

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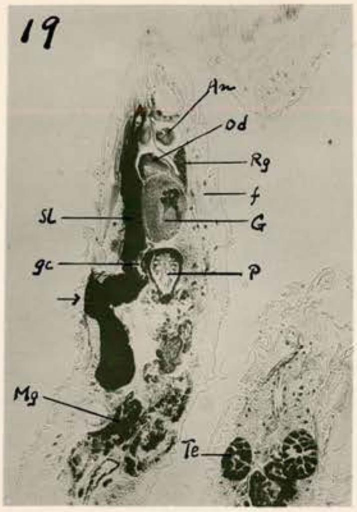
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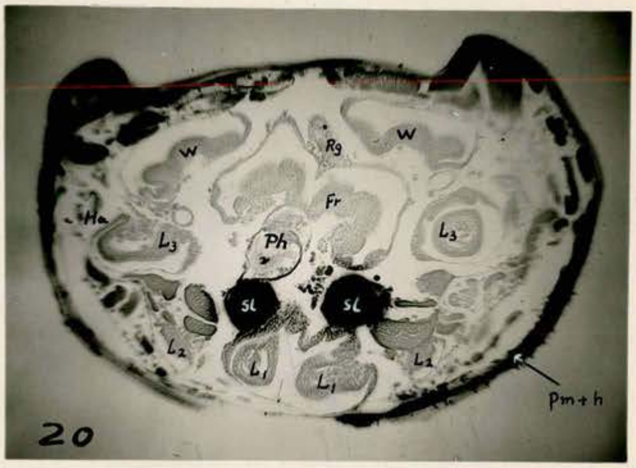
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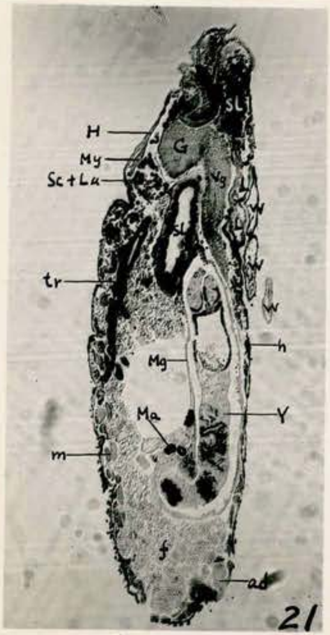
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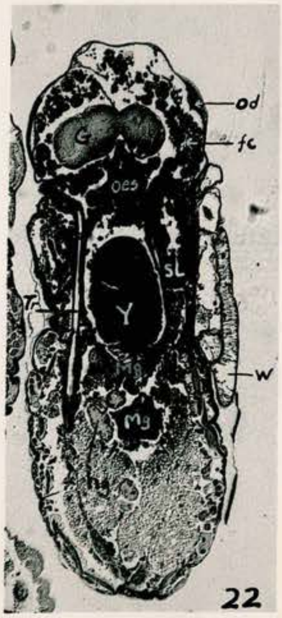
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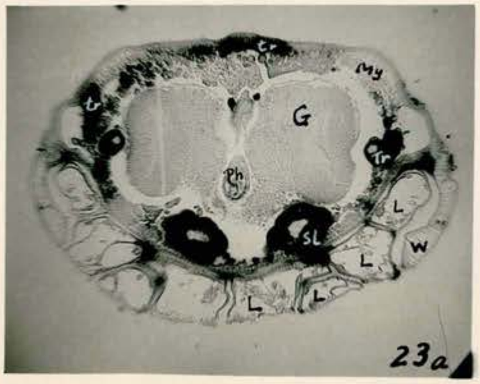
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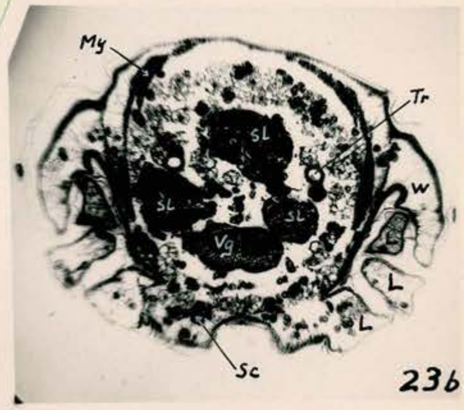
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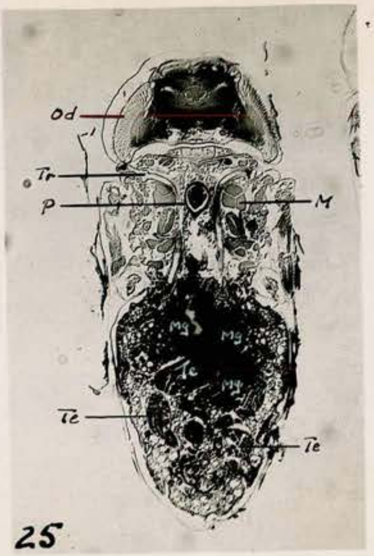

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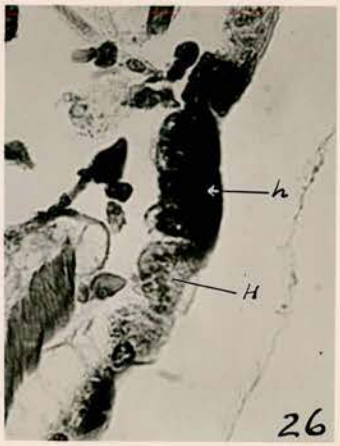

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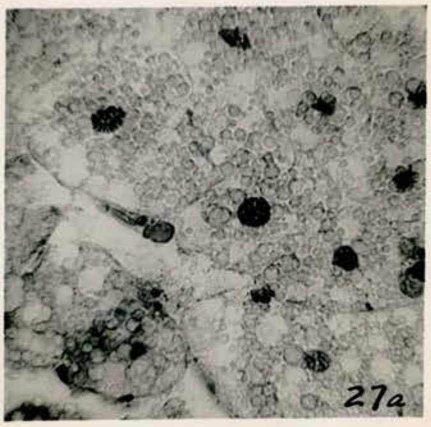

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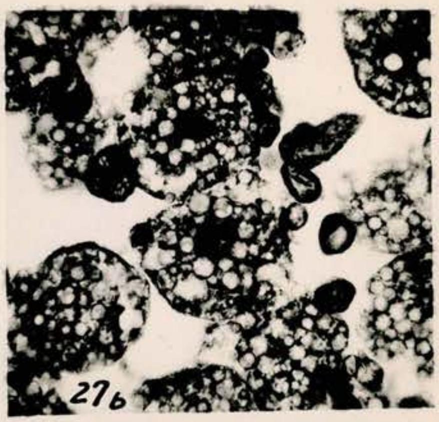

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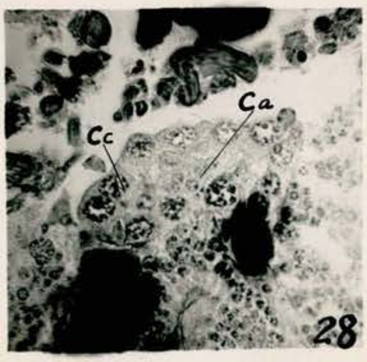

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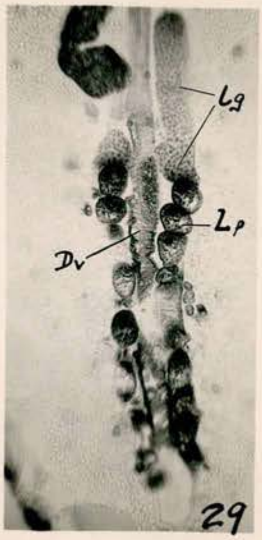

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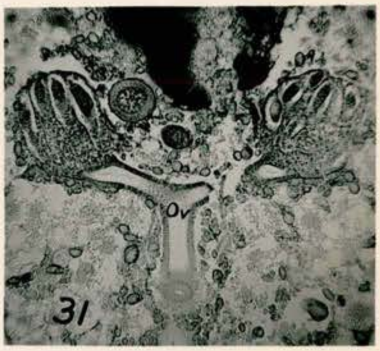
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