THE USE OF ULTRA-VIOLET MICRO-BEAM IRRADIATION IN A STUDY OF THE EMBRYONIC DEVELOPMENT OF DROSOPHILA MELANOGASTER

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by

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INTRODUCTION

A synthesis of evidence gained through experimental embryology and genetics has long been considered essential to an understanding of the relationship between gene action and the growth and development of form in an organism. The techniques employed in the former field of study, i.e. various forms of experimental interference with normal development, have unfortunately proved unsatisfactory as a means of elucidating this relationship. Certain objections on embryological grounds alone cast some doubt on the reliability of interference methods as a means of deducing normal developmental sequences (Waddington, 1942). However, even by overlooking such criticisms one easily sees the dubious nature of any further speculation attempting to correlate experimentally caused alterations with those of a genetic basis. The complexity of cell structure prohibits any confidence in the thought that external environmentally caused phenocopies might specifically resemble genetically caused characters in their biochemical basis, which, after all, is the ultimate aim of workers in both fields.

Instead, the most fruitful results have thus far been obtained in the study of the action of certain lethal genetic factors on critical phases of early embryonic development. In Drosophila numerous workers have analyzed different lethal mutants and have established accurately the critical developmental phases and the associated morphological characteristics if a pattern of damage occurs (Reviews: Hadorn, 1951; Imaizumi, 1958a). When the lethal action of the gene is expressed after gastrulation the complexity of the interacting components prohibits any more than a general descriptive explanation or understanding of the phenomena involved. Early embryonic and oogenetic

defects, on the other hand, render themselves accessible to further analysis by utilizing recently developed techniques.

Early embryogenesis, although still hindered somewhat by the small size and fluid characteristics typical of insect eggs, can be approached by vastly improved physical "tools", such as ultra-violet micro-beam irradiation; electron microscopy studies; and more refined cytochemical methods. Micro-beam irradiation with ultra-violet, which was employed in the present study, has two main advantages based on: (1) the precise method of treating the egg, and (2) the physical and biological properties of ultra-violet. For example, the beam itself can be focussed down to an area approximately 5-10 microns in diameter; the area of the egg to be treated can be precisely determined; the penetration of ultra-violet into the protoplasmic systems is small (approximately 95 per cent absorbed in 50 microns, Bachem and Reed, 1951), thus enabling localized surface damage of the treated area; and, with additional optical refinement, specified wavelength bands can be administered. These characteristics facilitate the "marking" of surface areas of a mosaic egg by inflicting histologically detectable damage on a specific area.

Oogenesis can also be studied more confidently and easily be electron microscopy (King, 1959), improved cytochemical methods (Hsu, 1952; Mulnard, 1954; Jacob and Sirlin, 1959; and Gorska and Sirlin, in press), nutritional control of certain physiological events of the ovary (King and Sang, in press), and genetically controlled experiments on ovary function (Robertson, 1957a and 1957b). An excellent general review of oogenesis studies has recently been published (Bonhag, 1958).

From the combined knowledge of oogenesis and early development it is possible that there will emerge a better understanding of the relationship

between metabolic synthesis and structural differentiation. The work presented in this paper was undertaken as a preliminary study of certain embryological aspects of the above problem, using ultra-violet micro-beam irradiation with the hope that further experimental exploration in a logically continuous manner would be possible.

The highly determinate nature of Dipteran eggs has long been a key problem for insect embryologists (Waddington, 1956). Earlier attempts to deal with the problem were not nearly as successful as similar work done in amphibian and invertebrates at approximately the same time because of the awkward size and fluidity of the egg. A brief summary of these earlier studies is listed below:

1. Cauterization - Reith, 1925 - Musca; Strasburger 1954, Calliphora; Howland and Robertson 1954, Drosophila; Haget 1953, Coleoptera;

2. Constriction - Pauli 1927 - Calliphora and Musca; Rostand 1927 - Calliphora;

3. Centrifugation - Pauli 1927 - Calliphora and Musca; Howland 1941 - Drosophila; Imaizumi - 1958 - Drosophila;

4. Ultra-sonics - Counce and Selman, 1955 - Drosophila;

5. X-rays - Sonnenblick 1940 - Drosophila; Sonnenblick and Henshaw 1941 - Drosophila;

6. α-radiation - Hanson and Heys 1953 - Drosophila;

7. Ultra-violet irradiation - Geigy 1951 - Drosophila; Aboim 1945 - Drosophila; Geigy and Aboim 1944 - Drosophila; Brauer, 1949, Callosobruchus.

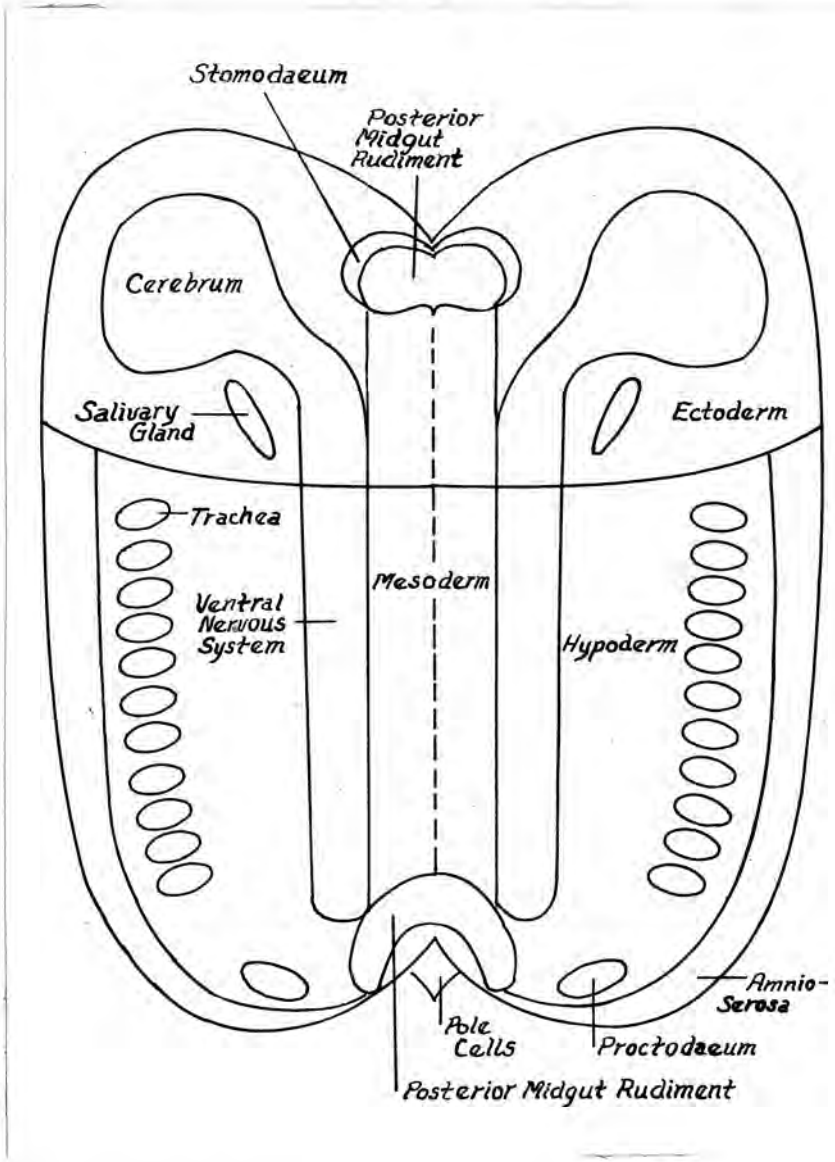
Needless to say, all this work has contributed toward the foundations necessary for the study presented in this paper. Certain works are

particularly relevant - especially in the light of the excellent studies in normal Drosophila embryology (Poulson, 1950; Sonnenblick, 1950; Ede, thesis; Ede and Counce, 1956).

The fine work of Geigy (1931) indicated that there were two determination periods in the eggs of Drosophila; the first being the mosaic nature of the freshly laid egg and subsequent early embryonic development, and the second governing the presumptive imaginal discs of later embryonic life, which in turn influence adult characteristics. This postulation was later substantiated by Waddington (1942). Such findings only emphasized the necessity of a more detailed analysis of the mosaicism of the egg. Geigy's technique of partial shielding and simultaneous ultra-violet treatment lacked the technical refinement necessary for an accurate mapping of the anlagen plan. Brauer's work (1949) on the larger pea beetle egg afforded somewhat more detailed information on the presumptive areas of that organism, but this was also done with shielding methods. The advent of the micro-beam finally seemed to afford the experimenter a sufficiently refined tool with which he might mark various localized regions of the surface of the egg and subsequently trace them histologically through their developmental patterns. Unfortunately, the relatively straight-forward methods of marking used in amphibian studies (Vogt, 1929) are not applicable to insect material because of the protective chorionic and vitelline membranes externally and the fluid nature of the internal constituents.

A certain amount of evidence has been accumulated and has made possible a hypothetical anlagen plan (Figure A, Poulson, 1950). Nevertheless, this needs experimental substantiation, and it was hoped that the micro-beam technique would be capable of doing just that.

Figure A.



The early blastoderm egg is seen from the ventral side, as though it had been split down the mid-dorsal line and then spread flat. (This Figure is a copy of that presented by Poulson, 1950, in Biology of Drosophila, ed., E. Demerec. p. 246. It represents that author's tentative presumptive fate map of the early blastoderm).

The second but equally important intended purpose of the work was to investigate the effectiveness of the apparatus used, the Beck reflecting Microscope, as an embryological tool. Designed on the principle outlined by Zirkle, 1953, it has numerous possibilities. Similar instruments have been used for a multitude of biological purposes (Zirkle, 1957), most of which deal with partial-cell irradiation and related biological effects. A discussion of the utility of the instrument shall be presented later.

A third area of interest which resulted from the present study deals with the general problem of radiation effects on biological specimens. A large amount of radiation work is presently being done on insect eggs because of their suitability as experimental material. They are easily obtained in large enough samples and are, under suitable conditions, relatively free of variables which might otherwise invalidate results. Genetic control is easily administered; contamination problems are almost non-existent; and the organic complexity, although still considerable, is minimized. In fact, because of the single, diploid nucleus present immediately after fertilization (and the exponential increase of nuclear material thereafter) much valuable information has been gained on the problem of nucleo-cytoplasmic interactions. Whiting (1949) has shown with X-ray treatment of Habrobracon eggs that smaller doses may have a chromosomal effect which may be genetically detected in surviving adults. Higher doses administered only to the cytoplasm by a process of shielding have lethal effects on developing embryos. In this case there is an "all or none" effect on the embryo, in that either death of the embryo occurs, or there are no visible mutations or reduced viability or fertility in the survivors.

Similar work with ultra-violet has also been done on Habrobracon by von Borstel (1957). He obtained clear-cut results on the relative sensitivity of the nucleus and cytoplasm to ultra-violet and the associated process of photoreactivation. Again the cytoplasm required a higher dosage and did not display the "single hit" inactivation characteristics of nuclear material. More refined work on the different effect of various wavelengths was also done on Habrobracon (Amy and von Borstel, 1957).

X-radiation of Drosophila eggs has produced similar information regarding nuclear and cytoplasmic sensitivity (Ulrich, 1955), and Goldman and Setlow (1955) obtained ultra-violet inactivation spectra as correlated with various developmental stages. Unfortunately, no detailed embryological evidence was presented. It was found, however, that nuclear irradiation at the blastoderm stage brought about early inhibition of development; whereas earlier treatment with only cytoplasm exposed resulted in either normal hatched larvae or abnormal unhatched larvae. This then parallels Whiting's, von Borstel's and Ulrich's findings on nucleo-cytoplasmic interaction. It is Goldman and Setlow's hope that micro-irradiation within well-defined spectral limits and at accurately timed developmental periods may provide useful information about sensitive compounds when correlated with absorption inactivation spectra and chemical analyses of irradiated eggs. Indeed, it does seem to be wishful thinking, for the biochemical complexity even of the relatively simple early egg would necessitate undue speculation.

The primary purpose of the present study is to obtain more precise information regarding the mosaic nature and subsequent developmental patterns of the egg of Drosophila melanogaster. The precision of the experimental tool should minimize the practical complications which previously have made

it impossible to distinguish between properties of the tool itself and the reacting components and characteristics of the biological system. Nevertheless, there will still be certain features of intra-organismic interactions which, if not approached with caution, can lead to tautological explanations of various developmental sequences. It is hoped that a study of this mosaic egg in its earliest stages will avoid such analytical traps.

MATERIALS AND METHODS

A. Stocks

The flies used throughout all the experimentation reported in this paper were from an inbred stock of Oregon K maintained in this Institute. The flies were always kept at 25 ± 0.5°C. Developing embryos were also kept at this temperature in accordance with developmental stages outlined by other workers (Poulson, 1950; Ede and Counce, 1956).

The viability of this stock was initially high, but it gradually decreased during the course of experimentation. This, however, was of no consequence in the interpretation of results, as adequate experimental controls were always maintained.

B. Egg Collection and Method of Treatment

Since the stage of development at the time of treatment was a crucial factor, great care was taken to standardize the egg collection procedure as much as possible. To ensure a continuous supply of eggs for whenever needed, cultures were kept so that four day old flies (at which time there is maximum egg production) were always available. Newly emerged flies were collected every 24 hours and were put up in fresh culture bottles; they were then aged for three days, and if egg collections were to be the next day, they were kept the third night on a 3 per cent, slightly acetic, agar medium with an abundant supply of fresh yeast available. This provided optimal feeding conditions for the twelve hours immediately prior to the collections. This acetic agar medium was in the form of small discs on a watch glass. The flies were placed in small creamer bottles and by inverting these bottles over the disc dabbed with fresh yeast the flies

were presented with optimal egg-laying conditions.

On the day of collection the discs were changed early in the morning and allowed fresh laying conditions for approximately two hours; these eggs were then discarded. This helped accustom the flies to the frequent shakings which would occur during the subsequent collections. After this two hour period the regular hourly collections were begun. Large numbers of eggs were obtained by this method, and the optimal feeding conditions helped ensure that oviposition would be regular and that very few fertilized eggs would be retained in the uterus of the female.

The flies were allowed to lay for periods of twenty minutes. These eggs were left on the agar disc, and set aside at 25°C. for the desired length of time, and then removed with a tiny platinum spoon and were dechorionated in a 2-3 per cent solution of sodium hypochlorite. This method of dechorionation was found to be much more satisfactory than any other; it was rapid, applicable to large numbers, and was not injurious to the eggs. After dechorionation the eggs were washed twice in distilled water and were at the same time checked by visual observation through a dissecting microscope. Any eggs which were not at the correct stage of development (Figures 4, 5, and 6) were discarded.

Two methods of treatment were used, depending upon the area of the egg to be irradiated. The first, used for pole cell irradiation, consisted of micropipetting the dechorionated eggs onto an improvised cavity slide (Figure 3) while it was submerged in a distilled water medium. While still submerged, a quartz coverslip of known thickness was placed over the cavity. The treatment slide itself was an ordinary glass slide with a piece of metal foil affixed to it. The foil had a circular hole of approximately 0.3 inch diameter cut into it, and its thickness was approximately the same as that of

an egg, i.e. 150 microns. Thus when the quartz coverslip was placed over the eggs which were in the hole, the eggs were not at all squashed or damaged. During the treatment the eggs were irradiated through the transmitting quartz coverslip and a very thin layer of water. There was only one disadvantage to this method; it was not possible to orient the eggs dorso-ventrally because of slight currents arising and thus moving the eggs as the coverslip was eased on. This was not important in pole cell treatments, however.

The other method of treatment used in surface irradiation of other parts of the egg was essentially much simpler, for the dechorionated eggs were placed on tiny agar discs in shallow cavity slides. By careful manipulation with a small brush the eggs could be oriented into any position. The eggs could then be treated as they lay on the surface of the agar. There was a practical disadvantage to this method, however. The eggs were exposed to the air and thus easily dried. Therefore, it was impossible to treat as large a number as in the slide irradiations.

It should be mentioned that pole cell irradiation was attempted on eggs adhering to the vertical surface of a tiny agar disc which had been cut transversely. This meant the pole cell cap as a whole was uppermost and could be treated directly. It was hoped that greater damage to the pole cells would result (Meyer, et al, 1949); but in fact, it was found that increased focussing difficulties occurred and the irradiation was not necessarily confined to the pole cells.

One of the most important points of procedure was the adequacy of the method of timing the developmental stage of the organism under observation. The procedure outlined above reduced undesirable variations

to a minimum. First, rapidly laying females produce a vast majority of eggs in which fertilization has just occurred. Second, visual observations of the transparent, dechorionated eggs enabled age determination to within one quarter hour at the times of treatment. Third, the entire procedure of treating a group of 15-25 eggs after they had reached the specific stage of development required just slightly less than one hour. This means that at least one hundred eggs can be treated in one day. This was particularly advantageous from the experimenter's point of view, because during the subsequent handling of the eggs in preparation for histological examination a fair number of eggs were frequently lost.

After treatment the eggs were allowed to develop for a specified period of time at 25°C and then fixed.

C. Fixation, Embedding and Staining

The process of fixation and embedding was as outlined below:

(1) Fixation with Kahle's fluid consisting of 95 per cent ethanol, commercial formalin, and glacial acetic acid (15:6:1), for a period of 4-18 hours. In order to facilitate penetration of the fixative, it was necessary to puncture very carefully the impermeable vitelline membrane of the egg with a tungsten needle sharpened to a delicate point in fused sodium nitrite.

(2) Three washes of 95 per cent ethanol during the course of one hour.

(3) Prestaining in 2-4 per cent eosin in 95 per cent ethanol for 2-5 minutes in order to aid the embedding procedure later.

(4) Two quick washes in 95 per cent ethanol.

(5) Overnight or more in 4 per cent phenol in butanol.

(6) Six hours in 1:1 medium of soft wax (48°C m.pt.) and the 4 per cent phenol in butanol mixture.

(7) Soft wax overnight.

(8) Hard wax (56°C m.pt.) at least one hour before embedding.

(9) Embed in hard wax and orient the egg with a warm dissecting needle.

All sectioning was done at 5 microns. Various stains were used, but the most satisfactory for general purposes was Heidenhain's iron hematoxylin method, allowing not less than twenty minutes in both the iron and the hematoxylin solutions.

It should also be mentioned that Poulson's modification of the Bodian staining technique (Poulson, 1945) was tried. Unfortunately, the results were unsatisfactory. This was probably related to the protargol (Gurr's, London) which was used, for Poulson emphasizes the importance of this substance and recommends that produced by Winthrop Chemical Company, New York.

Two other strains employed, principally for the study of cytoplasmic inclusions in the yolk, were borax carmine and azure B (Flax and Himes, 1952). The latter, usually used as a metachromatic stain for nucleic acids, was particularly satisfactory and could be administered relatively rapidly.

In most experiments a certain number of the treated eggs were allowed to develop to adulthood. For ovary studies of these flies King's (1957) method of Feulgen staining and squash preparation was used.

The number of pole cells incorporated into the embryonic gonad was determined by counting the nuclei and fragments thereof as they appeared in successive serial sections. In that most of these cells would be counted more than once in successive sections it was necessary to introduce a correction factor to derive an approximation of the actual number. The author is grateful to Dr. B. Woolf for deriving a formula which would serve this purpose.

$$\text{Correction factor} = \frac{1}{1 + X}, \text{ where } X = \frac{\text{nuclear diameter}}{\text{section thickness}}$$

In the case of the present work this factor equalled 0.42, and subsequently when data is presented it should be understood that the "corrected" value is derived according to the above method.

D. The Micro-beam Apparatus

The main piece of apparatus was the Beck Reflecting Microscope, of R. & J. Beck, Limited, London. It is shown diagrammatically in Figures 1 and 2. The essesntial features of the microscope and their relevance to the experimental procedure will be explained below.

(1) The reflecting objective (Figure 2) is the most important unit of the microscope. Its entire optical system (as in the whole microscope) consists of delicately adjusted mirrors with aluminised surfaces. Quartz optical refracting systems are commonly employed for ultra-violet microscopes, but there is appreciable loss of ultra-violet light at the numerous refracting surfaces (by absorption); and glass, of course, is completely absorbant. In addition, quartz transmission involves refraction differences, depending on the wavelength, so that the focal length is wavelength dependent. This reflecting system, therefore, avoids the transmission and refraction problems and thus provides the simplest and most efficient ultra-violet optical system.

As is seen in Figures 1 and 2, the light when it reaches the objective is reflected off a central stop; onto a semi-spherical surface composed of three finely adjustable mirrors; and from here focussed down on to the specimen, where a precise image of the aperture (seen to the left of the microscope in the diagram) is produced. Two objectives with magnifications of 52x and 74x were available for the present work. Depending upon the size of the aperture, the micro-spot can be focussed down easily to an area of 5 microns diameter.

These two objectives can be used simultaneously, one as the objective and the other as the condenser. This is advantageous when ultra-violet (instead of the visible light shown in the drawing) is being introduced through the base of the microscope.

(2) The vertically and horizontally adjustable stage is highly important for achieving precise focus of the image of the aperture. By means of a slide coated with finely powdered fluorescene one can first adjust the tube height and the position of the aperture so as to give a clear, perfectly centred image of the aperture on the slide. Once this has been done, however, further tube manipulation is impossible. Therefore, in order to facilitate focussing of the micro-spot on the specimen, it is necessary to have all adjustments independent of the tube itself. This is accomplished by the delicate vertical adjustment of the stage. Since the visible light beam and the ultra-violet beam are pre-arranged to be co-axial, visible light can be used to bring the desired area of the specimen into exact focus. Then one can administer the ultra-violet, knowing that its focal point is the same as that of the visible.

(3) The variable aperture and shutter mechanism (see diagram) permit different sized micro-spots to be projected onto the specimen. A cable release attachment to the shutter prevents any movement of the apparatus and thus allows carefully timed treatments with no chance of disturbing the pre-arranged optics.

(4) At the base of the microscope there is another aluminised mirror which reflects visible light (or ultra-violet if so arranged) into the condenser. It is then transmitted through the specimen and forms an image which is seen through the ocular. As mentioned above, this beam is pre-set

to be co-axial with the ultra-violet and thus allows visible focussing on the specimen.

(5) A 35 mm. camera specially designed for this microscope is easily attached to the top of the tube. It is parfocal with the objective, and by a quick shift of the tube mirror (shown in black in the diagram) the image is easily passed onto the surface of the film.

(6) The ultra-violet source throughout this experiment was an Osram 250 watt, high pressure mercury lamp, ME/D. It was fitted with a protective housing of metal with a small glass window, which for the present work had to be removed. This lamp has a wide spectral distribution extending well into the visible wavelength regions as well. However, the maximum energy output is in the ultra-violet, with principal emission occurring at approximately 3140, 3340 and 3660 angstrom units. This made the experiment very unspecific from the physical point of view, but since the biological results were of primary interest and could be achieved best with this source because of its high intensity (thus allowing a short treatment period), it was considered satisfactory. The most suitable source physically would be a low pressure mercury lamp emitting about 95 per cent of its total energy principally in the 2600 angstrom region. This is the region of maximum absorption for the nucleic acids. Those compounds showing maximum absorption in the 3000-3700 angstrom region include the carotenoids, flavins, and vitamins.

(7) The water filter, situated 6-8 centimeters away from the source, was used to absorb infra-red emission and thus prevent heat transmission. It consisted of a hollow brass cylinder, the open ends of which were covered with parallel quartz windows. It was filled with fresh distilled water before each usage. Encasing the cylinder were several coils of copper tubing through which cold water flowed continuously. The heat emitted by

the source was so intense that without a cooling device the distilled water contained in the filter would soon boil.

(8) The lens shown in Figure 1 was used to converge and focus as much of the ultra-violet as possible onto the aperture. It too was made of quartz, and had a focal length of approximately $6\frac{1}{2}$ inches.

(9) The source, filter, lens, and shutter mechanism were all mounted on a sliding optical bench, thus permitting stable conditions, on the one hand, and rapid alterations of the apparatus, on the other. This bench and the microscope were fixed into position on a large sheet of plywood. The author is grateful to the departmental workshop for preparing this arrangement.

In summary then, the experimental procedure was briefly as follows. The source image was first focussed onto the back of the aperture by careful adjustment of the lens. The visible light source was centred with respect to the optics of the microscope. The ultra-violet image was then focussed and centred onto a fluorescent surfaced slide by means of the tube adjustment. With the optics thus adjusted, the specimen could then be placed on the stage, and by means of the stage adjustment and using visible transmitted light the specimen could be brought into focus. By using the cable release the ultra-violet micro-beam could then be administered for the required time.

E. Photography

Ilford 35 mm. Micro-Neg Pan film was used for all photography of biological material. For studies of live specimens the camera and microscope described above were used. For photomicrographs of slide preparations an ordinary high-power compound microscope with a Leica camera attachment was used.

AN OUTLINE OF RELEVANT FEATURES AND THEORIES OF NORMAL DEVELOPMENT

Since the present study was primarily concerned with the developmental fate of the pole cells and various regions of the blastoderm, a brief summary of the early embryology and specified developmental stages thereafter will now be presented. This information was obtained from Poulson (1950) and Sonnenblick (1950), unless otherwise noted.

A. The Pole Cells

These cells are the first distinctly formed cellular units to develop from the fertilized egg. This occurs at the blastema stage, or approximately $1\frac{1}{2}$ - 2 hours at 25°C. The first indication of their formation is the arrival of an indefinite number of nuclei (derived from the freely migrating cleavage nuclei derivatives) in the periplasm (peripheral cytoplasm) at the posterior tip of the egg. These nuclei always number more than one (Huettner, 1923), but the results of various workers indicate a general variability. In fact, it has been shown that it is a matter of chance as to whether the cleavage nuclei derivatives are to assume roles as yolk, blastoderm, or pole cell nuclei (Parks, 1936) in later development. At this same time there is a growing accumulation of nuclei in the periplasm all around the egg, although no complete cell formation is yet evident in these other regions (Figures 5 and 9). The pole cells, on the other hand, actually bud off from the main body of the egg, and later when the blastoderm cells are completely delimited shortly after 2 hours, the pole cells are found to lie on top of the blastoderm cells (see Figures 5, 7 and 8). In addition, they are distinctly different in shape, spherical as compared to the columnar cells of the blastoderm.

Shortly after the blastoderm cells are formed there is a migration of some of the pole cells back into the yolk, passing in between the blastodermal cells (Figures 10, 11 and 12). Those pole cells not undergoing this inter-blastodermal migration remain at the surface of the posterior tip of the egg until gastrulation movements begin at 3 -$5\frac{1}{2}$ hours. During this "quiescent" period they exhibit no mitotic activity nor obvious spatial re-arrangements. In fact, the gonad destined pole cells do not show any further mitotic activity until approximately 16 hours, which is well after inclusion into the gonad at approximately 10 - $10\frac{1}{2}$ hours. At gastrulation the posterior midgut rudiment, (PMG)[1], which immediately underlies the pole cells (Figure 12), begins its migration along the dorsal surface and carries the surface pole cells with it.

At 5 hours the PMG is deeply invaginated, extending down into the yolk from the dorsal surface (Figures 13 and 14), and the pole cells are located within the cavity. The movements of the pole cells from this point onward are uncertain, although various workers have presented several different explanations. Since one of the main objects of the present study was to investigate this problem, a brief summary of the various theories and the methods of investigation will now be given.

Geigy (1951) first proved conclusively with ultra-violet pole cell irradiation that at least some of the originally segregating pole cells eventually were included in the embryonic gonad as adult sex cell precursors, and he reasoned that those carried into the PMG eventually migrated to the presumptive gonad rudiment in the mesoderm. No account

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[1] Henceforth the abbreviation, "PMG", shall be used to refer to "posterior midgut".

was given of the intra-blastodermal migrating pole cells.

Rabinowitz (1941) and Sonnenblick (1941) later proved with histological studies of a considerable number of normal embryos that by no means all of the original pole cells entered into gonad formation. Some of them, in fact, migrated back into the yolk passing between the blastoderm cells. The former author believed the inter-blastodermal migrating cells to degenerate in the yolk; the latter considered them to be "lost" in the yolk-filled gut.

Geigy and Aboim (1944) and Aboim (1945) did further work with ultra-violet irradiation of the pole cells, and with detailed histological studies provided circumstantial evidence that those pole cells carried into the PMG later assumed a role in gonad formation by means of a migration through the PMG wall into the dorso-lateral mesoderm. Here they eventually congregate in the mesodermal rudiment of the gonad in the tenth segment at approximately 10 hours. A photomicrograph showing this postulated escape from the PMG is seen in Figures 14, 15 and 16. These workers were unable to trace the earlier migrating pole cells.

Then Poulson (1947) presented a report of carefully studied histological evidence which suggested that both the interblastodermal migrating pole cells and some of those remaining in the PMG (which did not move dorsally and laterally out of the PMG and into the dorso-lateral mesoderm at 7 hours) were involved in the formation of the midgut epithelium at the point where the posterior midgut rudiment joins with the anterior midgut rudiment (Figures 17 and 18). He presented an additional theoretical argument which reasoned that since this section of the gut had marked physiological characteristics in larval function (Strasburger, 1952), it probably, therefore, had a distinctive precursive cell origin. He later obtained further evidence

of this physiological characteristic, i.e. high copper absorption (Poulson and Bowen, 1952), but he did not present any experimental verification of his theory regarding its cell origin.

However, later Poulson (1950) and Poulson and Waterhouse (1958) suggested that the earlier migrating pole cells are actually the gamete precursors and that those carried into the PMG play a role only in midgut epithelium formation. They claimed experimental proof of this on the basis of ultra-violet pole cell irradiations coupled with histochemical studies of the larval gut and histological studies of the embryonic gonad and gut. However, there is one obvious weak point in their argument. It was learned by personal communication with Poulson that all results were based on embryos treated no later than $2\frac{1}{2}$ hours of development. This is precisely the stage during which the pole cells are undergoing interblastodermal migration, i.e. 2 - $2\frac{1}{2}$ hours. In fact, it appears that in some cases the duration of this migratory period lasts considerably longer than $2\frac{1}{4}$ hours (Figure 12). Since the only way to get differential irradiation of the two separately migrating groups of cells is to treat after this stage, it seems a bit dubious to draw the conclusion which they did.

Also, it is probably worth mentioning that a shielding technique was used to achieve localized irradiation. This in itself introduces a certain amount of variability; because accurate timing of the developmental stages is more difficult owing to the relatively long period of time necessary to achieve proper orientation, and the shielding device probably allowed a certain amount of stray ultra-violet to fall upon other regions of the egg.

However, one bit of very questionable corroborative evidence for Poulson's theory has been presented. Counce and Ede (1957) in their study of the female sterility factor, nasrat^{A}, found that pole cell-containing

embryonic gonads were formed in this otherwise maldeveloping embryo in which the PMG invagination does not occur at all. Thus, no pole cells could be carried into the interior of the embryo via the normal gastrulation movements. The interblastodermal migration was observed, though. This led them to conclude that it was this first migratory pole cell group which was responsible for the normal gonad development. Although such an explanation certainly is plausible, a far more detailed histological analysis is required to substantiate this conclusion.

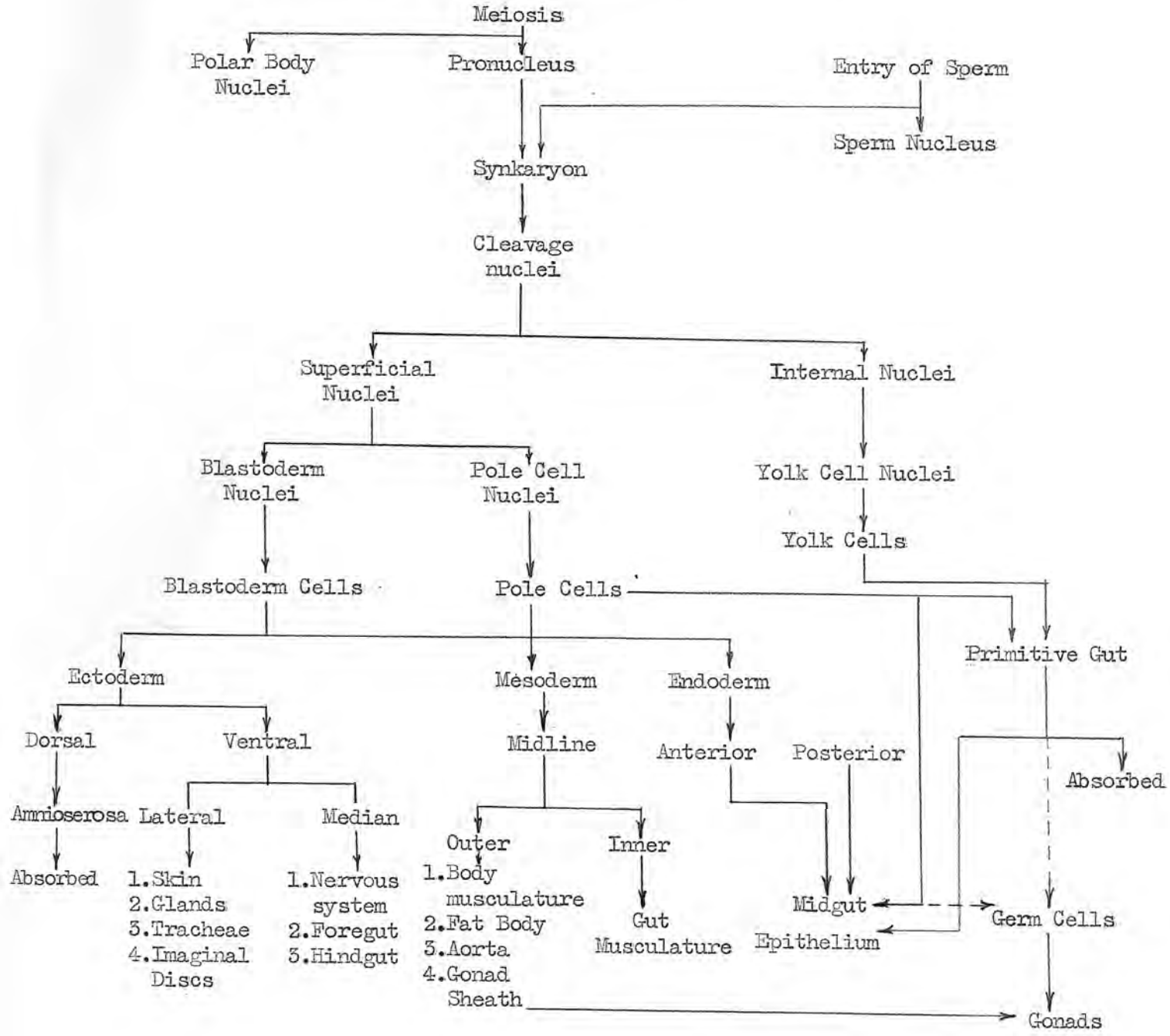
Thus, it remains unsettled as to what the actual migratory path is for those pole cells which are ultimately incorporated into the embryonic gonad.

B. The Primary Germ Layers and Theories of Cell Lineage

The problem of establishing definite homologies concerning germ layer formation throughout the embryonic development in the various families of Insecta has provoked considerable debate and still remains unsettled in some cases (Review: Johannsen and Butt, 1941). However, in the case of Drosophila there is little controversy on this broader issue, although certain minor points of cell lineage remain to be verified. For the purpose of clarity later in the paper (when descriptions of the normal morphological events will be immediately helpful) only the general diagrammatic postulations of cell lineage (Figures A and B, pp. 5 and 23 ; Poulson, 1950) and a brief explanation of germ layer formation will be presented now. The more detailed explanations will be given when needed to understand the experimental results of the present work.

Figure B. (Poulson, 1950)*

Cell Lineage Diagram



*
(A copy of the diagram presented by Poulson, 1950, in Biology of Drosophila, ed. E. Demerec. p. 243).

TIME CHART OF DROSOPHILA DEVELOPMENT

MORPHOLOGICAL EVENT

TIME	
0-15	Telophase of 2nd maturation division to conjugation of pronuclei.
15-30	1st division of cleavage nuclei.
1.00-1.30	Blastema.
1.30-2.00	Pole cell formation.
2.00-2.15	Blastoderm forming.
2.15-3.00	Migration of pole cells back through blastoderm cells.
3.00-3.15	Blastoderm formation complete, pregastrular movements.
3.15-3.30	Gastrulation begins.
3.30-3.45	Ventral furrow.
3.45-4.00	Cephalic furrow.
4.00-4.15	Anterior and posterior invaginations; extending germ band.
4.15-4.30	Neuroblasts 1st distinguishable.
4.30-4.45	Dorsal folds; flattening mesoderm.
5.00-5.15	Extended germ band; large neuroblasts.
5.15-5.30	Beginning stomodaeal and proctodaeal invaginations; neuroblast mitoses.
6.00-6.15	Deep stomodaeum and proctodaeum; mesoderm segmented.
7.00-7.15	Tracheal invaginations; salivary gland plates.
8th hour	3 small invaginations in stomodaeum roof - the origin of stomodaeal nervous system and ring gland. Termination of mesodermal mitoses - no further visible mesodermal differentiation. Demarcation of hindgut and midgut. Salivary gland indentation prominent. Segmentation of head and trunk; muscle attachments.
$8\frac{1}{2}$ hours	Rupture of PMG at tip and becomes confluent with yolk.
9th hour	Mesoderm fully separates into somato- and splanchnopleure. Shortening of embryo begins; salivary gland is internal. Demarcation of midgut.
10th hour	12 segments at $10\frac{1}{2}$ hours - including head. Beginning involution of head and dorsal closure.
$10\frac{1}{2}$ hours	Fusion ventrally of PMG and AMG by MMG (of yolk and/or pole cell origin?)
11 hours	Involution of head complete.
12 hours	Dark secretions appear in salivary glands.
13 hours	Midgut constrictions; larval musculature complete; gonads compact.

(1) Ectoderm - The ectroderm simply consists of those blastoderm cells which at approximately $3\frac{1}{2}$ hours are not invaginated along the median ventral line to form the mesoderm, or into the interior of the embryo to form the endodermal rudiments. During the course of embryonic development the ectoderm gives rise to those derivatives shown on the diagram through a series of invaginations and gradual cellular differentiation.

(2) Mesoderm - The mesoderm originates from the mid-ventral invagination occurring at approximately $5\frac{1}{2}$ hours. The ventral furrow is formed at this time, and thus the process is externally distinguishable. The furrow closes quickly and the invaginated cells briefly assume the shape of a hollow tube running almost the entire length of the embryo. The lumen is soon closed, however, as the germ band is extended, and there is much mitotic activity in these mesodermal cells. The regional differentiation of the mesoderm is thus completed, with organogenesis occurring gradually thereafter and mesodermal segmentation first visible at 5 - 6 hours.

(3) Endoderm - The endoderm arises primarily from the anterior and posterior midgut rudiments. The former of these invaginates simultaneously with the ventral furrow but at right angles to it at its most anterior point. Thus when viewing the ventral surface at this stage the ventral furrow and the anterior endodermal invagination appear as a giant "T" on the surface of the embryo. After the furrows close again the invaginated cells are completely internal and undergo many mitoses. During this period of high activity the endodermal rudiment separates from the adjacent mesodermal cells, and derivatives of the two layers are morphologically independent of each other throughout subsequent development. At $5\frac{1}{2}$ - 6 hours the stomodaeal plate (of ectodermal origin) begins to invaginate from its surface position and becomes intimately related to the endodermal element. This relationship

continues and eventually the lumen of the foregut (stomodaeal invagination) becomes confluent with that developing in the anterior midgut. The following gut-encircling movements will be described later.

The posterior midgut rudiment, in contrast, is a readily distinguishable structure. The rudiment itself is first noticed at $3\frac{1}{4}$ - $3\frac{1}{2}$ hours as a thickening and slight indentation of the cells immediately underlying the pole cells at the posterior tip of the embryo (Figure 12). Migratory movements during the next $1\frac{1}{2}$ hours carry the invaginating rudiment and the accompanying pole cells anteriorly over the dorsal surface to a point just posterior to the brain in the head region (Figures 13, 14 and 15). Subsequent development has been described previously in the discussion of the pole cells.

Thus at the completion of gastrulation the germ layers are established in the following form:

(1) The ectoderm completely surrounds the embryo. There is no visible morphological differentiation of any of its cells.

(2) The mesoderm, now comprising the majority of the germ band, immediately underlies the ventral ectodermal cells. It runs almost the entire length of the embryo on the ventral surface, and will spread laterally and dorsally during the subsequent extension of the germ band.

(3) The endoderm consists of an anterior and posterior rudiment. The former, having invaginated along with the mesoderm from the ventral surface but at right angles to it, is found adjacent to the mesoderm at its most anterior point. Its cells are rapidly proliferating.

The posterior endodermal rudiment has now invaginated well into the yolk with the invagination mouth immediately posterior to the cephalic groove on the dorsal surface. It contains some pole cells carried along from their original position at the posterior tip.

RESULTS

A. General Criteria

The general histological characteristics of the irradiated embryos as compared to the controls will first be described. In cases of limited damage, or where a long period of time had elapsed between treatment and fixation, the interpretation of the histological material was not straightforward. This difficulty was only emphasized by the unavoidable variation in staining which was found on different slides. However, familiarity with the techniques gradually minimised such variability, and after repeated observations one was able to distinguish quite confidently between the technically caused inconstancies and the experimentally caused alterations having a definite biological basis.

Also, it should be mentioned that the cytological specificity of the dyes considerably restricted the versatility of the specimens with regard to their interpretative value. The stain used most successfully in gonadal pole cell studies, Heidenhain's hematoxylin, was virtually unsuitable for studies of cellular inclusions in the yolk. This obviously prevented concurrent examinations of the same embryo for those two features; and because of the large number of embryos needed for gonad studies and the time-consuming treatment and histological preparations thereof, it was not possible to acquire an adequate number of properly stained specimens for conclusive yolk studies.

Depending upon the size of the area irradiated and the period of time elapsing between treatment and fixation, different manifestations of ultra-violet damage resulted.

(1) The most easily recognized damage is observed one hour after treatment

in cells which are normally segregated from environmental tissue, i.e. pole cells. The injured cells stain considerably darker, lack distinct nuclear structure, and begin to lose their customary cellular outline (Figures 19 and 20). Compare with Figures 7, 8 and 12.

(2) Damage observed several hours after treatment took a variety of forms and demanded painstaking study so as to avoid errors in interpretation. Again the best examples are found in pole cell treated embryos where at 5-7 hours the pole cells, or what remains of them, are morphologically segregated from their more dense environment. Pycnotic cells often result several hours after treatment (Figures 21 and 22). Also, in some cases only the nuclei were pycnotic, as one cell appears to be in Figure 24. It is possible that these darkly staining spheres, which resemble a cellular outline in size and shape, are not necessarily the remains of individual cells; they could well be a congregated mass of general debris, perhaps coming from several destroyed cells.

Swollen, vacuolated nuclei containing beaded chromatin adhering to the nuclear membrane are sometimes seen (Figures 23 and 24). This type of damage is much less distinguishable and demands careful study before one can conclude with certainty that this condition exists.. It is likely that cells thus effected have suffered less damage and may retain their cellular outline for quite a long period of time.

The type of damage most difficult to detect, however, was that shown in Figures 25 and 26. Here the remains of apparently lyzed pole cells are no more than a disorganized, diffuse volume of what appears to be cytoplasm. The problem of interpreting such evidence lies in its close resemblance to the cytoplasm of healthy cells. Only by a study of successive serial sections

can one tell if the cytoplasmic mass has any characteristics of form or has nuclei situated reasonably nearby; and if so, of course, one must conclude that this is not debris. Obviously, in regions of dense concentration it is impossible to distinguish between debris and the cytoplasmic portions of healthy cells. Such debris probably results from severe radiation damage.

It was common to find a mixture of healthy and damaged cells in the pole cell treated embryos. There was usually no grouping of the damaged cells apart from the normal ones.

(3) The study of the definitive embryonic gonad was much more straightforward, because the pole cells, if present, are quite conspicuous (Figures 27 and 28) in comparison to the gonad lacking them (Figures 29 and 30). To determine the number of nuclei present required careful study under high magnification of the successive sections. This, although simple in theory, was extremely tedious in practice and required a considerable length of time.

(4) A wider range of more severe defects were also obtained, although most of this material was of little value. Usually the most information which could be obtained from such specimens was an approximation of the age at which development ceased. This, of course, is interesting in light of the "critical periods" of development as established by other workers. This will be mentioned later in relation to specific results.

In Figures 31 and 32 is seen a good example of an embryo in which death has occurred shortly after gastrulation. Necrosis is very obvious, and yet has not advanced to such a degree that there is no semblance of general morphology. The outline of the PMG invagination is still quite evident in the form of the central lighter staining region. The former germ band (along ventral surface) has for the most part lost any remnants

of cell structure, and the yolk and general cellular debris have become one homogeneous mass. In the posterior area, however, a few swollen, vacuolated nuclei still are distinguishable.

Some eggs exhibited partial necrosis and complete, although disorganized, cell structure simultaneously (Figure 33). In the embryo shown it can be seen that development has probably reached a later stage of gastrulation, and although the majority of the egg is definitely necrotic there are still some healthy cells to the left. These closely resemble germ band cells, although they are less compact, and for this reason the embryo as a whole is rather interesting. It was fixed after 15 hours of development, with pole cell irradiation at 2 hours. Thus there seems to have been a complete retardation, but not death, of the embryo at approximately 5-6 hours, with some cells maintaining a healthy but undifferentiated appearance.

Other embryos underwent extensive cell proliferation but no differentiation (Figure 34). The embryo shown was not fixed until 15 hours, and although the general embryonic condition is completely abnormal proliferation of a mesodermal type cell has continued.

B. Pole Cell Studies

In order to obtain unequivocal evidence regarding the migratory movements of those pole cells eventually entering into gonad formation two experimental series, each consisting of carefully timed pole cell irradiations at specific stages of development, were carried out. The first of these series was conducted during the early period of all the work reported in this paper, and at that time the effectiveness of the micro-beam was still a matter of speculation. Consequently certain errors in procedure occurred which lessened the significance of the results. In

view of the different experimental conditions of the two series the work will be reported in two parts.

Series 1:

The two possible variable physical conditions, i.e. the size of the micro-spot and the period of administration, were 10-15 microns and 30 seconds per embryo, respectively.

The plan of experimentation, in accordance with the various postulations concerning pole cell fate (previously discussed on pages 18-22) was as follows: (1) to treat the embryo at that stage of development prior to pole cell delimitation and before the migration of the cleavage nuclei to the pole cell area of the periplasm, i.e. approximately 1 hour; (2) to treat the embryo after pole cell formation was complete but before the interblastodermal migration of some of them into the yolk (and perhaps eventually into the gonad, i.e. approximately 2 hours; and (3) to treat the embryo after the interblastodermal migration and before the gastrulation movements of the PMG and the overlying pole cells, i.e. approximately 3 hours.

In each of these differently treated groups some embryos were sectioned at each of five different stages of development, corresponding to: (1) one hour after treatment to determine the initial effect of the ultra-violet; (2) approximately 5 hours of development when the PMG invagination is in its most clearly defined form and thus enabling a study of the pole cells in the cavity, the PMG wall, and the relation of the two; (3) 7-8 hours of development when the pole cells included in the PMG cavity may migrate through the wall of the structure in passage to the dorsal mesoderm; (4) 11 hours of development, which is shortly after the completion of the midgut and thereby facilitating a study of that organ and the gonad; and (5) 13-14 hours of development, when the gonad destined pole cells

(whatever their migratory movements) should have assumed their definitive positions in the mesodermal rudiment of the gonad.

The main results of this series were found in the comparative gonad studies and mortality figures of the three groups of embryos, each treated at a different stage.

Table 1.

Pole Cells

Treatment Age	No. Gonads	P.C.'s per Gonad (Avg.)	Embryonic Mortality at 15 hours
1 Hour	11	16.0 (corr. = 6.7)	1/16 = 6.25%
2 Hours	5	16.6 (corr. = 7.0)	5/19 = 26.4%
3 Hours	14	14.2 (corr. = 6.0)	0/17 = 0.0%
Controls	10	20.6 (corr. = 8.7)	1/27 = 3.7%

In view of the small number of embryos which were studied and large intra-group variation in the actual counts the above figures by no means supplied sufficient evidence from which one could confidently draw any conclusions about the biological problem under consideration. Also, all the treated embryos which were expected to mature and provide material for the study of adult gonads died as a result of badly contaminated cultures. Thus the inconclusive nature of the little evidence obtained only emphasized the necessity of further study. Since the suggestions of these results were eventually substantiated in a much more satisfactory manner, they will not be considered further at this time but shall be referred to in later discussion. Also, supporting histological evidence will be presented then.

However, important information regarding the effectiveness of the apparatus and the technique was gained and thus made possible a much more

satisfactory approach to the problem in the next series. It was quite obvious that the micro-beam in this case (10-15 microns for 30 seconds) was too ineffectual to castrate the embryos completely with any degree of reliability, although one 1 hour and two 3 hour embryos were unilaterally lacking pole cells in the mesodermal gonad rudiment.

The mortality figures indicated that the micro-beam irradiation under these conditions was not prohibitively lethal and allowed one to continue experimentation with more confidence in the concept of localized damage.

Series 2:

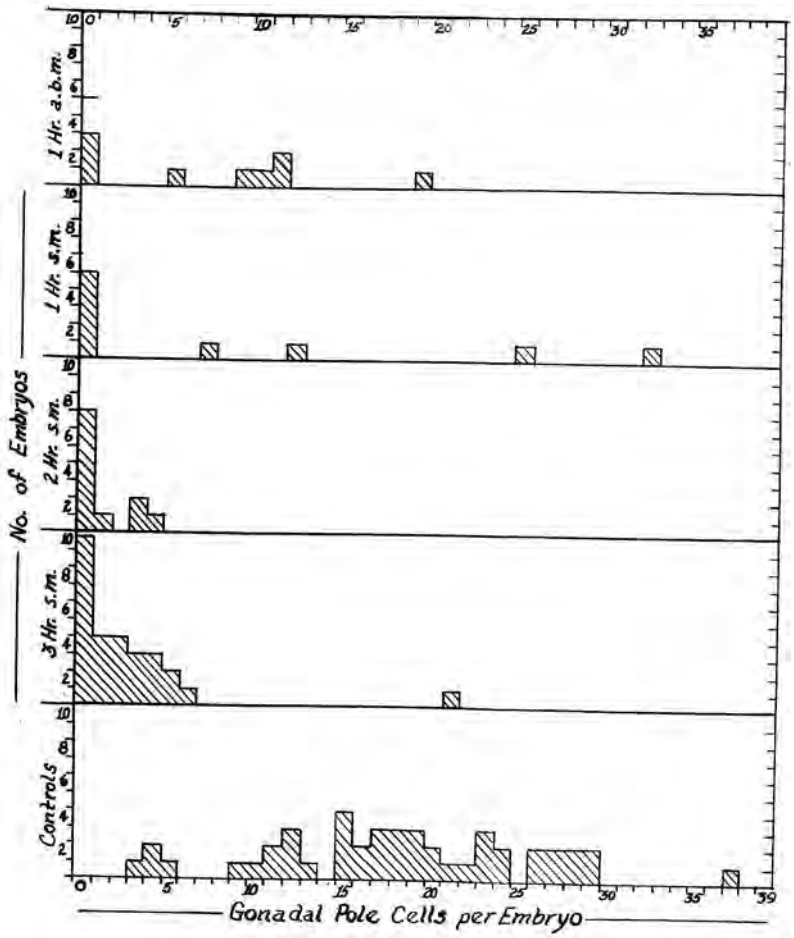
Gonad Development - On the basis of the above work the size of the micro-spot was increased to approximately 60 microns (Figure 35), and the duration of irradiation was kept the same, 30 seconds per embryo. Since it was found in the first series that Heidenhain's Hematoxylin, though particularly good for gonad studies, was unsuitable for detailed and yolk and gut studies at 8 and 11 hours development, the embryos were fixed at only one developmental stage, 15 hours. It was possible at this stage to observe the condition of the pole cells in the gonad and also to detect any pronounced abnormalities in midgut formation. They were treated, of course, in three groups at 1, 2 and 3 hours, respectively.

The results of this series are seen in Figure C.

By a comparison with the controls in Figure C it is obvious that irradiation at all three developmental stages has a marked effect upon the eventual number of pole cells incorporated into the embryonic gonad. This provides unquestionable evidence that those pole cells carried into the PMG invagination play a major role in normal gonad formation. The embryonic mesodermal gonadal rudiment, on the other hand, was invariably present,

Figure C.

Gonadal Pole Cells in Irradiated Embryos



Key: a.b.m. = agar block method of irradiation (see text).
s.m. = cavity slide method of irradiation (see text and figure 3)

TABLE 2.

Lethal and Castration Effects of Pole Cell Irradiation

Treatment Age	Embryonic Condition at 15 hours			Adult Lethality	Castration					
					Ovaries			Testes		
	Dead	Totally Abnormal	Total Embryonic Lethality		No. Flies	Uni-lateral	Bi-lateral	No. Flies	Uni-lateral	Bi-lateral
1 hr. (agar block method)	$\frac{27}{55}$ = 54.0%	$\frac{10}{55}$ = 18.2%	$\frac{37}{55}$ = 72.2%	$\frac{41}{56}$ = 73.2%	5	0	0	-	-	-
1 hr. (slide method)	$\frac{11}{39}$ = 28.1%	$\frac{2}{39}$ = 5.1%	$\frac{13}{39}$ = 33.3%	$\frac{28}{43}$ = 65.0%	4	0	3	7	4	2
2 hr. (slide method)	$\frac{22}{56}$ = 39.3%	$\frac{10}{56}$ = 17.9%	$\frac{32}{56}$ = 57.2%	$\frac{40}{63}$ = 63.5%	11	4	0	6	4	1
3 hr. (slide method)	$\frac{0}{45}$ = 0.0%	$\frac{0}{45}$ = 0.0%	$\frac{0}{45}$ = 0.0%	$\frac{19}{33}$ = 57.5%	8	1	5	7	2	3
Controls	$\frac{4}{68}$ = 5.9%	$\frac{2}{68}$ = 2.94%	$\frac{6}{68}$ = 8.8%	$\frac{36}{92}$ = 39%	13	0	1	10	0	0

(Figures 29 and 30), and thus corroborated Geigy's (1951), Aboim's (1945), and Haget's (1953) findings. Further evidence of this independently formed mesodermal rudiment was seen in the gonadal condition of those treated embryos raised to adulthood, but this will be considered later in more detail.

The more frequent occurrence of gonadal pole cells in the 1 hour group suggests that prior to the actual "budding off" and complete cell formation there is a possible recovery of the treated area. What this recovery might consist of biologically is purely a matter of conjecture.

A rather interesting bit of information regarding the treatment technique also emerged from the two groups treated at 1 hour. It was thought that the agar block method, with the eggs placed on end and thus making possible the direct irradiation of a greater number of pole cells, would destroy a greater number per embryo. However, the figures do not indicate this and only show that there was a much greater early embryonic mortality (Table 2). This was probably because of the inability to focus the beam accurately enough to prevent a certain amount of ultra-violet from hitting other regions of the egg. Also, there was no coverslip over the egg, and thus the intensity was greater.

It is worthwile to note that the results of the first pole cell experimental series (see Table 1, page 32) were, in fact, very similar, in spite of the small number of embryos examined and the large variability in the actual individual counts. Also, the mortality effects of the micro-beam were similar according to differential lethality with respect to age at treatment, although the smaller micro-spot seemed to have an overall proportionately reduced effect.

The adult survivors of treated embryos also supplied interesting

results. Unfortunately, there was only a small number of flies which were finally available for study after the various steps involved in preparation. Nevertheless, there was definitely a larger proportion of castrated, or partially castrated, flies in the experimental specimens than in the controls. The condition of these flies almost exactly resembled those depicted by Geigy (1931) and are shown in Figures 36 and 37. He concluded after careful histological study that the degenerate ovary stubs were indeed only that adult tissue originally derived from the mesodermal element of the embryonic gonad. Gametes were the only missing cell type in his sectioned material. Although serial sections of these defective ovaries were not made in the present study, their external characteristics were not distinguishably dissimilar to those shown in his drawings. The histological preparation of those shown in Figures 36 and 37 was bad, but it was readily apparent by microscopic study that they were indeed similar. Unfortunately, all the material so prepared was stained improperly, and they faded considerably during the delay until it was possible to photograph them. A very large scale attempt to reproduce such specimens was carried out on 280 flies, but this too met with unexplainable ill success in the form of such a high mortality rate that insufficient numbers survived for study.

The most that can be said about the castrating effect of pole cell irradiation is that castration definitely does result. Sometimes the effect is unilateral and sometimes bilateral. The figures suggest that there is primarily a bilateral effect when irradiated at 1 and 3 hours and a unilateral effect at 2 hours, although this certainly needs further experimental evidence. There was no consistent correlation between sectioned material and the adult flies with respect to this condition. Also, it appears that the healthy ovary of the unilaterally castrated adults has an unusually high number of

ovarioles; for in the five cases reported here they numbered 10, 14, 14, 16 and 16. The range in the normal flies included such high counts, but not consistently. A comparison of the number of ovarioles per healthy ovary per fly was impossible because of the small number of flies.

Degenerate testes were found as well as the defective ovaries just mentioned. These too stained improperly, and it was impossible to photograph them satisfactorily. They were, however, unmistakably defective, for in contrast to the coiled tubular organs of the normal flies, these were shortened, shrivelled, and uncoiled. The predominance of unilaterally effected testes occurred in the 2 hour group, similar to the ovarian studies.

Lethality (Table 2, p. 35) - A certain amount of data regarding the critical periods of embryonic development was provided by the lethality figures of the histological material. All the experimental material differed significantly from the controls, but before comparing the three different groups according to stage of treatment, it should be observed that the agar block method (Table 2) produced the highest embryonic mortality. Because of this different treatment method, these embryos should not be included in the comparison between groups. Probably the best method for ascertaining the embryonic lethality prior to 15 hours (at which stage they all were fixed) is to combine the figures of the two columns, "Dead" and "Totally Abnormal" for the latter in all probability would never fully mature. Those found to be "Dead" at 15 hours, incidentally, did in almost all cases give no indication of any further development after 6 hours, which corresponds morphogenetically to the period of tissue differentiation and early organogenesis.

A comparison of the three stages of irradiation shows the maximum

irradiation effect was at 2 hours (early blastoderm) with the 1 hour treated embryos (blastema) exhibiting a somewhat less severe reaction. The 3 hour group (complete blastoderm), on the other hand, very surprisingly showed no lethal response prior to 15 hours. This once again suggests an immediate protective mechanism afforded by completed cell structure, although on this basis one might assume that the blastema irradiations would be most harmful.

Also, in the 1 and 2 hour embryos a general developmental retardation was observed prior to the 15 hour fixation. Instead of being 15 hours old developmentally, most of them were nearer 11-13 hours. In that this retardation was not nearly so characteristic of the 3 hour embryos, the relative retardation insensitivity of the 3 hour embryos compares nicely with the low mortality prior to 15 hours, as reported above. There probably is a close connection developmentally between these two phenomena, although no pertinent intermediate and correlating stages of maldevelopment were observed.

One would expect to find a high correlation between the embryonic and adult lethality rates, but by comparing the two one sees the great discrepancy. The adult lethality was considerably greater. Admittedly, there was a high lethality rate in the normal stock, as shown by the controls; but the higher rate in the experimentals suggests that there was a delayed effect which acted sometime after 15 hours of embryonic development. If this was the case, it was particularly true of the 3 hour flies, where the adult lethality jumped more than 50 per cent over the 15 hour embryonic rate. No data is available regarding the exact time of this later lethal period, but it would be interesting to know. Perhaps there was a systemic defect somehow related to gonad function.

Histological Studies - The histological studies designed to trace the pole cells sequentially from their original definitive position at the posterior tip to their eventual participation in the formation of other organs unfortunately proved to be less successful. Heidenhain's hematoxylin, although excellent for gonad studies, stained the yolk so darkly that cellular inclusions were virtually undetectable. Thus the intermediate stages of pole cell migration were obscure in almost all of the preparations. This was especially true of the 8 and 11 hour stages from which it was hoped to deduce the location of the pole cells. It is at this stage that those carried into the PMG cavity must escape into the yolk and eventually migrate into their definitive positions in the dorso-lateral mesodermal gonad rudiments. Aboim (1945) claims to have observed this, but in view of the possible variations in interpretation of histological evidence and the alternative pole cell theory by Poulson (1950, 1958), it would have been desirable to judge this situation first-hand.

Nevertheless, the above-mentioned experimental results leave little doubt that Aboim was correct, and the migration of some pole cells out of the PMG cavity is probably shown in figures 15 and 16. Also, congregated around the yolk-facing surface of this 6-7 hour PMG can be seen a large number of yolk cells, whatever their origin. Whether these yolk cells are "primary" (derived directly from the original cleavage nuclei) or "secondary" (originally pole cells which during the inter-blastodermal migration moved into the yolk) is not certain.

It can be said, however, that damaged pole cells in any form were not detectable in the embryonic gonads. Aboim (1945) claimed that he had such evidence but admitted great difficulty in detecting it. The

last unequivocal observation of either damaged or normal pole cells was found in the 6-7 hour PMG cavities, and often a mixture of normal and variously damaged pole cells were seen in the same specimen.

In hope of finding evidence for the participation of the interblastodermal pole cells (secondary yolk cells) in midgut formation (Poulson, 1947) all the material was systematically analyzed along these lines. A minimum amount of such data was forthcoming, for in the 1 and 2 hour embryos (pre-interblastodermal migration) and not in the 3 hour embryos (post-interblastodermal migration) an occasional occurrence of blatant anterior midgut (AMG) malformation was observed. An example of this is shown in Figures 38, 39 and 40. There are two obvious discontinuities in the AMG epithelium each of which extended over several sections. The anterior one is located in the precursive caeca region, which, according to Poulson, is the area of primary yolk cell absorption during AMG formation. The posterior one lies at the point of fusion between the AMG and MMG (middle midgut). From this it appears as though the interblastodermal pole cells may play a role in both these areas of midgut formation. Also, there is a large accumulation of debris in these areas. These figures should be compared with Figure 17, where completely normal midgut structure is shown. In that this type of evidence was not found in the 3 hour irradiated embryos, there is a strong case to support Poulson's earlier theory.

The frequent observation of debris in the experimental 11-15 hour midguts and the deductions therefrom posed quite a problem. The gut pictured (Figures 41 and 42) actually belongs to an embryo treated along the posterior one-third of the mid-ventral region at the $2\frac{1}{2}$ hour blastoderm stage. However, since it closely resembles those of the pole cell

irradiations and will be referred to again later in a different context, it can double as an example of the vacuolated guts found in the pole cell series. These "holes" occurring in the yolk were also observed in the control embryos but at a lower frequency and covering smaller areas in section. Usually there was a fine network of cytoplasmic strands extending across the open area, but in the cases of the largest holes this was not so. Nevertheless, it can be reasoned that since such a phenomena occurs in the controls it probably is related to a normal degeneration of some of the yolk cells.

On the other hand, there was a definite increase in the occurrence of these holes in the experimental material over the controls, but the epithelium was never damaged. Furthermore, the experimental increase was found almost entirely in the 1 and 2 hour embryos. The 3 hour group closely resembled the controls. This suggests two possibilities; (1) either the lack of complete cellular structure of the blastodermal cells at 1 and 2 hours precipitates more extensive damage by the ultra-violet followed by a general dispersion of the irradiation debris which is eventually carried into the PMG and collected in the yolk, or (2) there is an inter-blastodermal migratory movement of even the pole cell debris at 2-2½ hours. In either case the 3 hour treatments would be free of such action, as, in fact, they are. The possibility of this debris being derived from damaged presumptive PMG rudiment cells underlying the pole cells is highly improbable, because the definite PMG epithelium was not defective.

C. Blastema and Blastoderm Surface Irradiation

In order to trace the developmental fate of various regions of the blastema and blastoderm, six different areas of the egg were irradiated

at these two stages of development. Three areas (Figure 43) of both the ventral and dorsal surfaces were treated at each of the two developmental stages, i.e. blastema (1 hour) and blastoderm ($2\frac{1}{2}$ hours). The numbers shown in Figure 43 will be used below to designate the various treatments, e.g. dorsal 1, ventral 3, *etc.*

The size of the micro-spot in these treatments was the same as that administered in the second pole cell series, 60 microns in diameter. The proportional area covered is shown in Figure 43, and a view of a living egg under treatment in the ventral 3 region is seen in Figure 44. Light diffusion makes the micro-spot appear a bit larger than it actually was.

Because of a shortage of time it was impossible to conduct an exploratory experiment to determine the most satisfactory dosage. Fortunately, a 30 second administration proved to be fairly satisfactory, although it is probable that a slightly shorter period would have reduced the high lethality rate (see below) and still have caused detectable damage. This 30 second treatment was administered directly to eggs resting on an agar block. No quartz coverslip was used. In this respect, the intensity differed proportionately from that of the pole cell irradiations.

(1) Survival Results - Approximately thirty eggs were treated at each of the six regions and then transferred to culture medium by a well-tested method. It was hoped that the adults obtained after this procedure would shed some light on imaginal disc development, but the survival rate was so low that insufficient numbers were available for study. Only in the ventral 3 blastoderm irradiated group was there more than one survivor; in this case 7 of 31 matured. These flies exhibited no external morphological irregularities. All other groups suffered mainly embryonic larval deaths, and in the ventral 2 blastoderm groups a very small number of unhatched

pupae were observed. This scattered type of evidence suggests very little, other than the fact that the ventral 3 region of the blastoderm is a relatively insensitive area to irradiation.

Embryonic lethality data, on the other hand, as obtained from histological material was much more complete. It is shown in Table 3 (p.45). Because of the severe effects of irradiation at the blastema stage, this material was sectioned primarily at 6 hours so as to prevent necrosis from completely destroying any remnants of morphological structure.

The embryonic ages of the material studied are noted in the table.

It is quite obvious from this data that irradiation at each of the six regions has severe effects when administered at the blastema stage. It caused either immediate death or incomplete gastrulation with death following shortly thereafter. A variety of degenerate conditions depicted these 0-6 hour deaths, some of which are seen in Figures 51, 52 and 53.

The blastoderm treated embryos were much more interesting. The ventrally treated embryos were least affected, as can be seen in the data, and provided informative material concerning cell lineage of that region of the blastoderm. This will be considered in more detail in reporting the histological studies of this material. The high incidence of lethality in dorsal 2 blastoderm embryos results from the interference with gastrulation which certainly is a critical phase in development. All this material exhibited PMG abnormalities and/or abortive gastrulation. The consistently low embryonic mortality of embryos treated in the head region (dorsal and ventral 1) is indicative of a mosaic nature of its development. Head irradiated embryos often displayed a general disorganization and retardation, since all of the head region participates in the involution process at 10-16 hours; but in these same specimens the rest of the embryo was usually normal.

TABLE 3.

6 and 15 Hour Embryonic Lethality* of Surface Irradiations

Stage of Irradiation	Dorsal Irradiation			Ventral Irradiation		
	1	2	3	1	2	3
Blastema	$\frac{5}{13}$ = 38.5% @ 10 hrs.	$\frac{10}{11}$ = 91.0% @ 6 hrs.	$\frac{2}{9}$ = 22.2% @ 6 hrs.	-	$\frac{8}{8}$ = 100.0% @ 6 hrs.	$\frac{9}{13}$ = 69.1% @ 15 hrs.
Blastoderm	$\frac{1}{7}$ = 14.3% @ 6 hrs.	$\frac{5}{9}$ = 55.5% @ 6 hrs.	-	$\frac{3}{13}$ = 23.1% @ 15 hrs.	$\frac{0}{10}$ = 0.0% @ 15 hrs.	$\frac{0}{15}$ = 0.0% @ 15 hrs.

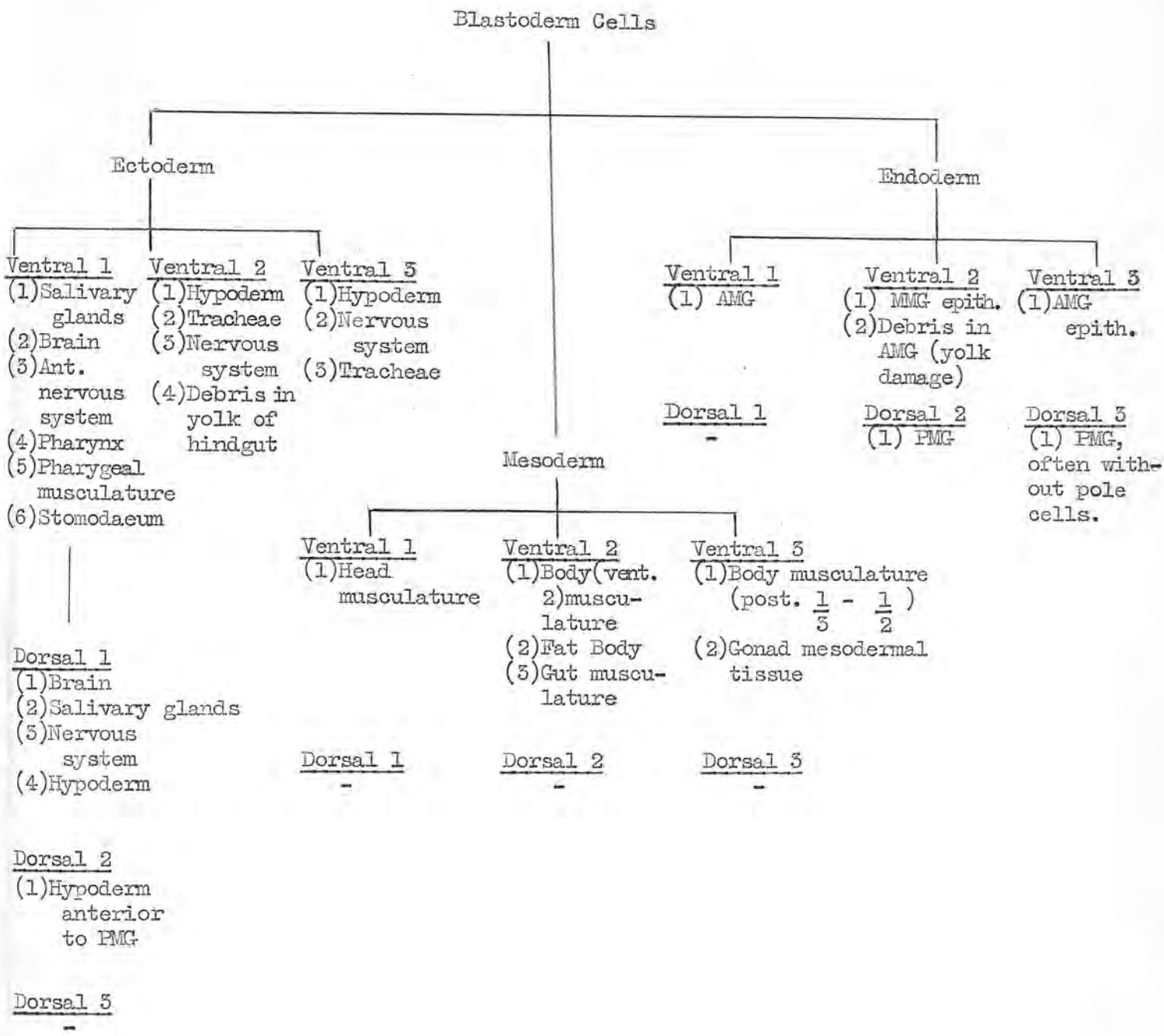
* Lethality here includes totally abnormal embryos which, although they may not be completely necrotic, obviously would never attain definitive larval structure.

Some of the similarly treated survival test embryos even reached the larval and pupal stages. These specimens probably were only retarded in embryogenesis, for if there was total disorganization of the head it is highly unlikely that the mouth hooks necessary for hatching out of the vitelline membrane would have been at all functional.

(2) Cell Lineage - The main results are shown in Figure D. This chart was compiled on the basis of the histological evidence which will be presented in detail below. Each of the six irradiated areas will be considered separately with accompanying photomicrographs.

Dorsal 1 - Both the blastema and blastoderm irradiation produced defects in two regions. The first, the head region as a whole, exhibited a variety of abnormalities which included both the ectoderm and mesoderm at 6 hours, and which usually led to a total head region disorganization of partially differentiated tissue at 15 hours. Since involution involves a coordinated action of all head ectoderm and mesoderm, a defect of any part is only magnified in later stages and leads to general malformation. Thus, the brain, pharynx, glands and head musculature are affected. Where the damage was less severe a retardation of involution of the head was observed in later development. Rather extensive damage is seen in Figure 45 (compare with Figure 14), where necrosis has set in, and where the PMG, which is the second region damaged, has an abnormal anterior wall. The cells of the latter are small and not as concentrated as in the normal embryo. In one case this PMG effect was still apparent at 15 hours. Probably severe PMG abnormalities resulted in death before a 15 hour gut structure could be reached. Often pycnotic debris was seen along the dorsal surface of the embryo between the head region and the mouth of the PMG. The amnioserosa membrane, on the other hand, was quite normal, indicating that its derivation is originally

FIGURE D



Experimentally Determined Cell Lineage of Blastoderm

from more posterior ectoderm cells which migrate forward during gastrulation (as explained by Poulson, 1950).

In that the pattern of damage was confined to the head region and the PMG, it appears that during gastrulation the dorsal 1 area normally contributes crucially to: (1) head morphogenetic movements and thus to brain, pharynx, gland, and musculature, and (2) PMG formation.

Dorsal 2 - The high mortality of these embryos emphasizes the importance of this area in gastrulation. Sectioned material showed that the primary effect of irradiation at both stages was a direct interference in PMG formation and thus in gastrulation. The damage was also seen to include the ectodermal tissue between the PMG mouth and the posterior most point of the head region. No 15 hour embryos were available for study, so it is impossible to say what the complete pattern of damage would be in those few which might have passed successfully through the period of regional differentiation and organogenesis following gastrulation.

Dorsal 3 - These embryos were not so seriously damaged as those of the dorsal 2 group, but the same type of abnormality occurred. Gastrulation was badly defected, and although a PMG invagination of some degree was usually observed, it was incomplete and spatially displaced.

Thus the evidence obtained from dorsal surface irradiation at both the blastema and blastoderm stages substantiates the postulated morphogenetic movements occurring during gastrulation. The posterior two-thirds of the dorsal ectoderm, in a series of apparently precisely coordinated movements, contributes to the invagination of the posterior endodermal rudiment and the extension of the germ band. The head region, on the other hand, seems to be little involved in this gastrulation process; and although damage inflicted at an early age has drastic consequences eventually, it does not

seem to alter greatly the morphogenesis occurring simultaneously in other parts of the 0-15 hour embryo.

Ventral 1 - The ventral series as a whole was much more interesting and informative in that early embryonic death was not too frequent and thus allowed a more complete analysis of the patterns of damage induced by the ultra-violet.

The ventral 1 irradiations, similar to the dorsal 1 treatment, produced a severe disorganizing effect on total head development. The "turning in" process of involution at 10-16 hours is completely disrupted by blastema and blastoderm irradiation. The most severe effect is evidenced by a complete absence of a majority of the ectodermal and mesodermal derivatives (see Figure C), and a disorganization and partial differentiation of the remaining tissue. The least severe effect took the form of retardation of involution. How extensive this retardation actually was is questionable, for no material older than 15 hours was available for study. Apparently in a very few cases development was successful enough so as to allow the embryo to hatch into larval form, but development ceased shortly thereafter, which may be attributable to brain and thus hormonal malfunction. This latter point, however, is purely a matter of speculation.

The relatively localized nature of the damage allowed further development to proceed in the rest of the embryo. However, often a twisting around the longitudinal axis was observed. In that the nervous system is a continuous structure extending the length of the embryo, it is possible that the damage inflicted to the brain and anterior portion of the ventral nervous system sets off an intra-dermal abnormality which in turn upsets the overall axial orientation of body parts.

The gross head malformation produced by ventral 1 irradiation prohibited any detailed studies of individual organ development. It is probable that a smaller micro-spot administered at various stages would not induce such totally disruptive effect on head involution and would enable one to trace histologically more detailed aspects of development.

Ventral 2 - Blastoderm irradiation of this region induced a large variety of defects in the ectodermal, mesodermal, and endodermal derivatives by 15 hours; but these were inconsequential with regard to total embryonic development at that stage, for mortality was nil. Blastema treatment, in contrast, was very lethal. This may well be related to the postulated "differentiation centre" (Geigy, 1931), and will be considered in the discussion.

Blastoderm irradiation did not interfere with the normal gastrulation movements, although it is probable that during this extension of the germ band (when normally a large number of mitoses occur in ventral mesoderm cells and thus aid in their spreading around the internal surface of the ectoderm) the ventral area of damaged mesoderm spreads somewhat posteriorly. This postulation would be fitting in either of two possible cases; (1) that at $5\frac{1}{2}$ hours the normal mesodermal tube invagination of the now defected cells is still carried out, (as in all probability it is, for associated ventral and lateral ectodermal structure thereafter displays no spatial defects), or (2) that the invagination does not occur in this region and thereby a large proportion of mesoderm precursive material is never available for further development.

The pattern of damage resulting from ventral 2 treatment (Figure C) included hypoderm, tracheae, and nervous system (ectoderm); body musculature, gut musculature and fat bodies (mesoderm); and midgut epithelium

(endoderm). It is probable that hypoderm defects resulted from that portion of the blastoderm irradiated by the periphery of the micro-spot, for when the mesodermal tube is invaginated a certain amount of more lateral ectodermal cells migrate medially toward the invagination. The tracheal absence would be expected, of course, for they are invaginated segmentally in the form of small pits at 6-7 hours from the ventro-lateral ectoderm. It is interesting that apparently in all cases there was no regulating mechanism which allowed damaged tissue to be replaced by proliferation of similar neighbouring tissue of equipotentiality. This was most obvious in the case of mesodermal tissue. Such an event does not necessarily imply no cellular equipotentiality, however, for it is possible that the rapid succession of developmental events prohibits any delay in development which would allow "competent" neighbouring cells to undergo compensatory differentiation.

The most significant ectodermal derivative abnormality was the common occurrence of a malformed and incomplete nervous system in the ventral 2 region (Figures 46 and 47). The lack of nervous tissue as a whole and incomplete fibre formation are evident in Figure 47. The normal tissue of Figure 46 is obviously more complete and displays the well-organized segmental ganglion more anteriorly. The interesting feature of this and the rest of the ventral 2 pattern of damage is that there is no evidence of ectodermal or mesodermal migration posteriorly during the PMG invagination on the dorsal surface. All damage inflicted in the ventral 2 region remains localized to that area. In other words, there is no complete ectodermal migration around the entire gastrular surface as the PMG rudiment moves anteriorly along the dorsal surface

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and eventually invaginates down into the yolk, as seen in Figures 12, 13, 14 and 15. Just how this somewhat independent migration of the PMG can take place will be considered later.

The mesodermal pattern of damage included body musculature, gut musculature, and fat body deficiencies. The area of defective mesodermal development was localized primarily to the ventral 2 region, both dorsal and ventral, and sometimes extended slightly into the posterior third of the embryo. Comparable examples of this type of damage will be presented in microphotographs of the ventral 3 embryos, which exhibited similar mesodermal characteristics.

The occurrence of posterior and middle midgut maldevelopment was quite unexpected and certainly has no immediately evident interpretation. It is possible that yolk and yolk cell alone damage may account for incomplete epithelial formation in these two structures, but this is probably only part of the story. It seems far more likely that some yolk cells and yolk were damaged by penetrating ultra-violet (and thus the debris in the region of defective epithelium). Assuming that those yolk cells were destined to become part of the yolk encircling "primitive gut" (Poulson, 1950) and would eventually participate in the gut formation of that particular area, there would thereby be a localized deficiency of midgut epithelial precursors. Then the failure of gut musculature (normally derived from the adjacent splanchnic mesoderm on the ventral surface) because of radiation damage would only magnify the extent of malformation, leaving a defective epithelium and debris. Unless one assumes a direct participation of mesoderm cells in the epithelium itself, this seems to be the only plausible interpretation.

Briefly then, the presumptive potential of the ventral 2 region

of the blastoderm seems to be mesoderm (and later fat bodies, and gut and body musculature) from the median cells; ectoderm (and later hypoderm, tracheae, and nervous tissue) from the more lateral cells along the midventral line; and perhaps indirectly endodermal tissue, as related to "primitive gut" mesoderm development.

Ventral 5 - Irradiation of this area provided a considerable amount of information regarding gastrulation and cell lineage. A brief summary of Sonnenblick's (1950) account of some of the more detailed aspects of the posterior endodermal invagination will be presented, for the experimental results cast some interesting light on this phase of gastrulation.

After the mesodermal invagination, or the first phase of gastrulation, has been completed the stage is set for the posterior endodermal invagination. Immediately prior to the beginning of the antero-dorsal migration of the PMG rudiment a sudden burst of mitotic activity has been observed in the extreme postero-ventral ectoderm. This region is, in other words, that group of cells immediately beneath (or "around the corner from") the PMG rudiment (Figure 12). This seems to initiate the migration of the rudiment and has been postulated by Sonnenblick as providing enough physical force to push the rudiment around to the dorsal surface. As the total ectodermal cell volume increases several smaller invaginations appear anterior to the PMG invagination, which is progressively deepening. Eventually there is a participation of cells lying anterior to the mouth of the invagination, and they form part of the anterior wall (as was shown in the dorsal 1 and 2 embryos).

As has been mentioned above, irradiation of the dorsal 1, and

ventral 1 and 2 blastoderm regions do not seem to alter the morphogenetics of the posterior endodermal invagination. This was at first somewhat surprising, for it obviously suggested the localization of all participating factors in the postero-dorsal two thirds of the embryo and the extremely posterior ventro-lateral area. Thus this phase of gastrulation would simulate a rippling effect passing enteriorly over the dorsal surface with no relative movement in the other regions of the ectoderm.

The ventral 3 embryos did, in fact, complete this picture, for blastodermal irradiation in only one case affected the endodermal invagination, and the rest of the time the damage was purely restricted to mesodermal tissue. Since the micro-spot was administered before mesoderm formation, those cells lying along the midventral line would be presumptive mesodermal tissue. The slightly lateral and unirradiated cells of this region, on the other hand, would be presumptive ectodermal cells and would migrate to the midline during mesodermal invagination. The ectodermal cells of the subsequent midline would therefore be undamaged and completely capable of rapid mitotic activity just before antero-dorsal migration of the PMG rudiment. This also accounts for the lack of defects observed in the hindgut and anus, which apparently develop from this region (see Figure A, p. 5).

Blastema irradiations, in contrast, invariably prevented gastrulation and often resulted in early death even before the first phase of gastrulation, i.e. mesodermal invagination. When early death was the case any number of factors could be responsible, but when only the PMG dorsal migration phase of gastrulation was affected it is likely that the typically more extensive effect of ultra-violet in the non-cellular blastema stage had injured a more extensive area of the postero-lateral periplasm

as well and thus destroyed the presumptive lateral ectoderm.

The ectodermally derived nervous system frequently was abnormal in the treated material. A discontinuity of the tissue is seen in Figures 49 and 50, and in the successive serial sections shown in Figures 51 and 52. For comparison, see Figure 48. The lack of fibre formation is first seen (Figures 49 and 50), and then the complete absence of any nervous tissue. The loose connection existing between the nervous system and the hypoderm is depicted by the separation of the two. Although this could be considered an artifact resulting from embedding, in this case it almost certainly is not, for haemocytes can be seen between the two tissues. The abnormal hypoderm is also shown in these figures. It is a single layer of cells which may or may not be complete. About this latter point one cannot be certain because a loss of such a delicate bit of tissue may well have occurred during embedding and sectioning.

Another type of nervous system defect was a spatial displacement of the intact tissue. This is seen quite clearly in oblique sagittal section in Figure 58 (compare with Figure 57). In this case it has lifted up and away from the underlying hypoderm (not seen) and has coursed slightly laterally. As mentioned previously, such nervous system displacements may account for slight torsions of the embryos around their longitudinal axes, as was sometimes observed.

Mesodermally derived body musculature defects are particularly well shown in Figures 53-56. It should be mentioned that those described under "ventral 2" were identical in kind to those now mentioned. Figure 53 shows both normal and abnormal conditions, in that on one side well-formed muscles can be seen spanning from segment to segment. On the opposite side, however, none are present. Figure 55, showing normal PMG, hindgut,

and anus formation, at the same time completely lacks body musculature in this posterior region. The single layer of cells comprising the hypoderm is again pictured in Figure 56.

Without doubt the most interesting mesodermally traced defect was the absence of the mesodermal gonad rudiment (Figures 41 and 42). This occurred several times unilaterally, and once bilaterally. In view of the fact that several workers, including the author, have found this rudiment to form independently of pole cell presence, this finding provided further proof of its independent mesodermal origin. In fact, in one embryo what were believed to be pole cells were seen free in the body cavity in the normal gonad vicinity.

An extremely disappointing accident occurred, however, which prevented the study of the totally "gonadless" embryos in subsequent development. Those few adults which developed from ventral 3 blastoderm treated eggs were lost. Needless to say, this would be an interesting point to investigate.

The pattern of damage of endodermal defects was found to include the PMG epithelium (Figures 49-52). It can be seen that immediately dorsal to the malformed region of the ventral nervous system there is a break in the PMG epithelium from which some yolk and what may be yolk cells (primary or secondary?) are escaping. It seems reasonable that some cells of the PMG rudiment were partially damaged during treatment by the very periphery of the micro-spot. These cells may have remained functional until the gut formation had been completed; and then, dying, they opened a small hole in the epithelium. Since there may have been defective gut musculature as well, because of the mesoderm irradiation, there was little resistance to the dense yolk. There does appear to be

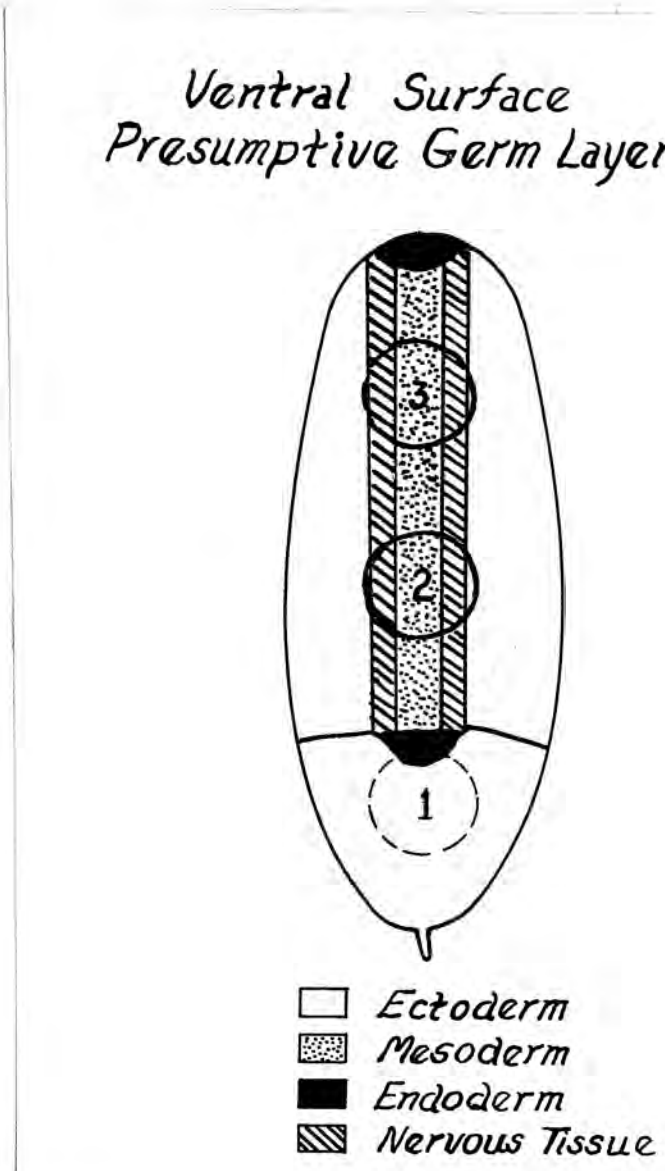
gut musculature in other regions, however, so this latter possibility probably was not the case.

Thus, ventral 5 irradiation of the blastema prevents proper gastrulation and PMG invagination. Similar treatment of the blastoderm results in ectodermally derived hypoderm, trachaeal, and nervous system defects; mesodermally derived body and gut musculature, and gonad defects; and endodermally derived posterior midgut malformations.

The ventral 1 region contributes crucially to the entire development of the head by virtue of the involution process. The midventral 2 and 3 regions of the blastoderm are responsible for mesoderm formation in the posterior two-thirds of the embryo, and the regions slightly lateral to the median line later become ventral ectodermal tissue.

A diagrammatic representation of the general presumptive character of the ventral surface of the blastoderm is shown in Figure E, p. 58. This compares with Poulson's postulated anlagen plan, (Figure A, p. 5).

Figure E.



Ventral Surface Presumptive Germ Layers just Prior to Gastrulation. The egg is seen as though viewed from the ventral surface, and the three areas of irradiation are shown by the circled regions.

DISCUSSION

A. Pole Cell Fate and Gonad Development

The carefully timed irradiations of pole cells at various stages and the subsequent study of the definitive embryonic gonad proved beyond any doubt that in normal development the gamete precursors (Geigy, 1951) are derived from those pole cells carried into the posterior midgut invagination during gastrulation. Thus Poulson's hypothesis that the interblastodermal migrating pole cells entered into gonad formation was shown to be incorrect. The fact that post-interblastodermal migration irradiations had the most pronounced effect not only pinpoints the PMG pole cells as being those entering into the gonad, but it also discounts the possibility of a general equipotentiality of both groups of cells. Seemingly, if this equipotentiality existed a deficiency of PMG pole cells would be compensated for by the earlier migrating pole cell's incorporation into the gonad. This characteristic would account nicely for a theoretical explanation of curious gonad development in the two mutants, Lff 11, (Ede, 1954) and nasrat^{A} (Counce and Ede, 1957), where normal gonads formed in the absence of a normal PMG invagination. However, numerous alternative theories could be put forth which would explain this finding; but in view of the extreme complexity of a developmental system such speculation is fruitless without experimental evidence as a basis.

Repeated occurrences of mesodermally derived gonads without pole cells was unquestionable evidence of the mesodermal rudiments' independent formation. No inductive influence by the pole cells was needed, as Counce and Selman's (1955) interpretation of their ultra-sonic results suggested. They found in a small number of cases that a complete disorganization of the egg's constituents resulted in a displaced but complete gonad. From

this they deduced that it was the pole cell influence acting upon competent mesoderm from any one of various regions which initiated the gonad's development. However, it could also be argued that a few competent pole cells by chance became situated next to gonadally determined mesoderm after the chaotic effects of ultra-sonics.

The independent mesodermal derivation of the gonadal sheath was even more graphically demonstrated by its absence after irradiation of the posterior third of the presumptive mesoderm cells of the blastoderm. In fact, free pole cells but no mesodermal rudiment were seen in the body cavity near the normal gonad region. These results were very similar to those of Haget (1953), who by microcautery and micro-vivisection produced experimental evidence of the mutual independence of the pole cell and mesodermal rudiments of the gonad; of the regional origin of the mesodermal rudiment; and of the lack of endodermal influence on the competence of that mesodermal rudiment.

The adult condition of both sexes of the pole cell irradiated embryos took one of three forms; two normal reproductive organs, one normal and one defective, or both defective. The defective condition resembled exactly that illustrated by Geigy, but his observation regarding the correlation between the time of treatment and the resultant adult condition were not fully duplicated. He found, as did Aboim (1945), that irradiation before pole cell formation led invariably to bilateral "castration". In this regard the present results were similar, because some of both the histological and the adult specimens were bilaterally "agametic", but none unilaterally so. However, it was here that the similarity ended. To obtain unilateral castration Geigy claimed the irradiation only need be <u>any</u> time after pole cell formation, and Aboim

claimed it must be after the beginning of their migration along the dorsal surface. Contrary to both of these, the present experiment produced unilateral adults from 2 hour irradiations and a mixture of both at 3 hours. To complicate the issue further, there was no correlation between the adult and embryonic condition, except in the above-mentioned 1 hour treatment. Thus, since only small numbers of such adults were available it was impossible to draw any conclusions. All that can be said is that the pre-pole cell irradiated embryos were sometimes bilaterally agametic as embryos and as adults, and in this respect corroborated both Geigy and Aboim; but contrary to the results of those two workers, a mixture of conditions were obtained from irradiations after pole cells had formed. A large scale attempt to reproduce the unilaterally affected embryos failed because of an unaccountably low viability of the treated embryos, so no conclusive results on these later irradiations were forthcoming. Geigy and Aboim both presented elaborate theories regarding the morphogenetics underlying the bilateral or unilateral effect, but until repeatable results are obtainable from eggs treated at the same stages such efforts are in vain.

When there was a normal ovary unilaterally, there appeared to be a higher number of ovarioles in that ovary than in flies with two normal reproductive organs. Geigy also reported similar results in the form of a "larger" ovary. This suggests a systemic compensating aspect of development, probably occurring during the larval period. Saveliev (1928) and Kerkis (1933) observed that ovariole number was determined during larval life. Also, it is known that nutritional and genetic factors affect ovariole number (Saveliev, 1928; Robertson, 1957a and b). It is probable that the micro-beam will be a great aid in further study of ovary

development and function, for it should provide a neat method to induce experimentally the occurrence of flies with unilaterally functional reproductive organs. In fact, it was hoped that the above-mentioned large scale attempt to obtain such flies would provide material for that very purpose.

The puzzling fate of the interblastodermal migrating pole cells remains an open question; although, a minimum amount of evidence was obtained to support Poulson's earlier suggestion that they played a key role in midgut epithelium formation where the anterior and middle midgut rudiments fuse. To account for the relative infrequency of such embryos (previously described in detail in "Results") two possible explanations exist: (1) There is an abundance of interblastodermal pole cells which are capable of participating in midgut formation, and it is only in rare cases that a sufficient number are damaged to such an extent that there is none available for this function, or (2) slightly damaged pole cells undergo the normal interblastodermal migration and actually assume their midgut role before degenerating completely. In either case unambiguous histological evidence would only rarely be obtained, because the development of such a defect could occur over a rather extensive period of time and its morphological manifestation would be transitory. A more complete histological study of properly stained irradiated specimens is obviously required to provide a satisfactory answer to this question.

B. Cell Lineage and Morphogenesis

(1) Cell Lineage - The presumptive areas of the blastoderm, as deduced from tracing histologically the ultra-violet "marked" cells, substantiate in general the anlagen plan put forward by Poulson (see Figure A, p. 5). Furthermore, there was no evidence of changes occurring in the location

of these presumptive areas between the blastema and blastoderm stages. The size of the micro-spot in the present work completely confirmed all evidence regarding the formation of the primary germ layers (Figure E, p. 58), and in a general way confirmed the theories on organ differentiation thereafter (Figure D; compare with cell lineage diagram of Poulson, Figure B, p. 25). It is readily evident from this exploratory work that a smaller micro-spot would enable even greater refinement in the tracing of these presumptive areas and could be used most successfully in the study of the mosaic nature of the egg.

(2) Gastrulation - The localized nature of the blastoderm irradiations, coupled with Sonnenblick's (1950) systematic description of all available normal gastrulation data, provided suggestive evidence regarding the physical forces involved in the posterior endodermal invagination. This evidence has been carefully described in "Results". There is no doubt about the localization of the participating factors to the dorsal 2 and 3 and ventral 3 regions, and thus discounts any consideration of a general shifting of the entire blastoderm. Instead, Sonnenblick's postulation that the initiating force for the PMG rudiment migration arises in those extreme postero-ventral cells immediately beneath the rudiment (Figure 12) seems entirely the case. It was not clear as to whether the force and compression effect actually preceded the differentiation of the PMG rudiment, although it would seem likely, in that the elongation of those cells could be caused at least partially by physical forces. The several shallow invaginations subsequently appearing anterior to the advancing PMG rudiment are also probably caused by the forces resulting from this rapid cell proliferation and resultant increase in volume of surface cells.

Further evidence for this "force concept" of endodermal formation was found in the different effects of ventral 3 irradiation at the blastema and blastoderm stages. The results of the latter treatments are discussed above, but the blastema results were of a completely different nature. The typically more extensive effects of blastema irradiation were once again obvious, but there was a distinct morphological characteristic of those which survived until at least 6 hours embryonic age; there was a ventral hernia accompanied by only a slight anterior migration of the PMG rudiment. In the region of the hernia there was no ectoderm and thus probably a less compact cell concentration around the entire surface of the embryo. In such a condition an increasing cell volume resulting from the postero-ventral ectoderm proliferation would certainly not cause a compression and movement of the adjacent endodermal rudiment, for any force developing would meet with no resistance.

Another aspect of morphogenesis which seems to be particularly related to gastrulation is the ultimate orientation of body parts. This, of course, is not unexpected, for the subsequent tissue relationships occurring in the embryo would undoubtedly be related to the original spatial distribution of the primary germ layers. The experimental evidence which contributes to the understanding of the primacy of the germ layers in overall embryonic orientation was found in the slight embryonic torsions along the longitudinal axis following ventral irradiations. In all three regions it was found to be an occasional irradiation consequence. Certainly in the two posterior regions this could be interpreted as having a mesodermal basis; but in view of the similar effects arising from the ventral 1 region, (where mesoderm invagination is less extensive), it is more probable

that the nervous system is the primary influencing factor since it extends the length of the entire embryo. Of course, by going back one step further in this causal analysis one sees immediately that the definitive nervous tissue was, in fact, the ectoderm lying just lateral to the presumptive mesodermal cells at the time of irradiation (Figure E, p. 58). Therefore, it can be reasoned that in all probability the micro-spot was slightly displaced to one side, and that the major contribution of presumptive nerve cells was made by the more complete opposite side. This obviously would result in an early lateral displacement of a part of the nervous system.

(3) Activation and Differentiation Centres - On the basis of experimentally induced lethality there was no evidence of an activation centre (or "formation centre", Seidel, 1935). This region, often found in the "indeterminate type" of insect eggs, strongly suggests the presence of a biochemical centre at the posterior tip of the egg which, upon activation with nuclei, emits a diffusible chemical substance. This substance then seems to activate a region on the ventral surface lying about one-third of the egg's length from the anterior end, or the presumptive thorax region. This is the region of the first distinguishable differentiation, the thickening of the germ band, and thus has been termed "the differentiation centre". Considerable evidence for the existence of this latter centre has been obtained for almost the entire range of indeterminate to determinate eggs (Waddington, 1956).

Geigy (1931), Yao (1950), and Imaizumi (1958) have described various forms of developmental phenomena in Drosophila which seem to have their origins in the ventral region of the blastoderm just posterior to the head. Imaizumi observed in centrifuged eggs that blastoderm formation proceeds both backward and forward from this point, and Yao discovered by histochemical work that acid and alkaline phosphatase

appear first in this region just after the mesodermal invagination and then spread gradually throughout the rest of the ectoderm. Geigy observed a sensitivity gradient to ultra-violet which had its centre in this presumptive thorax area and proceeded posteriorly as a function of age. An examination of the embryonic lethality effects of the surface irradiations in the present work (Table 3, p. 45), shows the concurrence of these results with Geigy's.

It is tempting from the embryologist's point of view to try to extract from these various bits of corroborating evidence a primary mechanism which might initiate the onset of differentiation. Seidel first attempted this in the postulation that it was associated with a simultaneous yolk contraction which thus allowed, if not directed, the morphological distribution of the rapidly proliferating cells of the germ band. Yao's investigation was a more refined attempt at seeking an answer, but in essence probably provided little more than a confirmation of what would be expected biochemically in highly active cells. A high concentration of phosphates seemingly indicates only that a respiratory increase takes place. His attempt to find correlated RNA and -SH concentrations was unsuccessful and thus provided no theoretical evidence for localized protein synthesis, which might account for differentiation characteristics. There is the possibility that the available techniques are too insensitive to detect small gradients. Seidel's yolk contraction hypothesis although not too convincing, cannot be disproved on the basis of the present work. Also, ultra-violet penetration through protoplasm (50 microns, Bachem and Reed 1951) is probably sufficient to pass through the periplasm (approximately 40 microns) in appreciable amounts.

(4) The Mosaic Nature of the Egg - The rapidly accumulating evidence dealing with oogenesis emphasizes the developmental continuity from the newly synthesized egg to its first steps as an independent organism just after fertilization. The mosaicism soon apparent certainly is not explainable in a knowledge of early embryogenesis. The present results demonstrate that this mosaicism is nearly complete by one hour after deposition. It is possible that slight regulative powers exist during this first hour (Howland and Sonnenblick, 1956), but certainly not to any considerable degree. The only such evidence obtained in the present work was not very convincing. The one hour pole cell irradiations (see Figure C) produced a higher number of embryos with pole cells incorporated into the gonad than did either the 2 or 3 hour treatments. There was no difference in treatment technique which might explain this, and in addition, one would be inclined to believe that the lack of cell structure at one hour would facilitate even more widespread damage because of no "protective" cellular sub-units having yet formed. Nevertheless, the fact remains that there appeared to be less damage inflicted at this 1 hour stage, although the 2 hour mortality was higher. It can be suggested, therefore, that there was a recovery mechanism, or regulative power, of that presumptive pole cell area. Poulson (personal correspondence) feels that his results suggest the same thing. In spite of the plausibility of this interpretation, the present author remains sceptical, because this is the only case in the present work to support "non-mosaicism", whether it resembles recovery, regulation, or whatever. Unfortunately, no very early micro-spot irradiations were administered, but the refinement of the apparatus would make such an experiment very possible

and would probably provide worthwhile information regarding the onset and extend of the mosaic qualities of the egg.

With sound results from this very early "marking" of the egg, one could then hope for a logically continuous correlation with relevant aspects of oogenesis. The work of Jacob and Sirlin (1959) with auto-radiographic methods has already provided many interesting results suggesting the DNA and RNA relationships, and Gorska and Sirlin (in press) have obtained data pertinent to protein synthesis. This type of work coupled with the knowledge of the sub-microscopic structure (King, 1959; Waddington and E. Okada, in press) might well lead to an improved understanding of the synthesis of the mosaic egg.

C. Critical Periods in Embryogenesis

Before examining the embryonic lethality results it is first necessary to differentiate between two types of death which probably occur in the embryo. In experiments using external interference in the normal developmental pattern, it is helpful to have a classification of the biological responses according to their relation to the interference. For the present purposes the two general types of embryonic death can be termed "direct" and "indirect". The former refers to an immediately lethal reaction in the embryo with its obvious causation being the external interference. In the present experiment this apparently took the form of a toxicity resulting from ultra-violet damage and suggested a spreading effect. As mentioned previously, this was particularly evident in blastema treatments where there was a pronounced susceptibility. This susceptibility was probably related to the lack of cell formation, which would seemingly serve as a protective barrier to the diffusion of the toxic substance.

The indirect death response would be a later lethal effect based upon abnormal morphogenetic and epigenetic relationships within the embryo. In the present work the ultra-violet damage was later manifested primarily in abnormal gastrulation and early tissue differentiation. In other words, the early damage was not significant developmentally until certain epigenetic crises. These epigenetically critical periods have been well established by previous workers (Review: Imaizumi, 1958a). They are blastoderm formation (0-$2\frac{1}{2}$ hours); mesodermal and endodermal tissue differentiation, or gastrulation ($3\frac{1}{2}$-5 hours); shortening of the germ band (9-11 hours); and eruption of gas into the tracheae (20 hours).

The gastrulation defects were usually directly correlated with the role of that particular area which had been irradiated. This has been mentioned previously in "Results" and the above discussion of gastrulation. Briefly it can be said that the major gastrulation abnormalities arose from the malfunction of a small region of irradiated cells which upset the biophysical factors occurring and participating in normal gastrulation.

It should also be mentioned in this context that the exceedingly high lethal effect of ventral 2 blastema irradiations probably was a combination of direct and indirect responses on the part of the embryo. It was direct, in that the toxicity arising probably would be particularly widespread in the blastema; and it was indirect in that the apparently highly significant action of the differentiation centre was damaged in its formative stages.

The higher lethality of the 2 hour pole cell treatments versus the 1 and especially the 3, is not easily explainable. One might postulate that the presence of nuclei (in comparison with the blastema stage) is

significant, but this is incompatible with the 5 hour results, where an even greater number of nuclei are present. Also, it could be suggested that damage was inflicted to the postero-ventral ectoderm cells which are responsible for the important rapid proliferation and possibly the physical force behind the PMG migration; damage to this area, as was explained above, halts normal gastrulation. In spite of all the possible explanations, none is any more convincing than the other, and it remains an open question.

The delayed lethality of the 5 hour pole cell treated embryos also is unexplainable. The absence of any indirect effect prior to 15 hours of embryonic development, but the very high lethality prior to emergence, certainly is interesting and could be much more thoroughly investigated with careful observation of late embryonic and perhaps larval and pupal stages.

D. The Applicability of Micro-beam Irradiation

The present experiment demonstrates the value of the micro-beam apparatus as a "tool" in insect embryology. It provides the most precise method for experimental interference with the developmental events of the early embryo. Regarding Drosophila in particular, it could be used satisfactorily in more detailed and less "regional" analyses of the presumptive areas, and especially in studies of imaginal disc formation and function.

Also, development of unilaterally agametic adults from pole cell irradiations suggests a means for imposing an isolated variable upon the adult ovarian condition. These unilaterally affected flies could then be subjected to genetically controlled studies of egg production and related phenomena (Robertson, 1957a). This same approach might be used

in the study of hormonal roles in insect morphogenesis (Bodenstein, 1954).

The highly refined techniques of tissue culture render the microbeam a suitable tool for physical interference in studies of tissue growth and differentiation. A certain amount of this type of work has already been done (Zirkle, 1957), but the exploitation of the possibilities of the apparatus is far from complete.

For any of this work the introduction of a monochromator and photoelectric dosimeter into the apparatus is highly desirable and would enable a much more refined approach to the problems. These additions would provide a physical specificity which was lacking in the present work.

CONCLUSION

(1) The utility of ultra-violet micro-beam irradiation as an effective tool in the study of insect embryology has been demonstrated.

(2) Experimental verification of the postulated presumptive germ layers of the blastoderm of Drosophila melanogaster has been presented (Figure E, p. 58). Particular organ development was traced to various regions of the respective germ layers (Figure D, p. 47).

(3) a. The precursive gamete cells of the adult reproductive organs are those pole cells carried into the posterior midgut invagination during gastrulation.

b. The mesodermal rudiment of the embryonic gonad is formed independently of these precursive gamete cells; it is derived from the posterior half of the midventral mesodermal invagination.

c. The interblastodermal migrating pole cells do not become incorporated into the embryonic gonad as gamete precursors. Instead, they probably assume a role in the formation of midgut epithelium.

(4) The condition of the adult reproductive organ of pole cell irradiated embryos varies and appears to be at least partially dependent upon the stage of development at which they are irradiated. Both bilateral and unilateral defects were observed in these adults, but only those embryos treated at the blastema stage were of only one kind, i.e., bilateral.

(5) Detailed information was obtained regarding the morphogenetic factors of the endodermal invagination (see "Discussion").

(6) Corroborative evidence was found for the presence of a "differentiation centre" in the presumptive thorax region of the blastema and blastoderm, although it was not possible to gain further information about its functional

character.

(7) The embryos exhibited a much greater susceptibility to ultra-violet damage at the blastema stage than at the blastoderm stage. This apparently is related to the incomplete cellular formation found in the blastema.

(8) Gastrulation was a critical period in the development of irradiated embryos. The fault in morphogenesis was easily traced to the irradiation damage and that particular role in gastrulation which the damaged cells would normally assume.

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KEY TO FIGURES

A	-	anterior
AMG	-	anterior midgut
B	-	"pressure buckle"
BN	-	blastoderm nuclei
BR	-	brain
CYT	-	cytoplasm
CS	-	cytoplasmic strands
D	-	dorsal
DE	-	debris
EC	-	ectoderm
ES	-	esophagus
G	-	gut
GB	-	germ band
GO	-	gonad
H	-	haemocytes
HEG	-	head ectoderm
HG	-	hindgut
HY	-	hypoderm
MG	-	midgut
MP	-	Malpighian tubule
MS	-	mesoderm
MSV	-	visceral musculature
MUS	-	body musculature
NF	-	nerve fibre
NEC	-	necrosis
NS	-	nervous tissue
NUC	-	nuclei
P	-	posterior
PC	-	pole cells
PH	-	pharynx
PMG	-	posterior midgut
PMGR	-	posterior midgut rudiment
PMUS	-	pharyngeal muscle
PV	-	proventriculus
SLG	-	salivary gland
SP	-	spiracle
ST	-	stomomodaeum
TR	-	trachea
VAC	-	vacuole
VM	-	vitelline membrane
VNS	-	ventral nervous system
YK	-	yolk

Figure 1. Diagrammatic representation of the experimental apparatus. All mirrored surfaces are shown in solid black.

The ocular of the microscope is actually rotated 90° in practice, such that it would be extending outward toward the viewer with respect to the surface plane of the diagram.

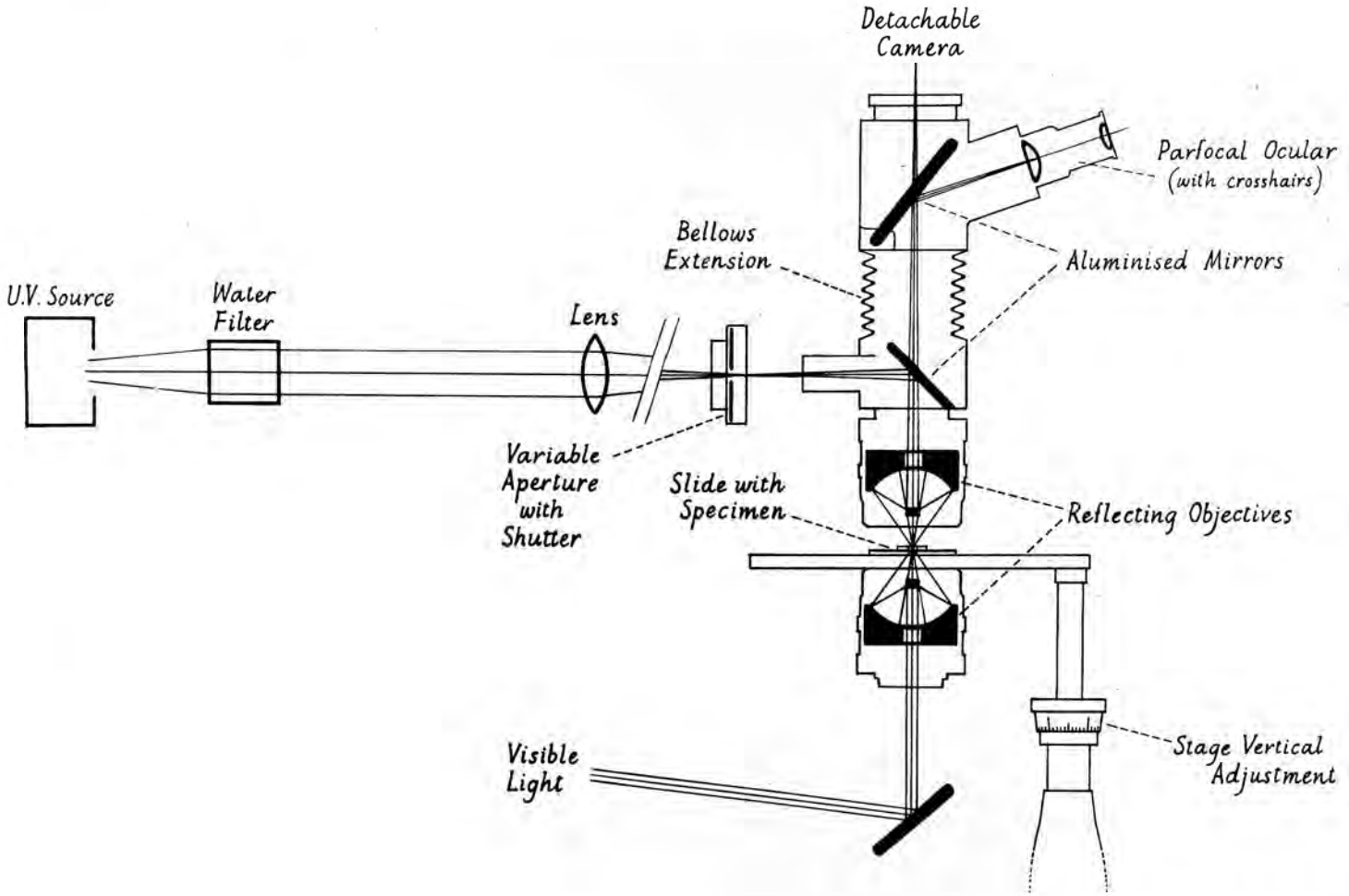

Detachable Camera
Parfocal Ocular (with crosshairs)
Bellows Extension
Aluminised Mirrors
U.V. Source
Water Filter
Lens
Variable Aperture with Shutter
Slide with Specimen
Reflecting Objectives
Visible Light
Stage Vertical Adjustment

Figure 2. The reflecting objective of the miscroscope.

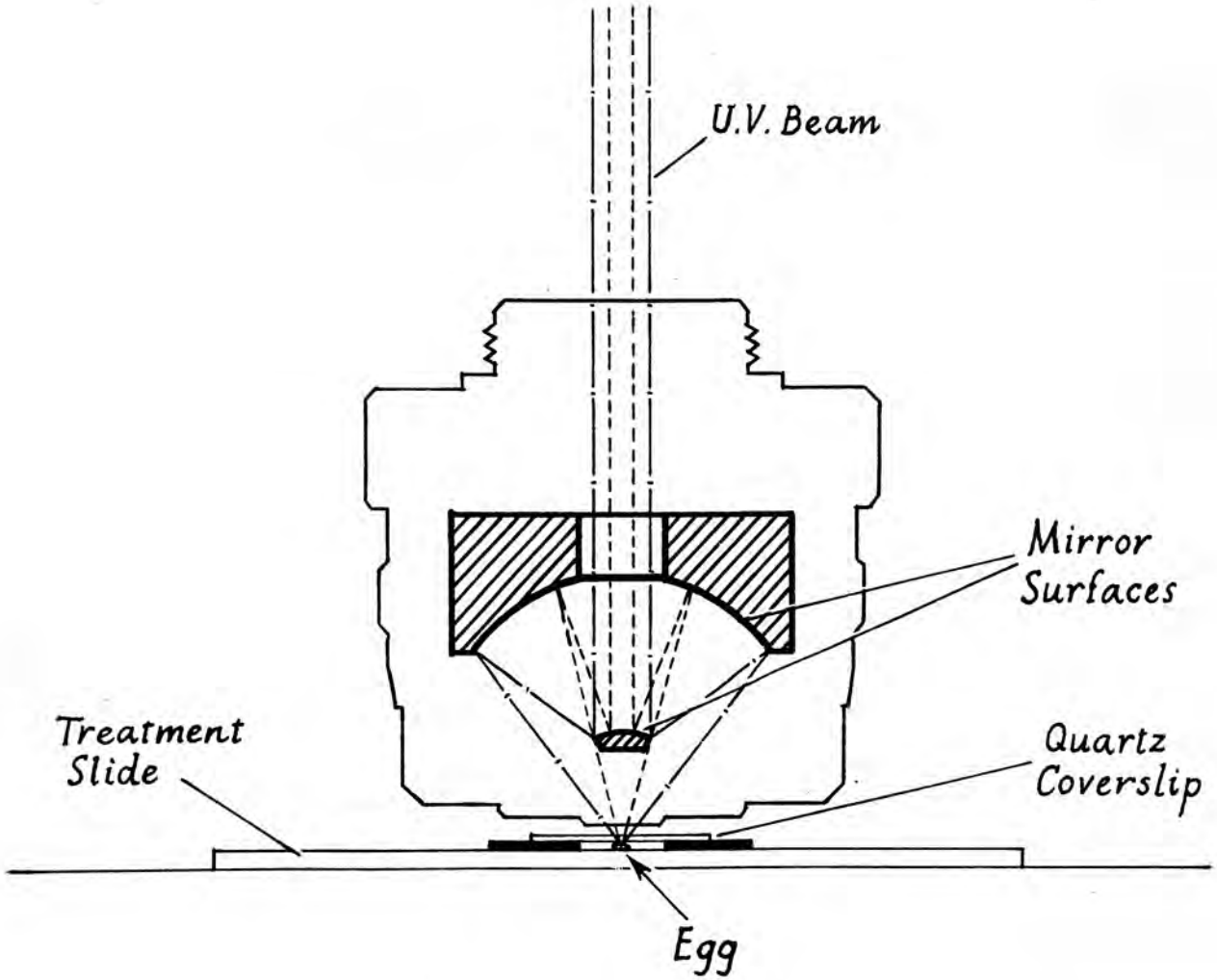

U.V. Beam
Mirror Surfaces
Quartz Coverslip
Treatment Slide
Egg

Figure 5. The improvised cavity slide used for pole cell irradiation.

The centre piece with the hole in its centre was a thin piece of metal foil which was approximately the same thickness as the egg. The eggs were then placed in the hole, and a quartz coverslip was eased over them. All this procedure took place while the entire slide was submerged in a distilled water medium.

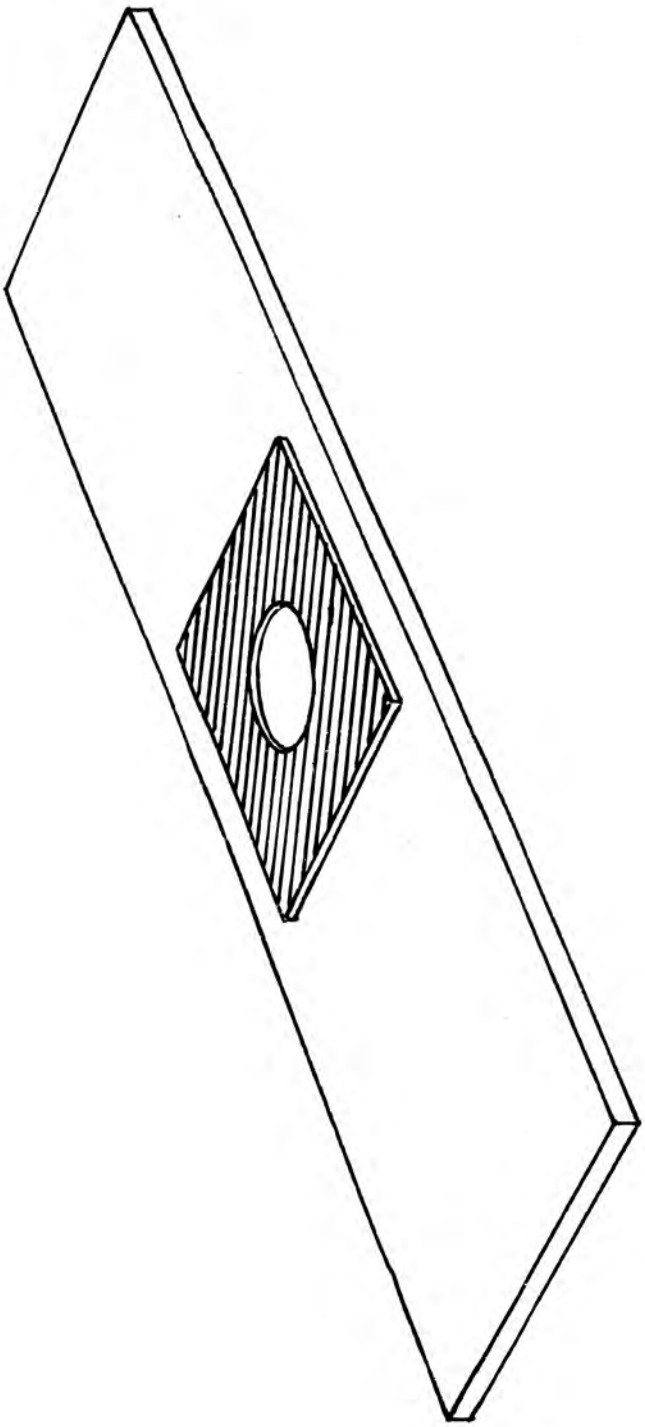
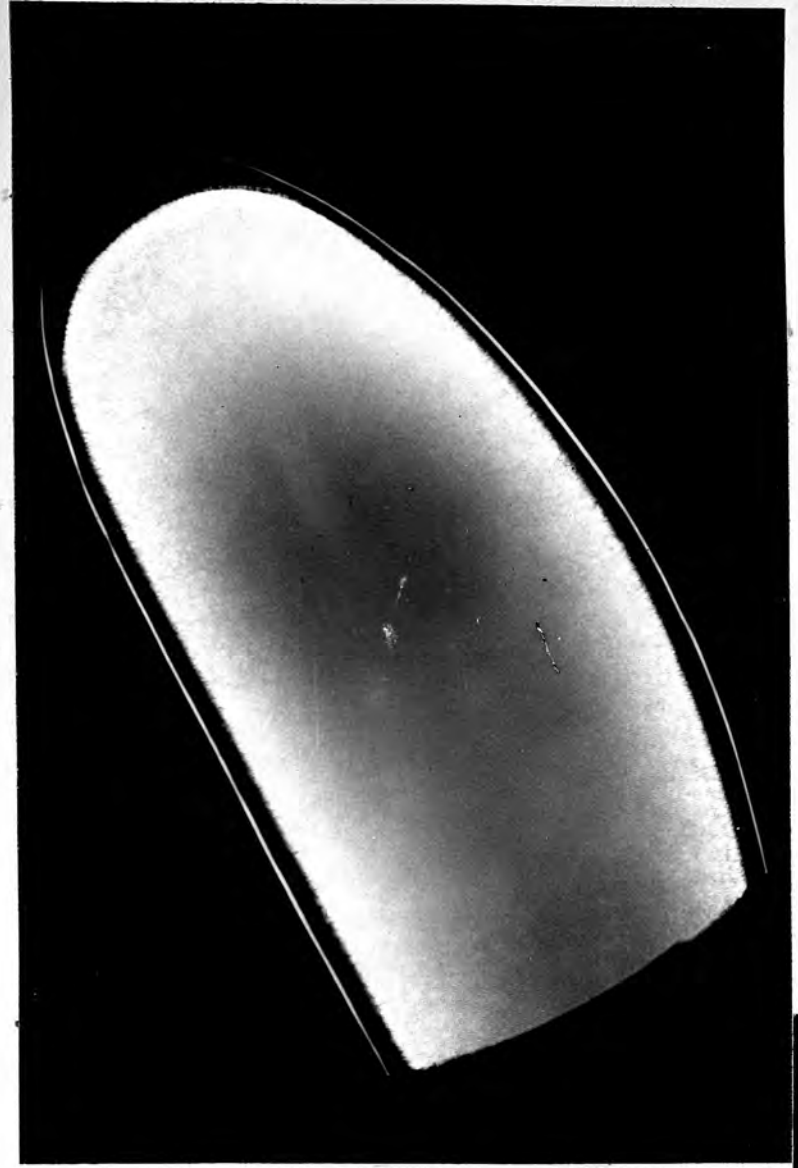


Figure 4. The posterior two-thirds of a living egg at 1 hour after deposition (blastema). Notice that the pole cells are not yet formed. x 72

Figure 5. The posterior two-thirds of a living egg ca. 2 hours after deposition. Notice the well formed pole cells at the tip, and the presence of a few nuclei around the entire surface of the egg in this early stage of blastoderm formation. x 72.



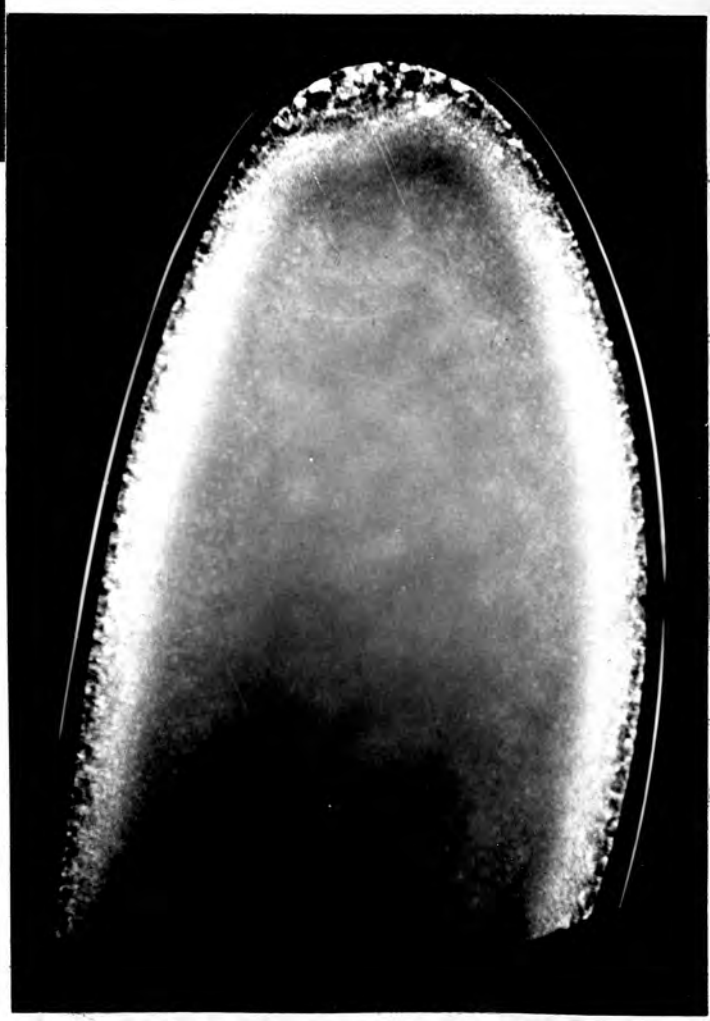
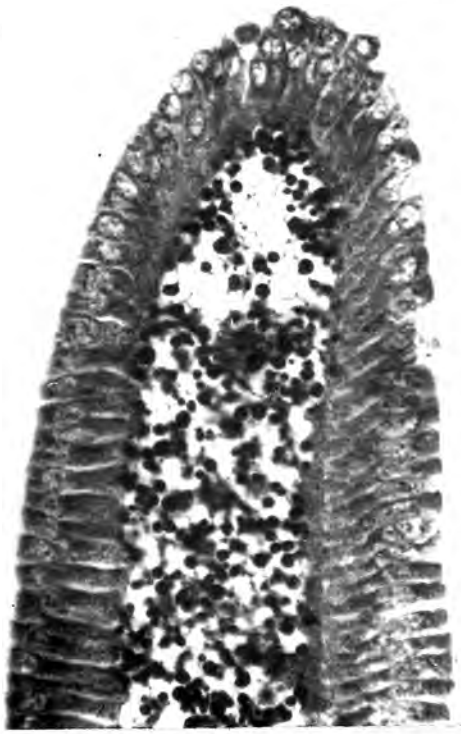


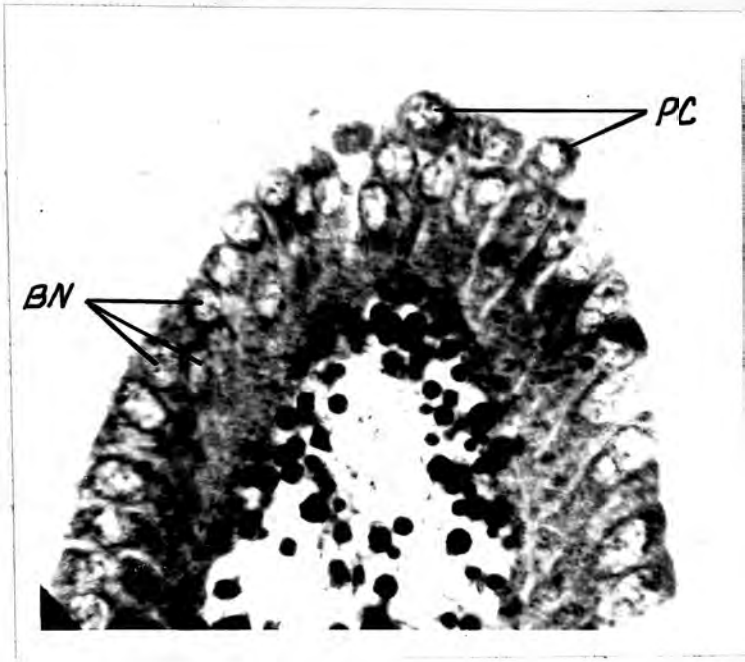
Figure 6. The posterior two-thirds of a living egg 3 hours after deposition (complete blastoderm). The pole cells at the tip are obviously distinct from the columnar and densely packed blastoderm cells underlying them. x 72.

Figure 7. A 3 hour egg (blastoderm) seen in sagital section. Notice the compact nature of the blastoderm cells, and the large spherical pole cells above them at the tip. x 400.

Figure 8. Same specimen as seen in Figure 7, but at higher magnification. x 950

Figure 9. Dying egg at 1-1½ hours. The vacuolation of most of the cleavage nuclei which are now located in the periplasm indicates a recent death, although their spatial distribution is typical of eggs this age. x 400

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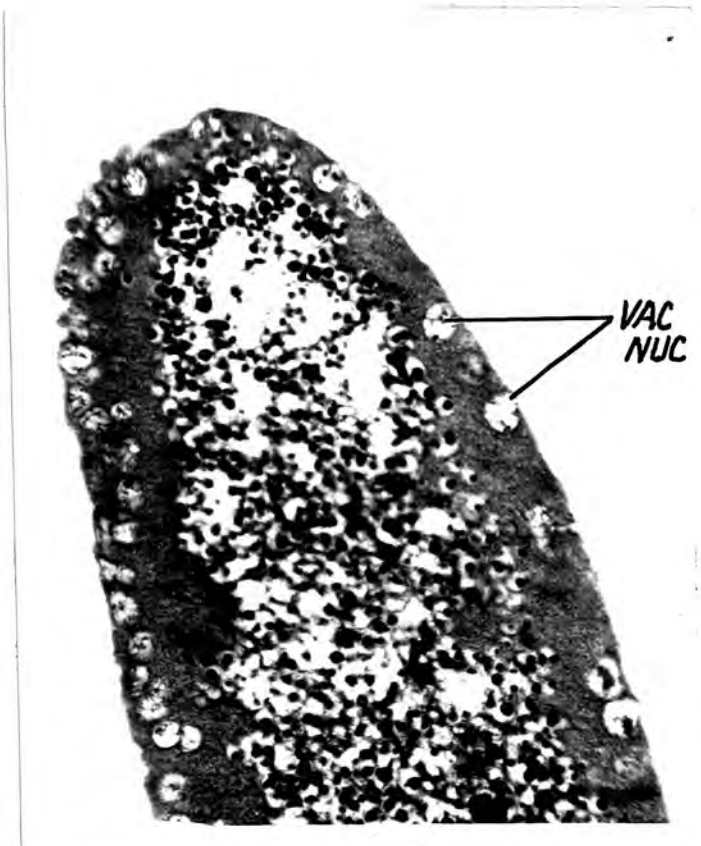
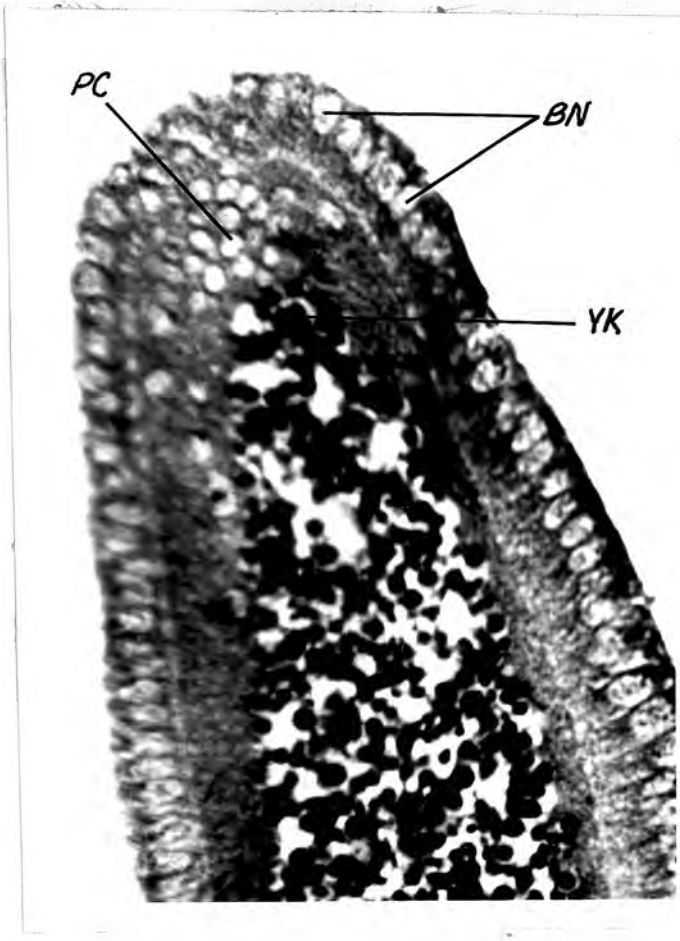

VAC
NUC

Figure 10. Egg of ca. 2-2½ hours. The pole cells are seen during the interblastodermal migration. Their spherical appearance makes it easy to distinguish them amongst the blastoderm cells, and there is a particularly large number just beneath the periplasm in the yolk. x 400.

Figure 11. Same specimen as seen in Figure 10, but at a higher magnification. x 950

Figure 12. Completed blasoderm just prior to gastrulation. Pole cells still appear to be migrating interblastodermally, however. The slight indentation just along the dorsal surface (on the left) is not the PMG rudiment, but one of several "buckles" which arise temporarily during the PMG migration. This latter tissue is not prominent in this section because the section slightly lateral of the midline. x 400.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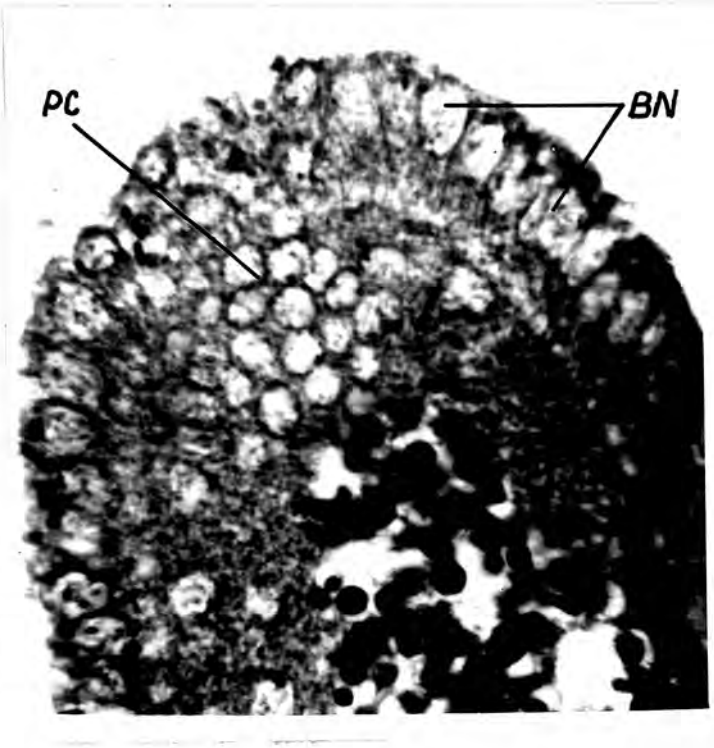
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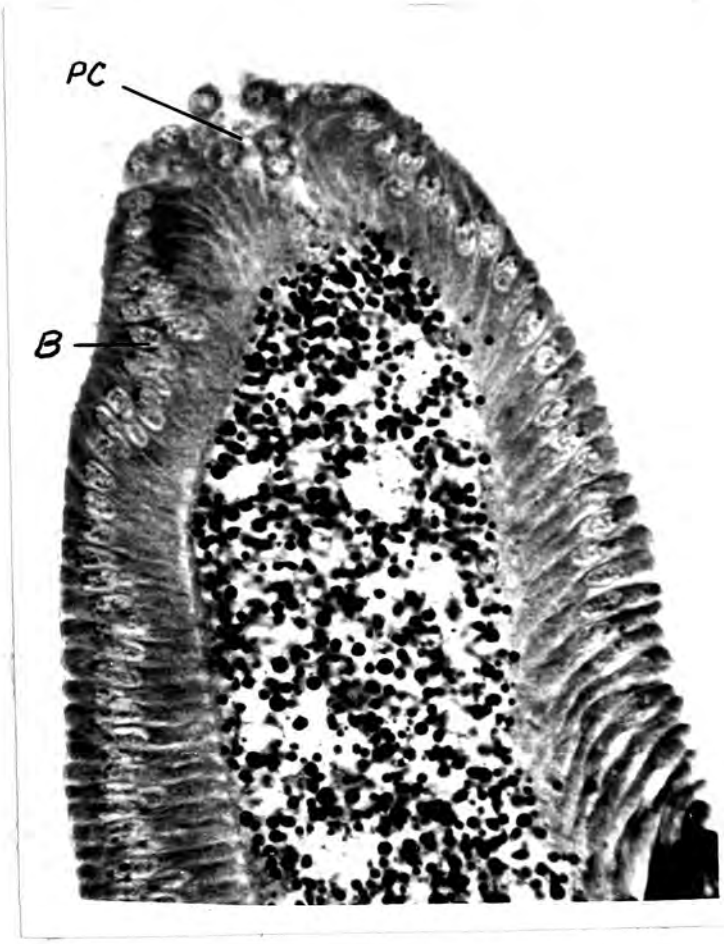
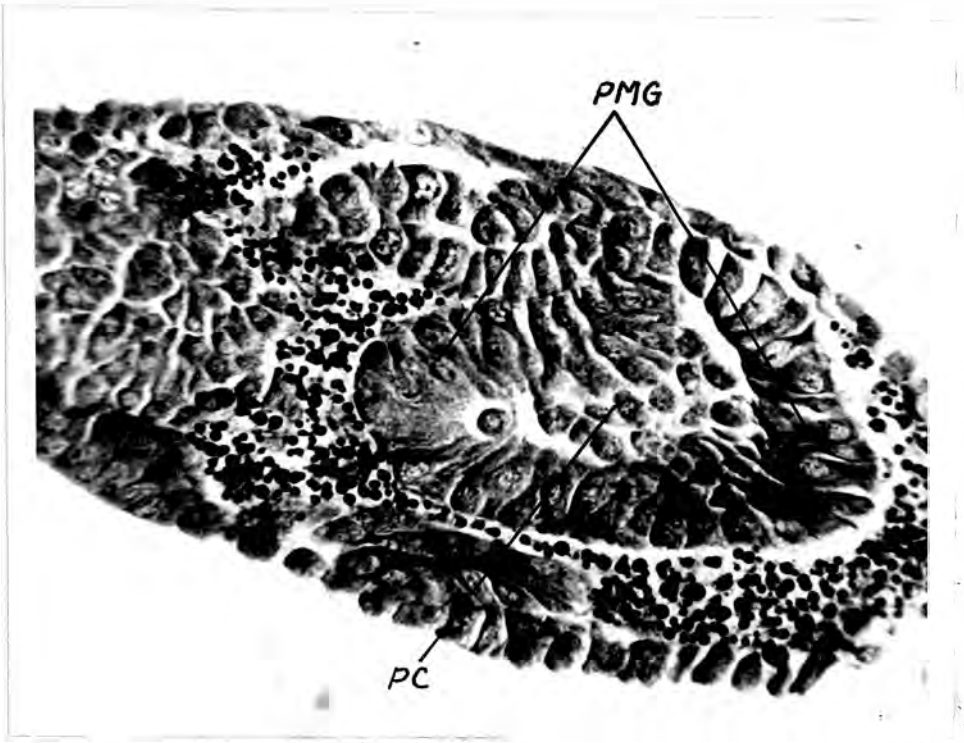

PC
B

Figure 13. A 5 hour embryo showing the PMG invagination (sag. section, with the anterior to the right). The pole cells are easily distinguishable in the cavity of the invagination.

The egg as a whole is somewhat swollen as a result of the fixation puncture, but there is little distortion of the PMG invagination.

x 400

Figure 14. An embryo of ca. 6 hours. The general morphology is well shown in this sag. section. The pole cells are seen in the PMG invagination; the stomodaeum at the anterior ventral surface; and the mesoderm as opposed to the ectoderm along the ventral surface. x 200


PMG
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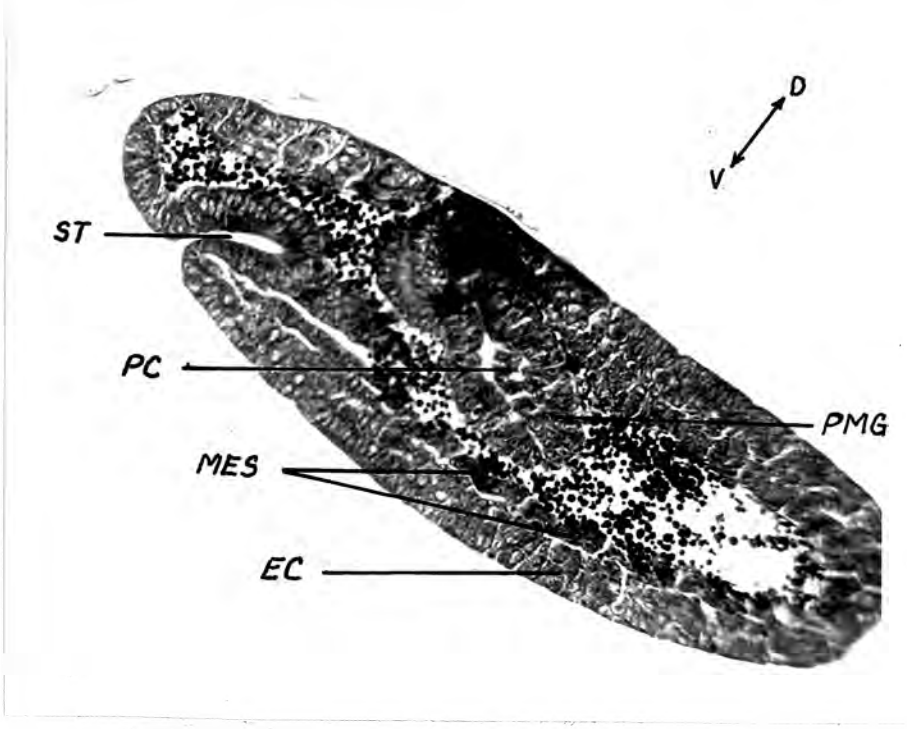
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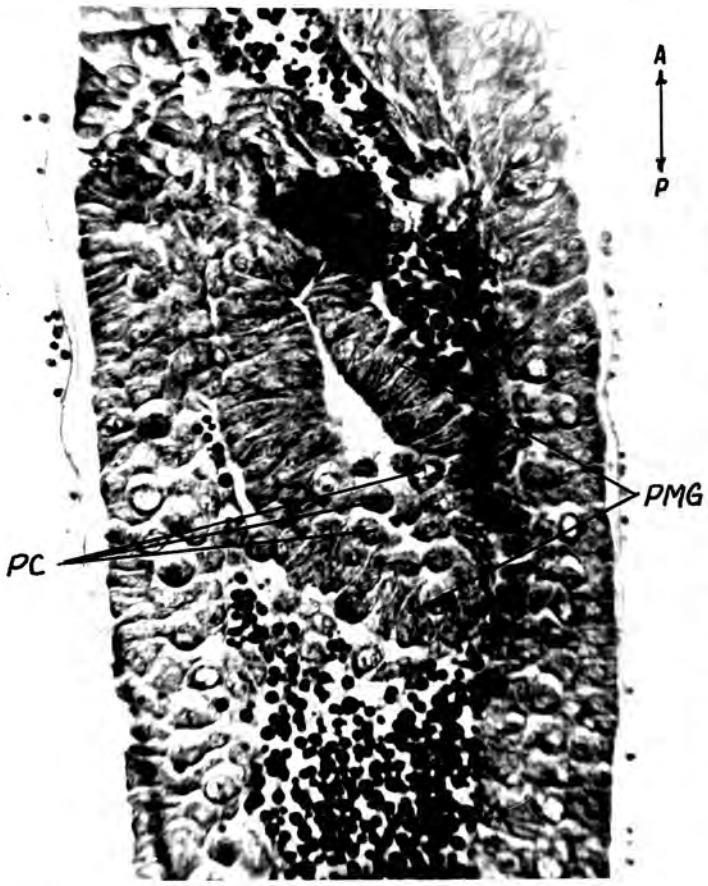
Figure 15. A 6-7 hour PMG invagination. The pole cells appear to be migrating through the PMG wall and into the yolk. See Figure 16. (sag. sect.)

x 400

Figure 16. The same embryo as seen in Figure 15. The pole cells are intermingled with the columnar cells of the invagination and are probably undergoing a migratory movement through the PMG wall. x 950

Figure 17. A frontal section of an embryo of ca. 12 hours. A congregation of spherical cells can be seen on either side of the midgut, situated amongst the columnar cells of the epithelium. These are the points of fusion of the AMG and MMG epithelium, and the groups of cells may be pole cells. x 400

Figure 18. The same specimen as seen Figure 17. x 950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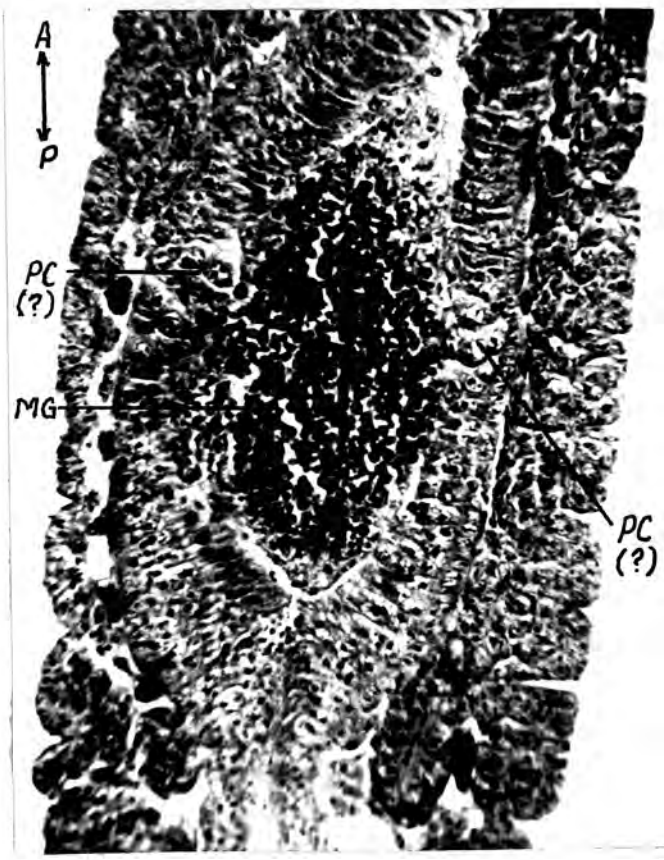
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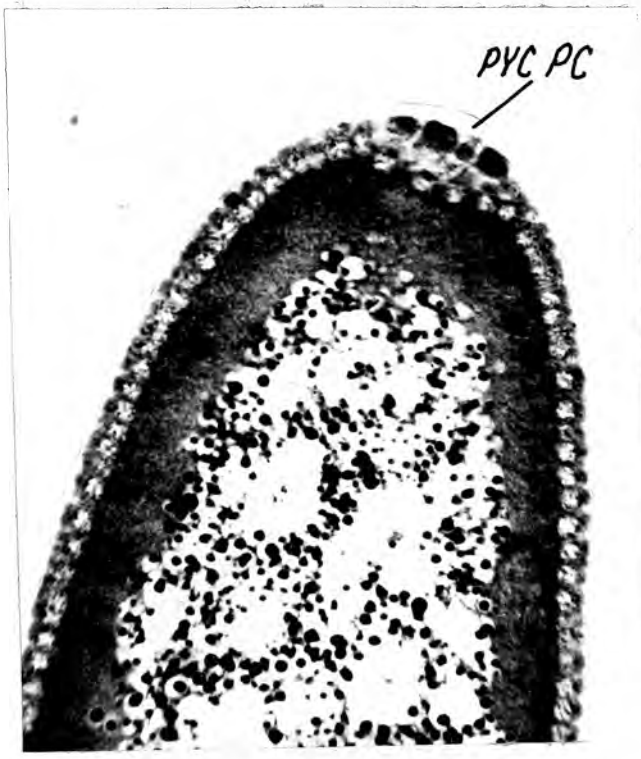
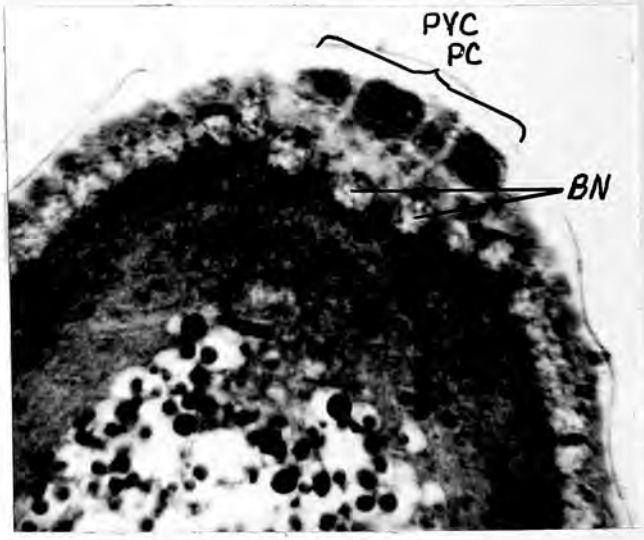
Figure 19. Pycnotic pole cells seen one hour after irradiation. Notice their lack of distinct cellular outline, but their continued segregation from the uninjured pre-blastodermal, nucleated periplasm immediately beneath. x 400

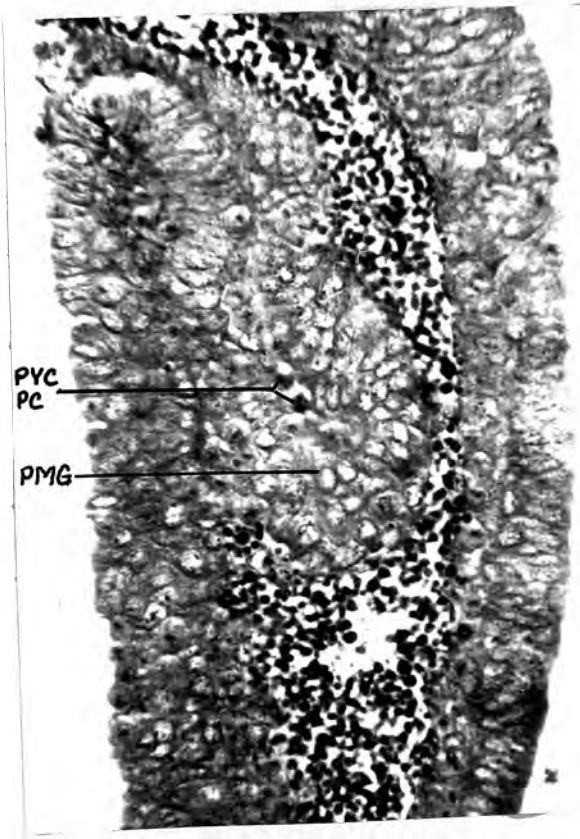
Figure 20. The same embryo as in Figure 19, but at a higher magnification. Notice the localized effect of the ultraviolet in this embryo, which was treated in the pole cell area at ca. 2 hours. x 950

Figure 21. Pycnotic pole cells at a later stage of development than those in Figures 19 and 20. This embryo is ca. 7 hours old, x 400

Figure 22. The same embryo as in Figure 21. x 950


PYC PC


PYC
PC
BN


PYC
PC
PMG

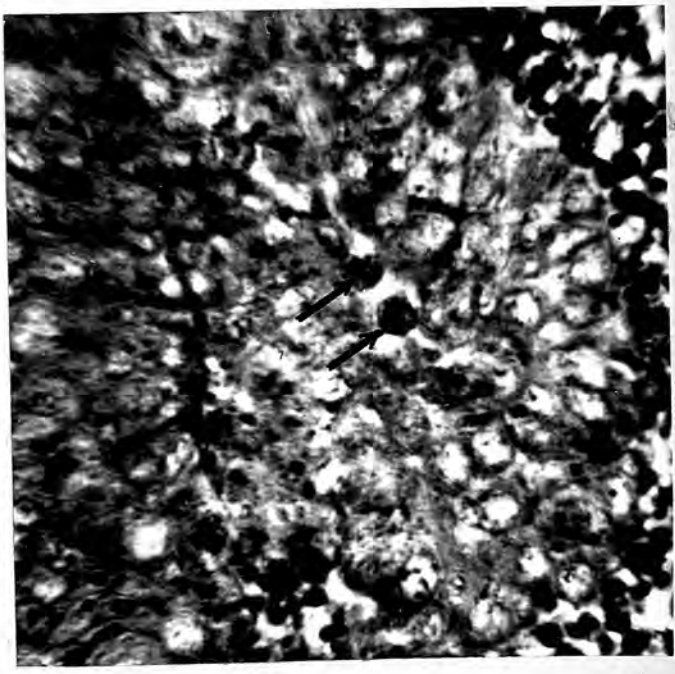


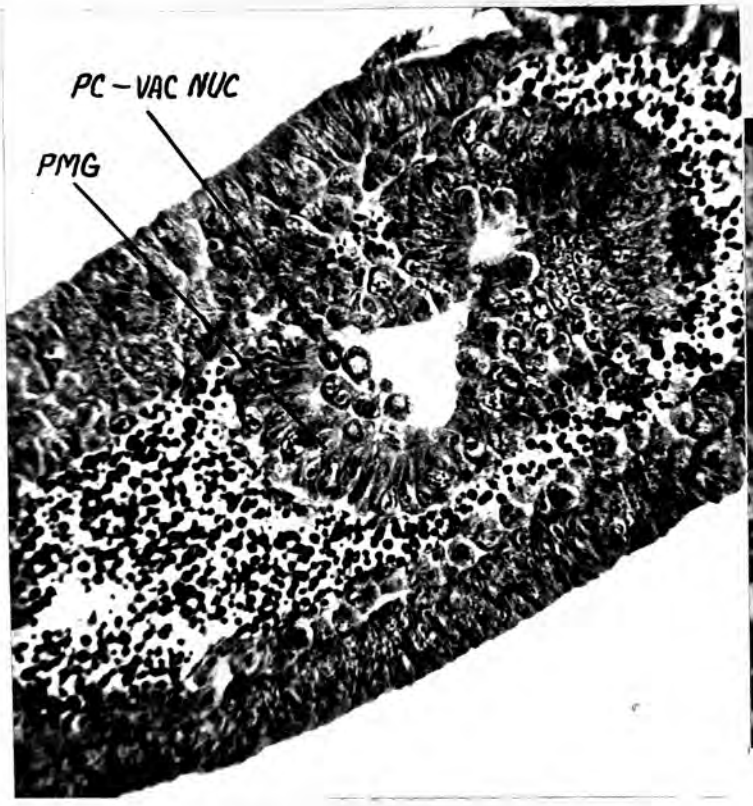
Figure 23. Irradiation damaged pole cells in the form of vacuolated nuclei. They are seen in the PMG cavity of an ca. $5\frac{1}{2}$ hour embryo (frontal section). Notice the beaded chromatin adhering to the membranes of the vacuolated nuclei. x 400

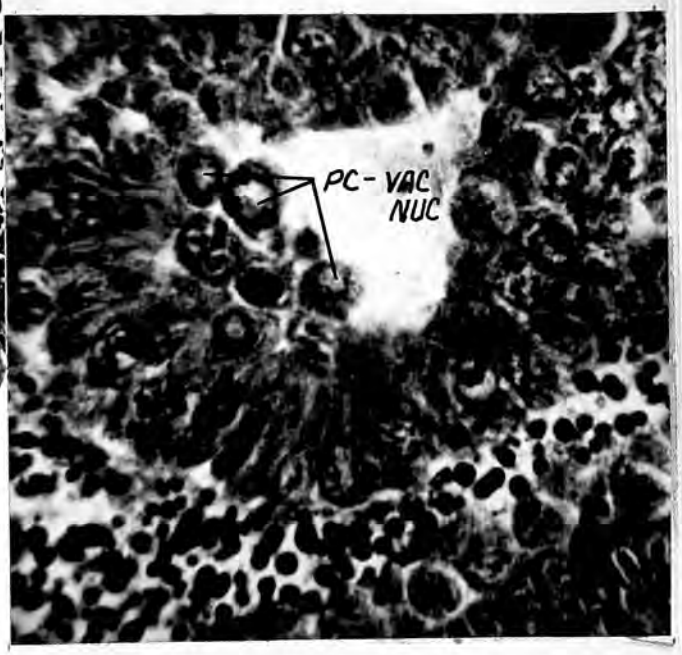
Figure 24. The same embryo as is Figure 23, but at a higher magnification.

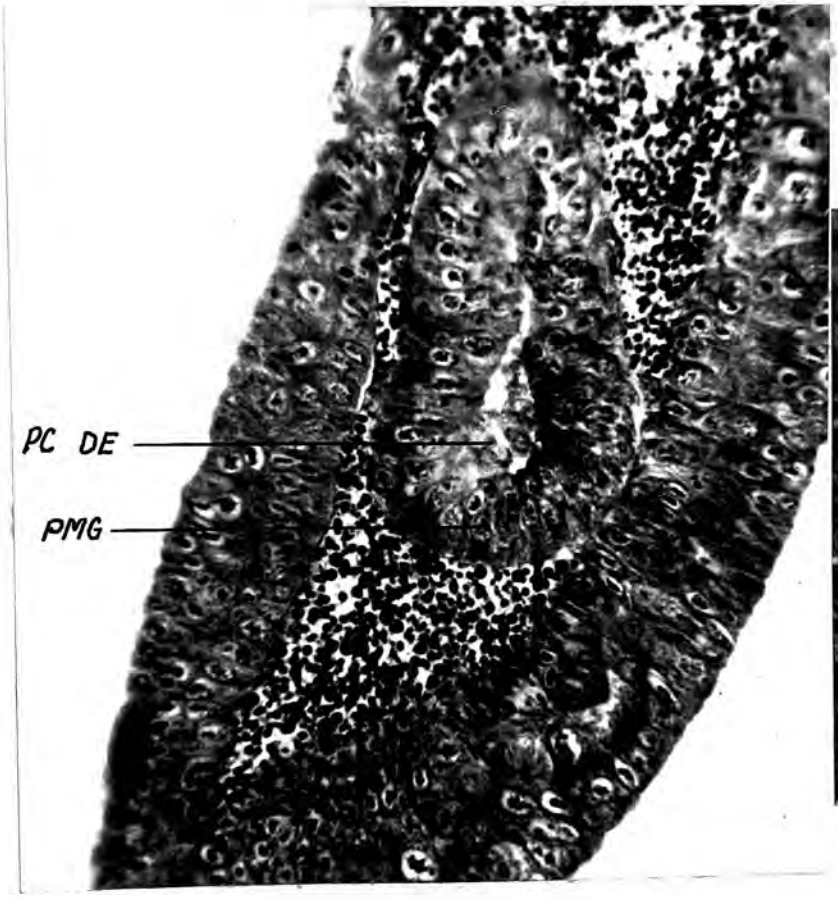
Figure 25. Another manifestation of radiation damage, general cell debris. The lack of cellular form and normal staining capacity distinguishes the diffuse debris in the PMG cavity.

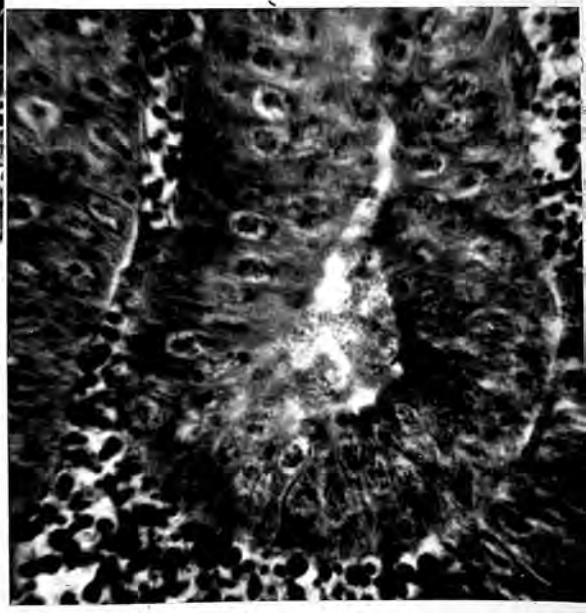
x 400

Figure 26. The same embryo as in Figure 25. x 950







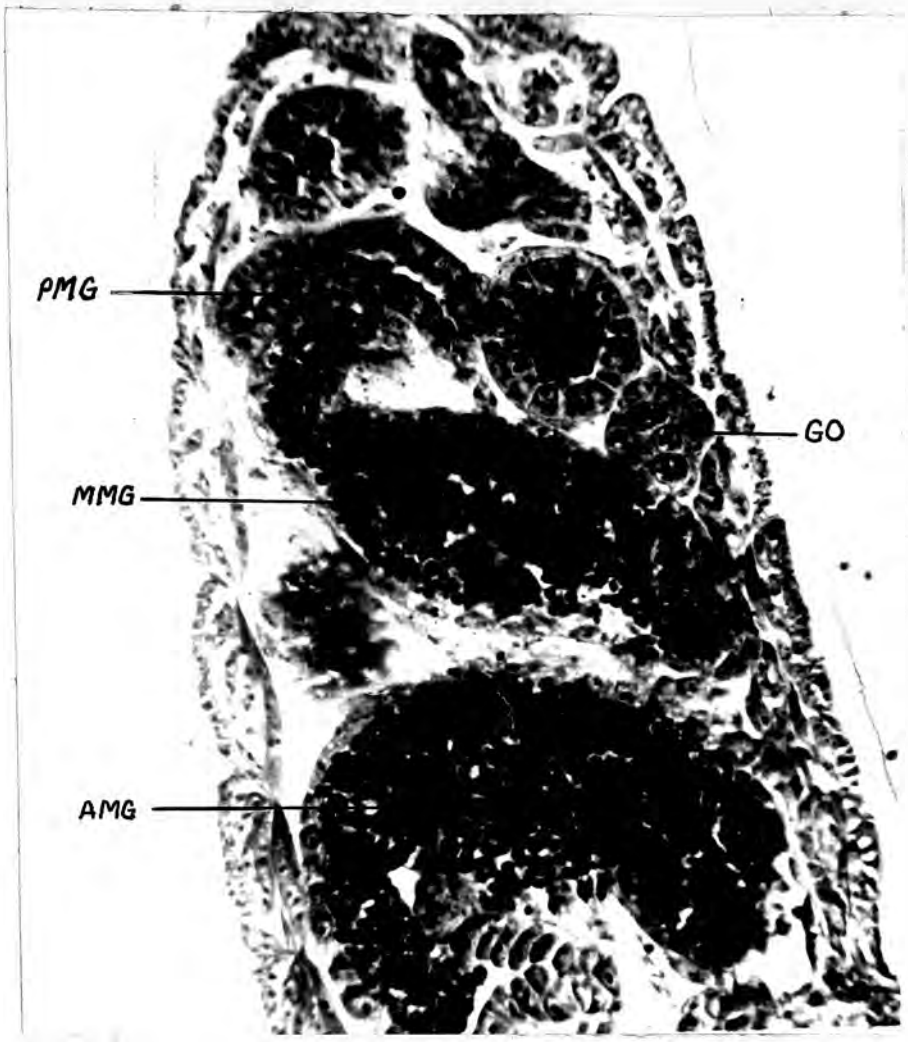


23 – 26

Figure 27. The posterior part of a 15 hour embryo showing the normal gonad (horiz. section). x 400

Figure 28. A view of the gonad of the same embryo as seen above. Notice the pole cells, their large spherical nuclei, and the dark clumps of chromatin. 4, perhaps 5, nuclei are clearly visible.

The small dark nuclei interspersed throughout the cytoplasm surrounding the pole cells are characteristic of the mesodermal tissue of the gonad. x 950


PMG
GO
MMG
AMG

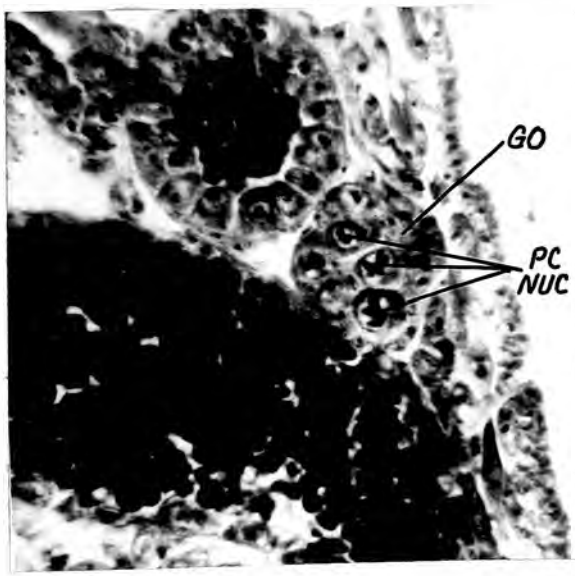

GO
PC
NUC

Figure 29. An "agametic" gonad in 16 hour embryo. The pole cells are absent, but the characteristic mesodermal gonadal cells are still congregated. Compare with Figures 27 and 28. x 400

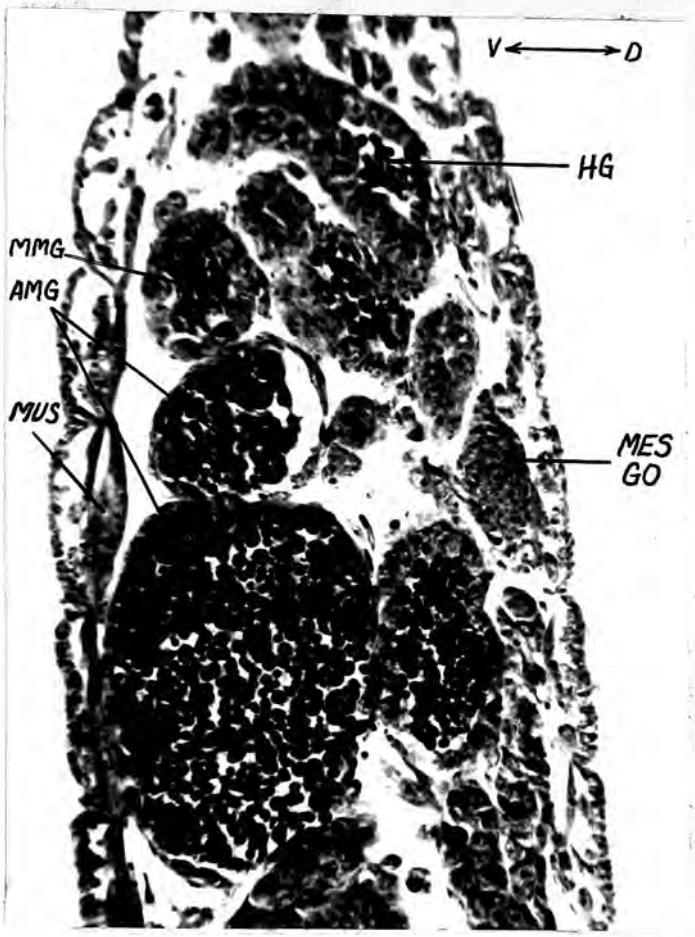
Figure 30. The "agametic" gonad of Figure 29 under higher magnification. Compare with Figure 28.

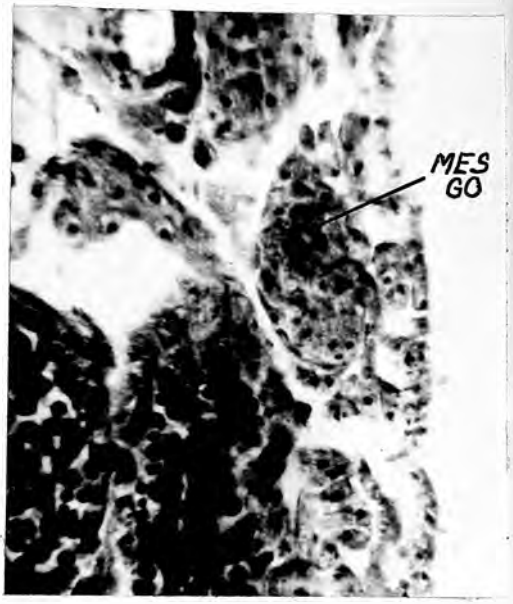
x 950

Figure 31. Necrotic embryo having died shortly after gastrulation and fixed soon thereafter. Outline of the PMG (lighter staining area) is the distinguishing factor. x 200

Figure 32. Same view as in Figure 31, but under higher magnification. Yolk and cell debris a homogeneous mass along ventral (right) surface, while PMG area is free of yolk.

x 400


V ⟷ D
HG
MMG
AMG
MUS
MES GO


MES GO

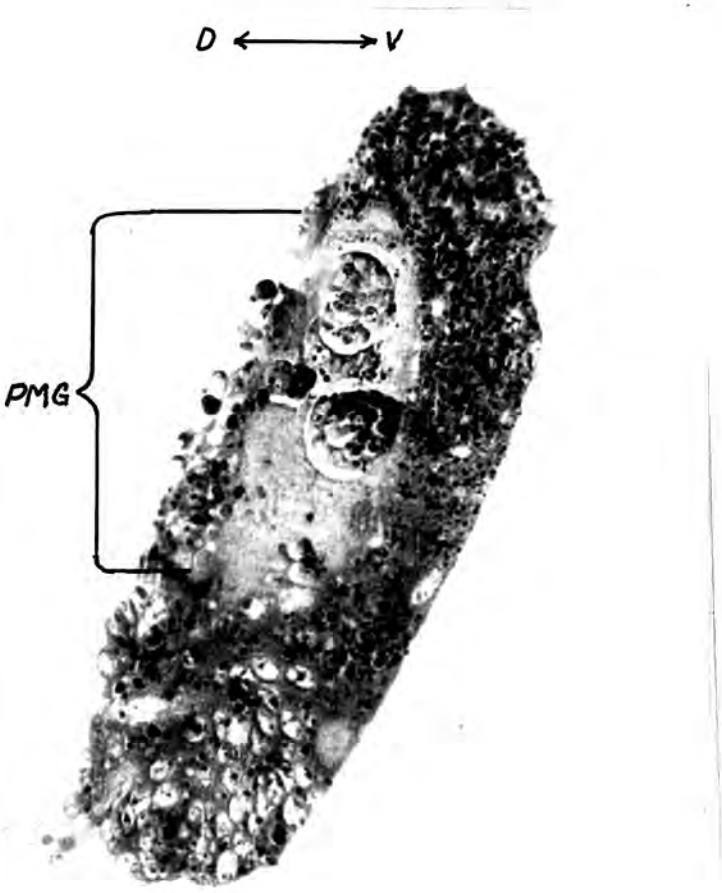
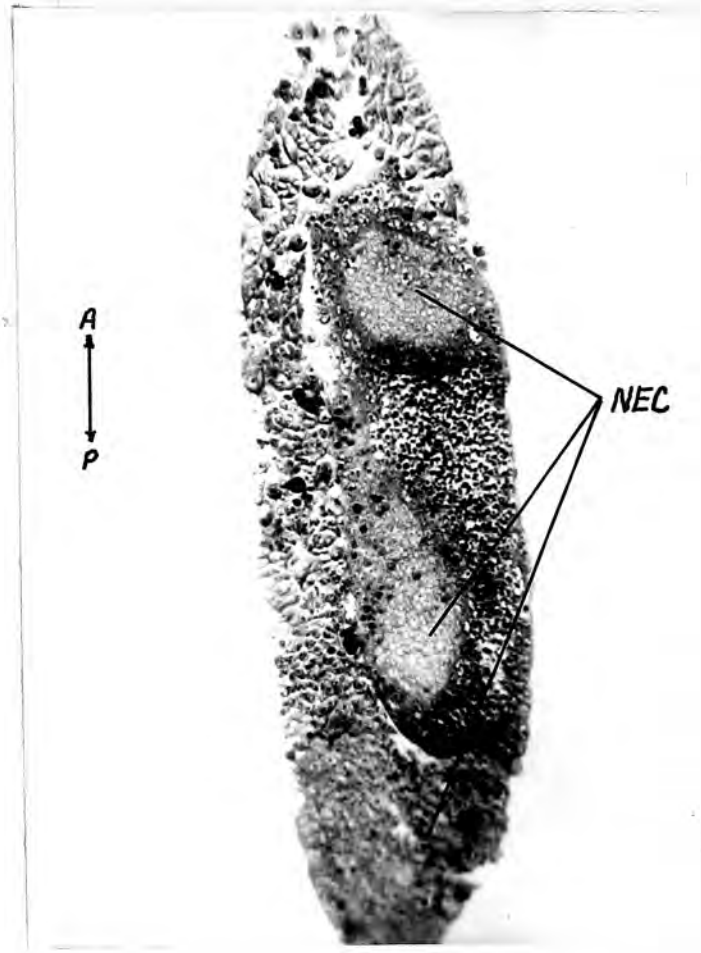

D ⟷ V
PMG

Figure 33. Necrosis and complete, although disorganized, cell structure co-existing in earliest stages of embryonic death. Normal morphogenesis probably reached gastrulation.

x 200

Figure 34. Cell proliferation without differentiation. Normal development has ceased probably no later than 6 hours, but this egg, fixed at 15 hours, has had continued cell proliferation. x 200


A
P
NEC

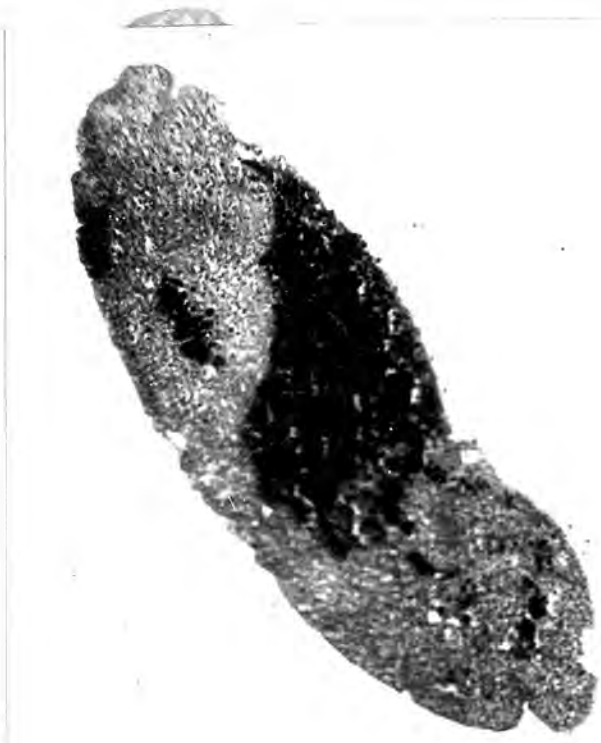


Figure 35. The ultra-violet micro-spot being administered to the polar region of an egg $1\frac{1}{2}$-2 hours. The "spot" actually was no more than 60 microns in diameter, but the light scattering in the protoplasm makes it appear larger. The scale at the top of the photograph is in divisions equivalent to 10 microns. x 72

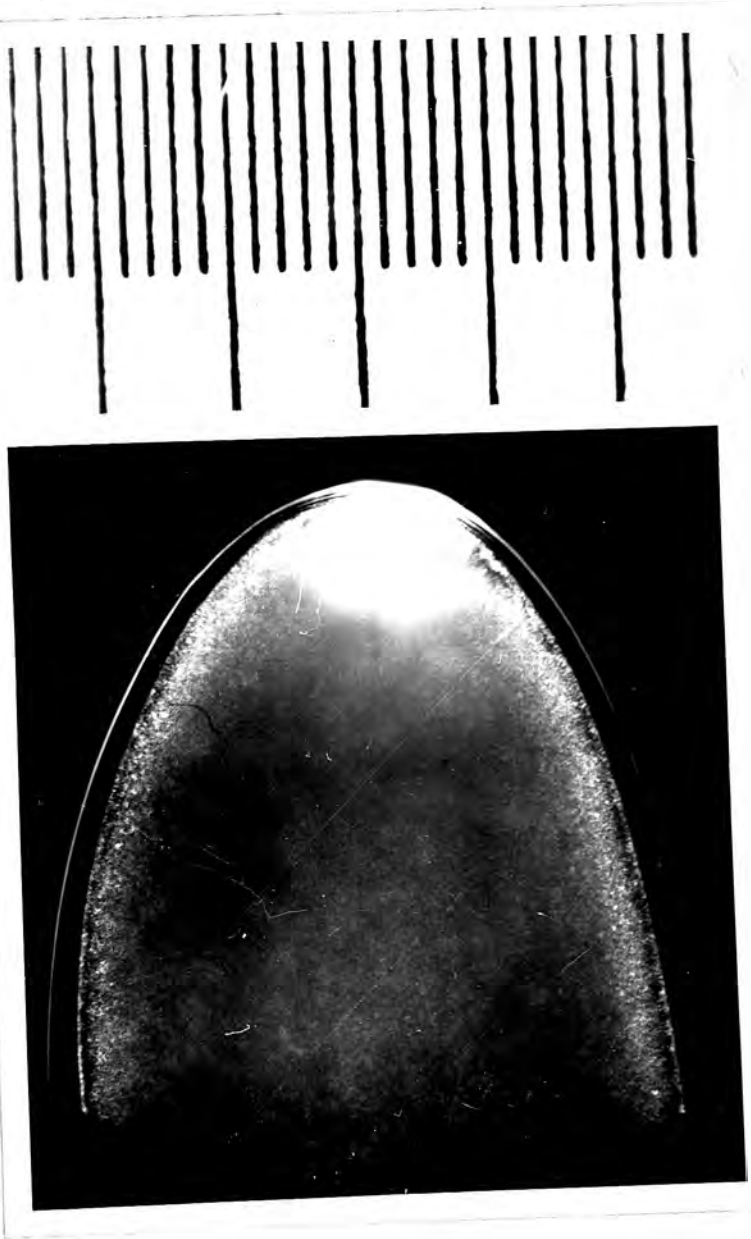


Figure 36. The circled area is a defective ovary which developed from a pole cell irradiated embryo. It has no gametes and is composed only of mesodermally derived tissue.

The ovary on the opposite side was normal, and in fact, had a high number of ovarioles, 15-16, as seen in this squash preparation. This latter fact is determined by counting the germaria (GMA). x 16

Figure 37. Same as above, but at a higher magnification.

x 40


GMA

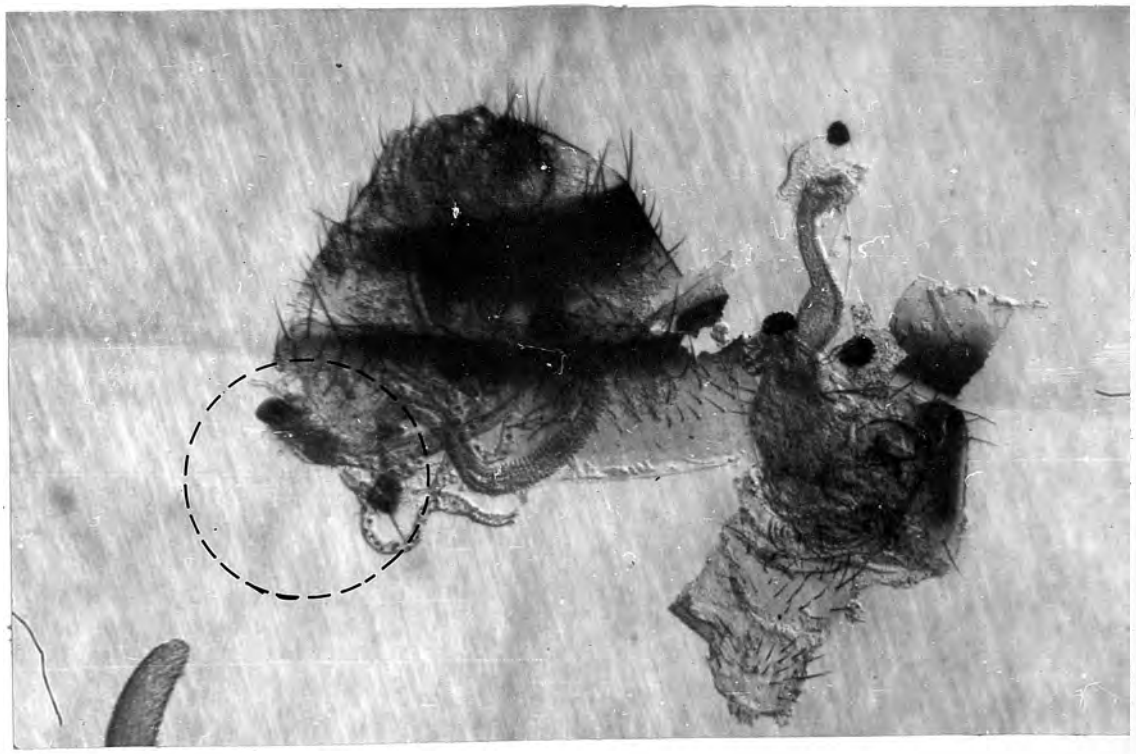
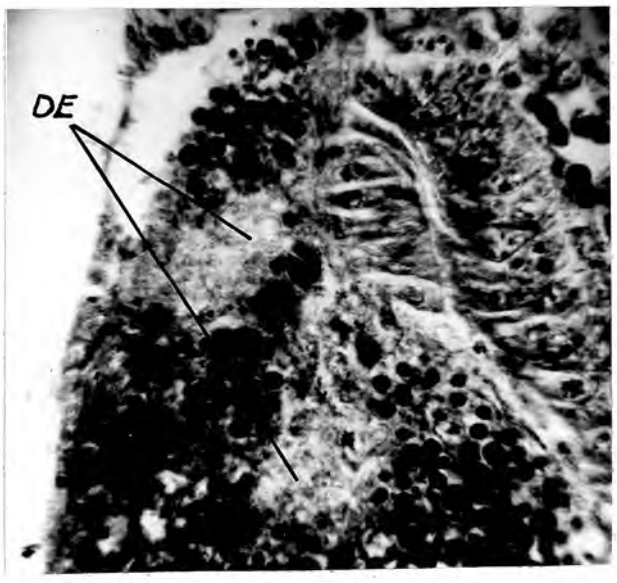
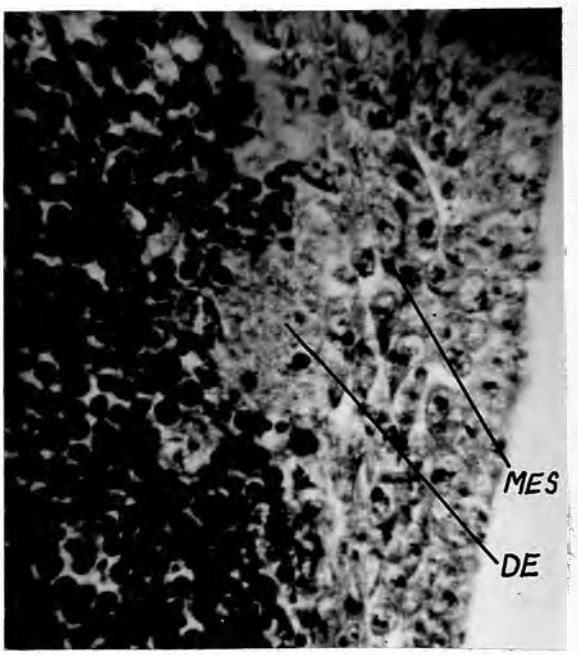


Figure 38. Defective midgut formation in a 1 hour pole cell treated embryo. The circled areas indicate the epithelial abnormalities in this ca. 12 hour embryo. See below. x 400

Figure 39. The anterior epithelial defect shown above. This is normally the region of a caecum invagination from the AMG epithelium, but as can be seen here, cell structure is totally absent on this one side of the proventriculus. Only debris is apparent. x 950

Figure 40. Lateral defect of midgut seen in Figure 38. A study of successive serial sections has shown that the debris seen here extends over a wide area, and in the absence of any well-defined epithelium formation. x 950

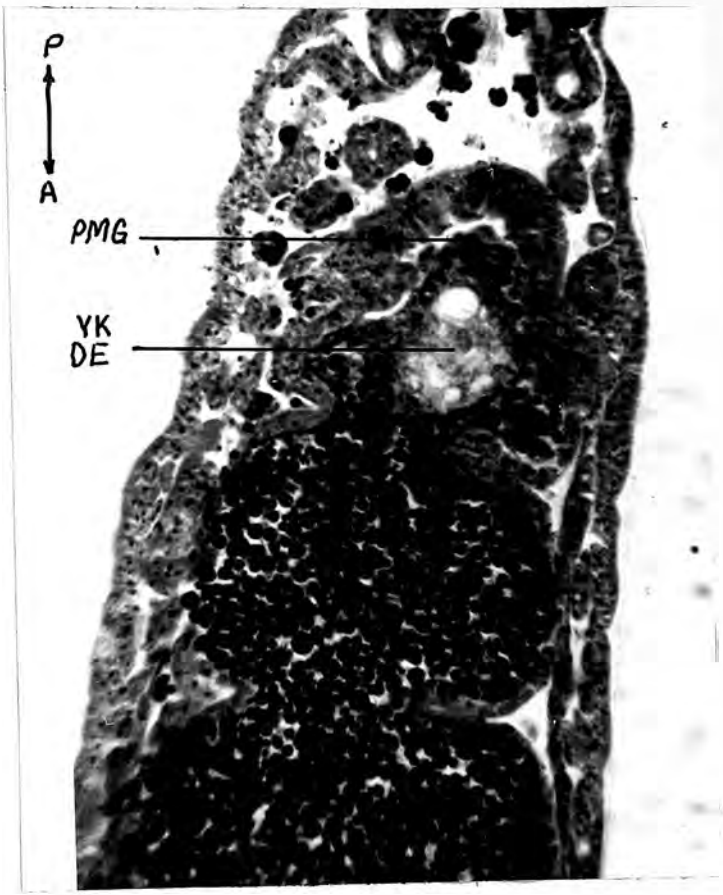




38 39, 40

Figure 41. Yolk debris. Often seen in yolk of both experimental and control embryos. In this case it is located in the PMG of a ca. 14 hour ventral 3 embryo. (Horiz. sect.) x 400

Figure 42. The yolk vacuole and debris seen above. Under this higher magnification, the cytoplasmic stands are quite apparent. x 950


P
A
PMG
YK
DE

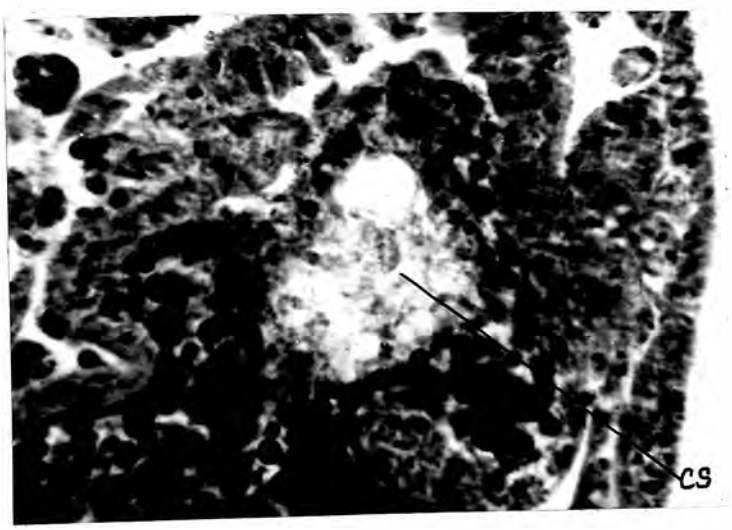

CS

Figure 43. A diagrammatic representation of the areas of the blastema and blastoderm, both ventral and dorsal, which were "marked" with ultra-violet for the purpose of tracing cell lineage.

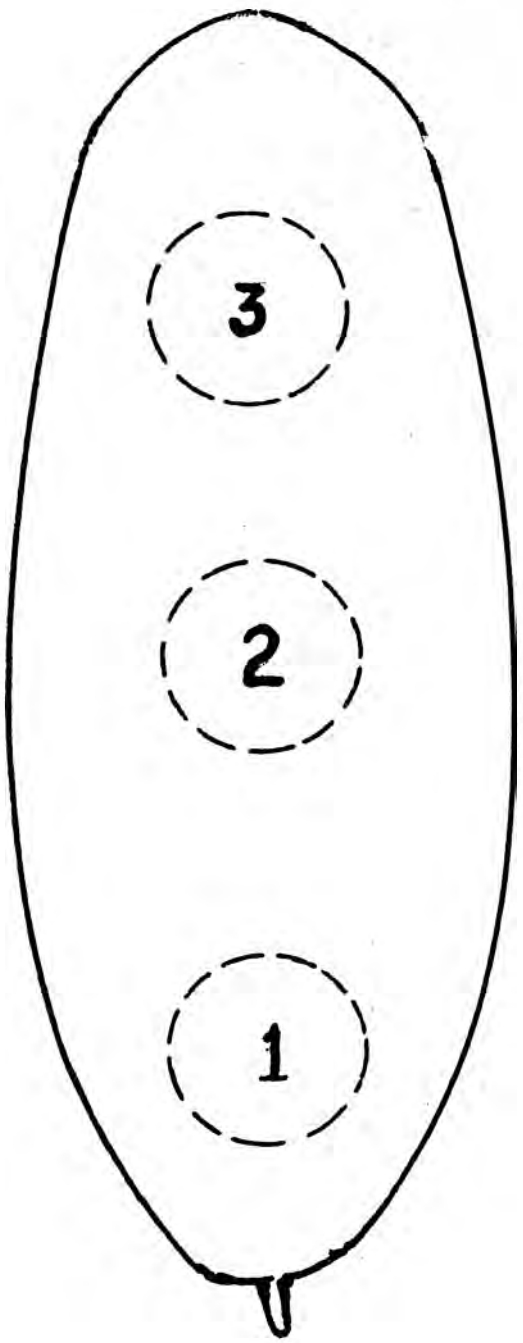
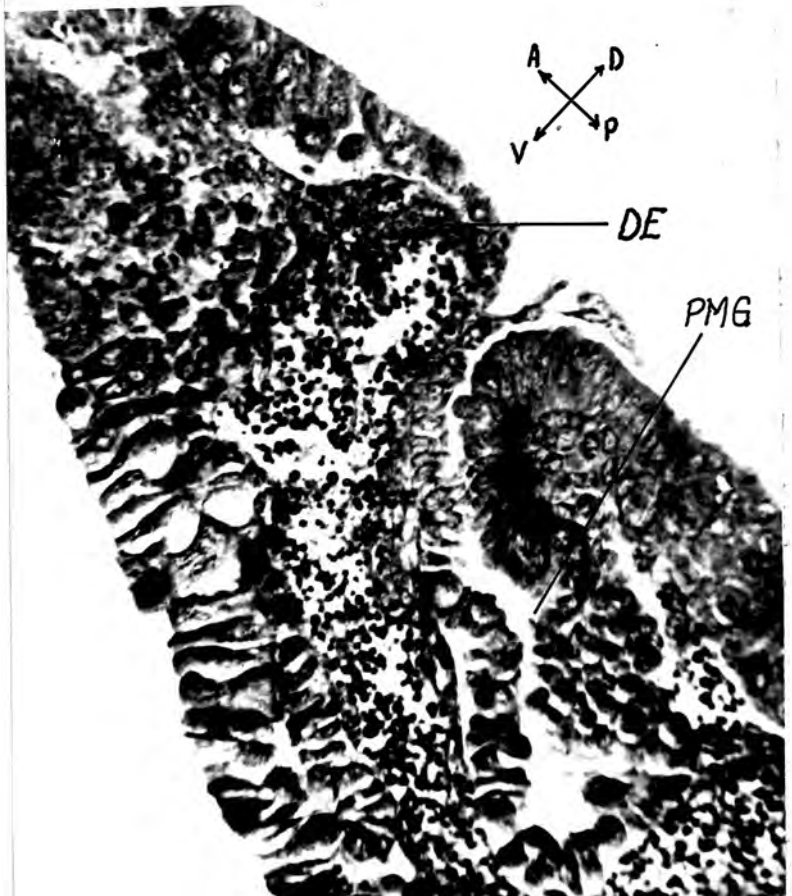
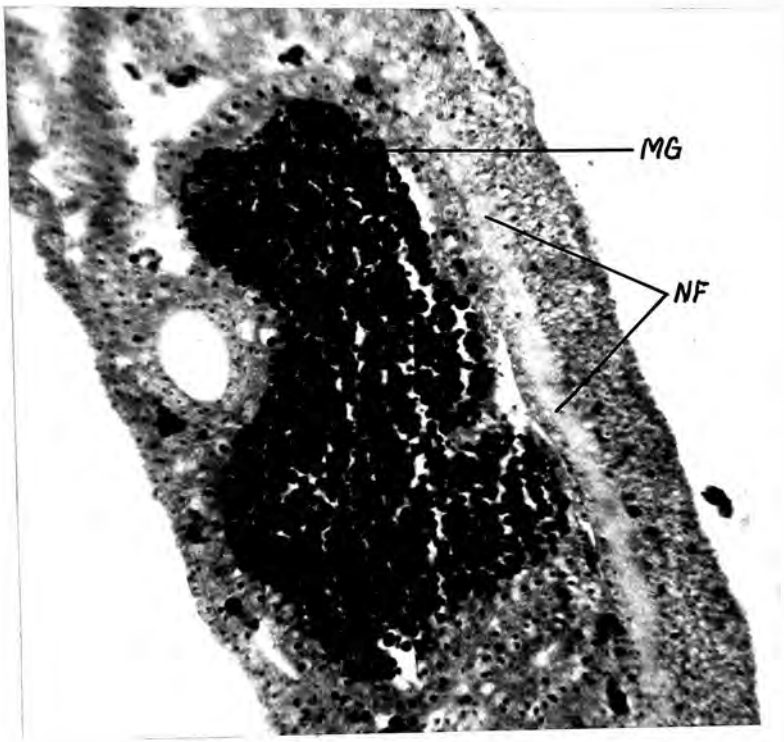

3
2
1

Figure 44. The micro-spot in surface irradiations. In this case it was administered to the dorsal 3 region (see Figure 43). The "spot" had a 60 micron diameter, although light scattering in the protoplasm makes it appear slightly larger. x 72

Figure 45. Dorsal 1 damage in an embryo of ca. 6 hours. Debris and lack of cell structure can be seen antero-dorsally, and the anterior wall of the PMG invagination is malformed.

The embryo as a whole is swollen ventrally, but this is an artifact caused by the fixation puncture. x 400


A
D
V
P
DE
PMG


MG
NF

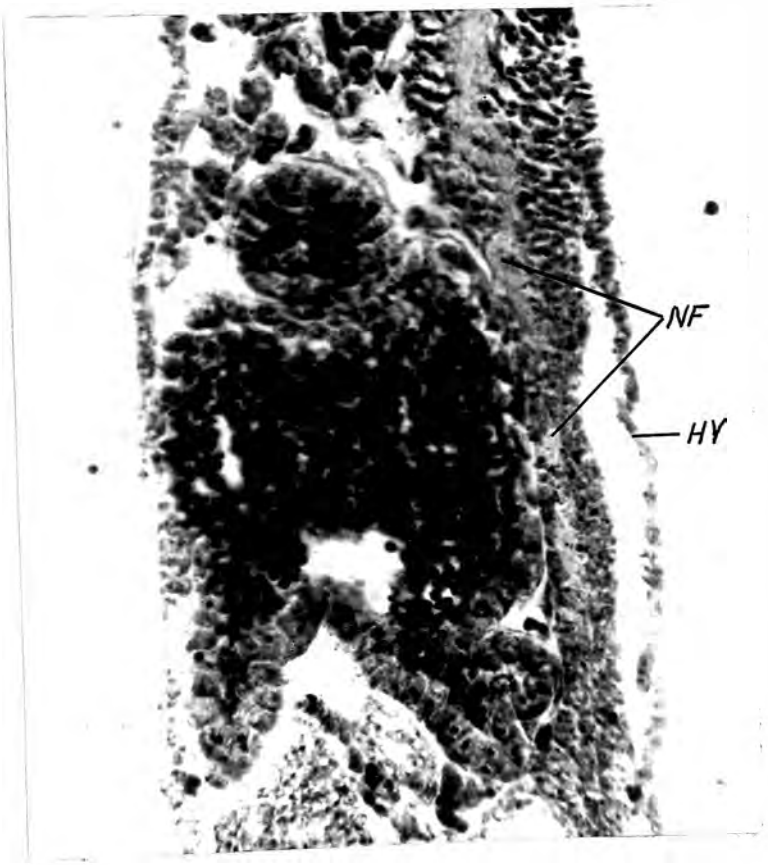

NF
HY

Figure 48. Normal ventral nervous system of 14 hour embryo. The ganglia are particularly evident. x 400

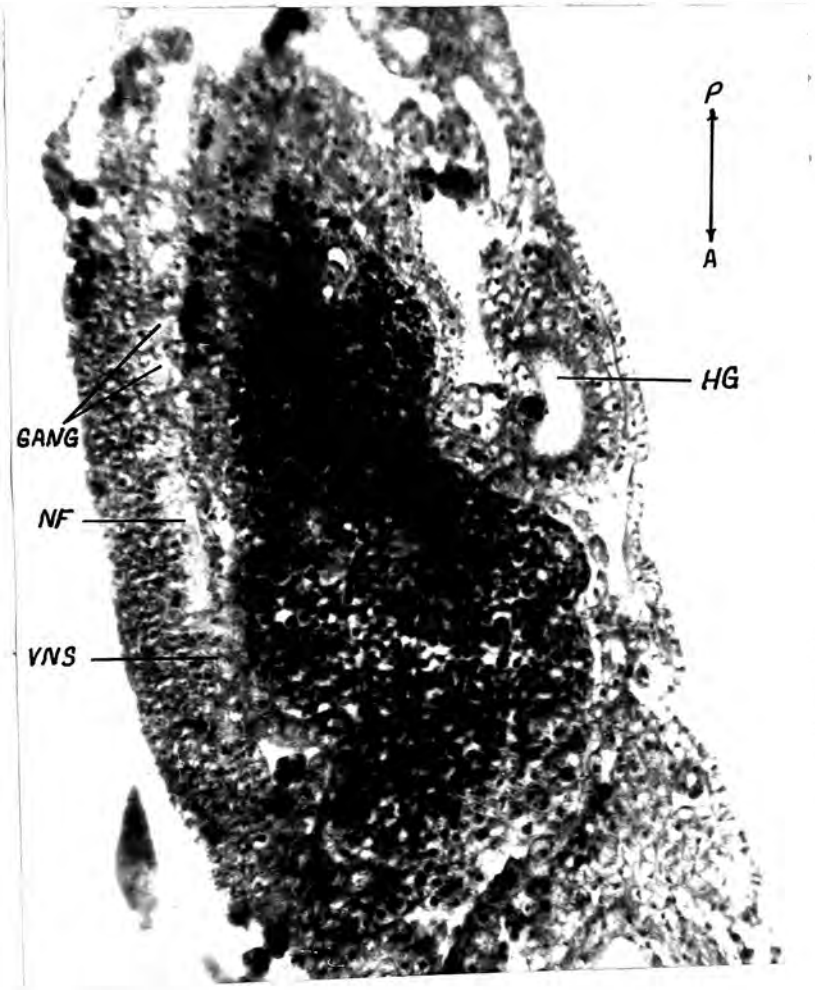
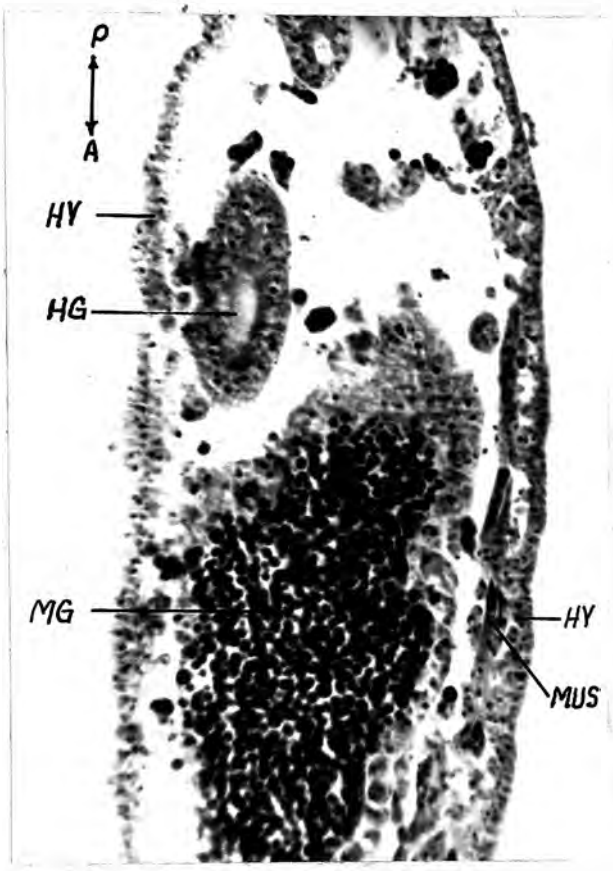

P
A
HG
GANG
NF
VNS

Figure 53. Mesodermally derived defects in ca. 13 hour ventral 3 embryo. The body musculature is only unilaterally and not at all posteriorly present in this embryo (frontal sect.) x 400

Figure 54. High magnification of the normal musculature seen unilaterally in Figure 53. x 950


P
A
HY
HG
MG
HY
MUS

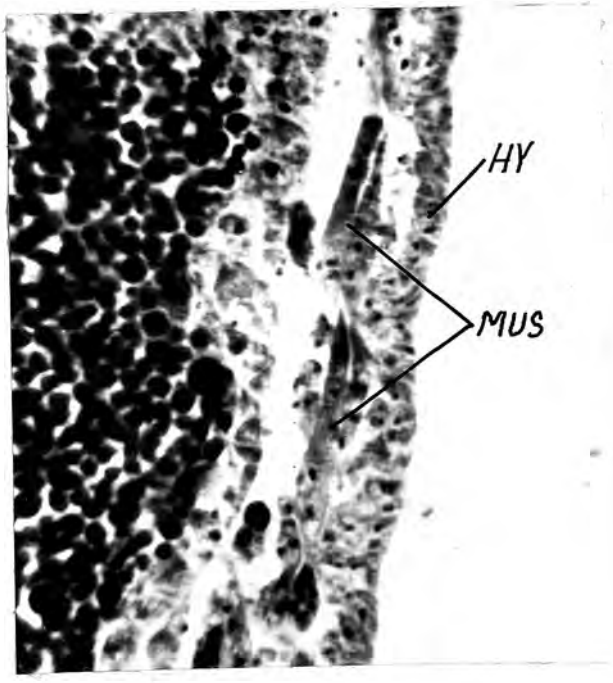
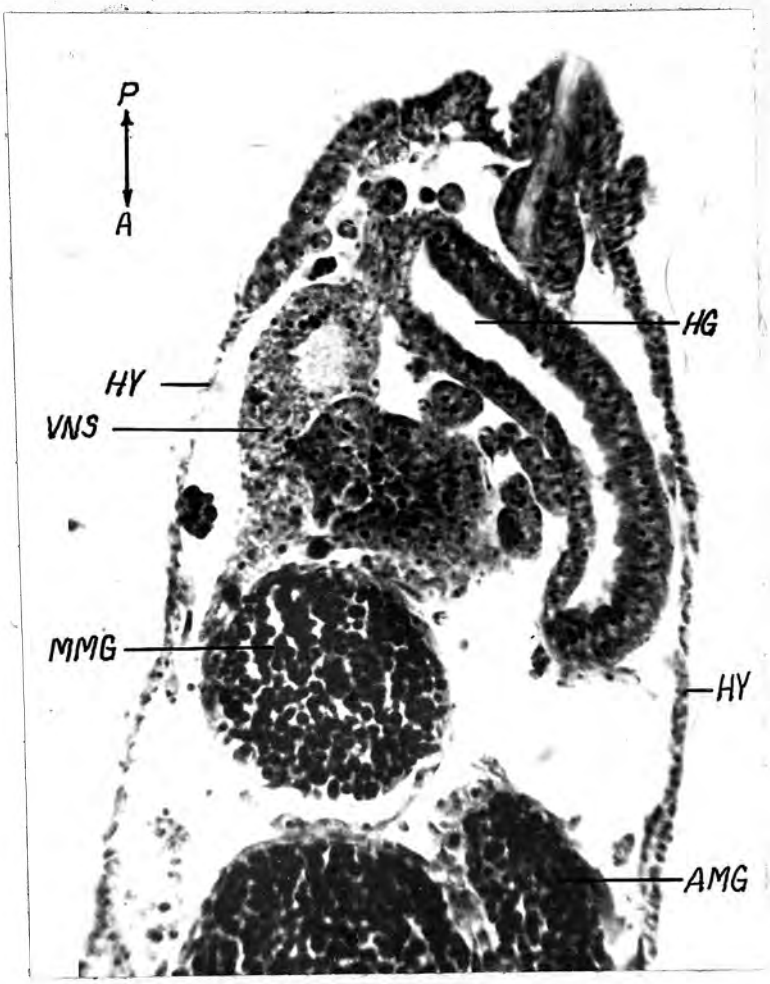

HY
MUS

Figure 55. Abnormal hypoderm, and a complete absence of mesodermally derived tissue in a 15 hour ventral 3 embryo. (horiz. sect.) x 400

Figure 56. "Naked" hypoderm of above under higher magnification. No mesodermal tissue is present.

x 950


P
A
HG
HY
VNS
MMG
HY
AMG

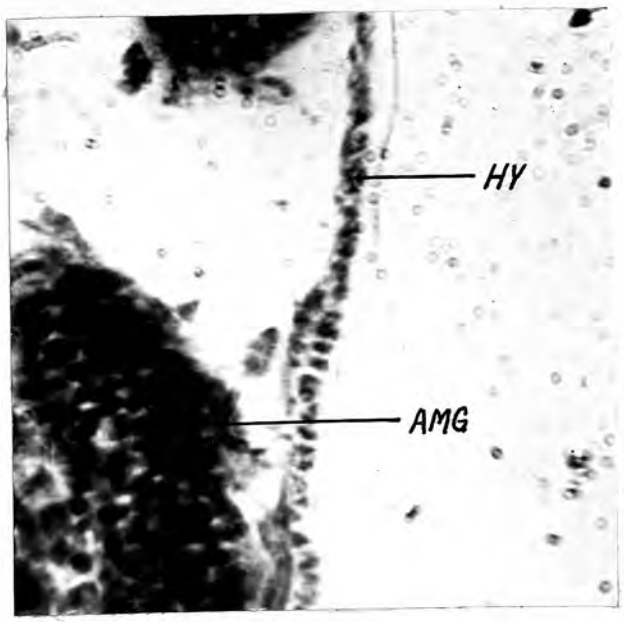
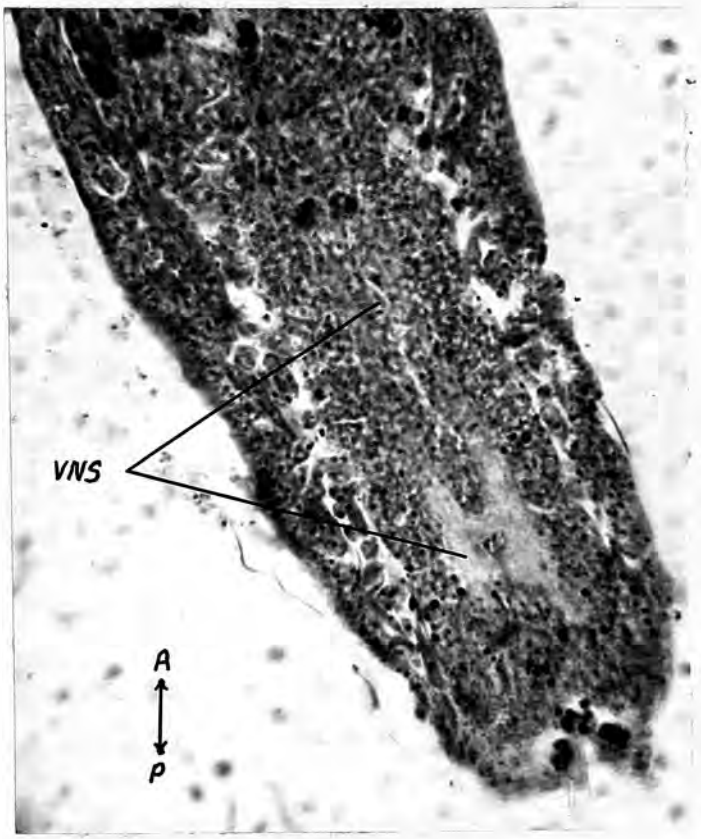

HY
AMG

Figure 57. Normal ventral nervous system in posterior region of ca. 14 hour embryo. Notice the bilateral fibre tracts and their courses parallel to the longitudinal axis. (horiz. sect.) x 400

Figure 58. Abnormal ventral system of a 14 hour ventral 5 embryo. A spatial displacement has occurred which has disturbed the symmetrical pattern along the axis (compare with Figure 57). (slightly oblique horiz. sect.) x 400


VNS
A
P

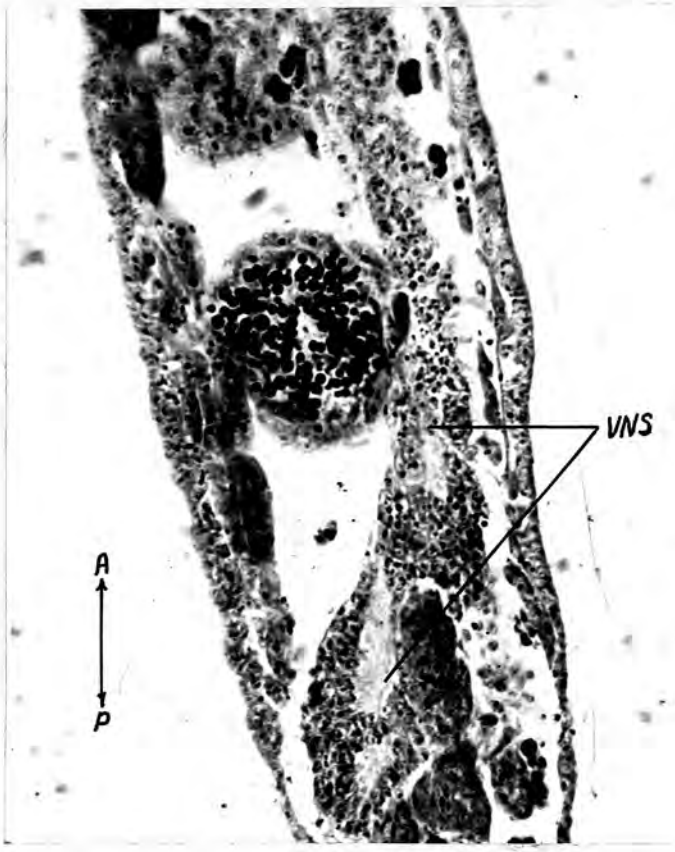
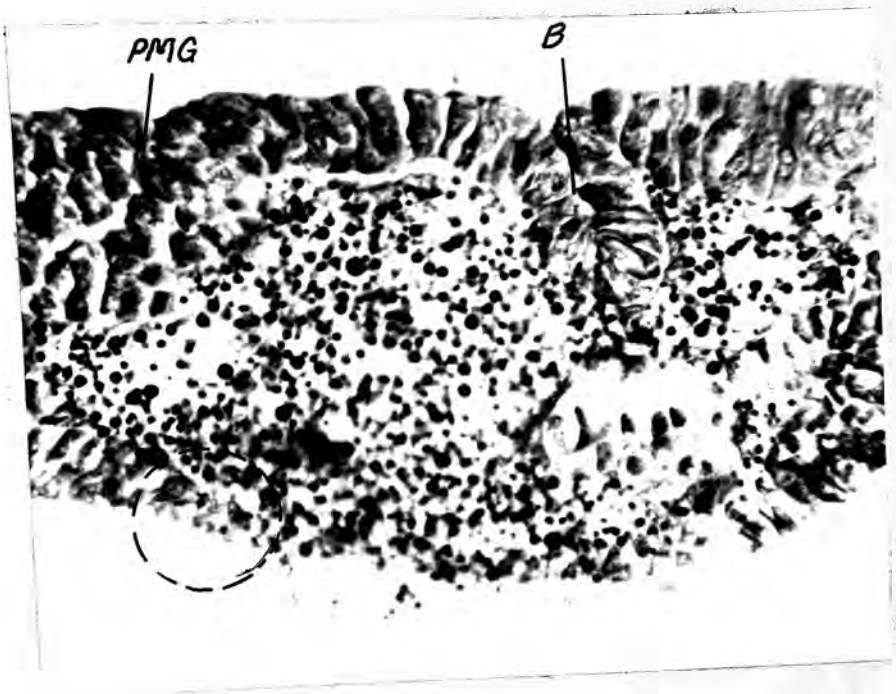
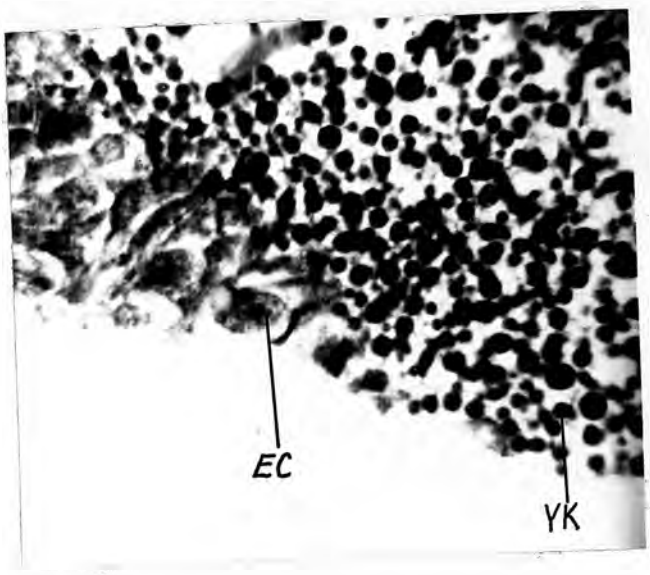

VNS
A
P

Figure 59. Ventral hernia and abortive PMG invagination of ventral 3 embryo at ca. 6 hours. The latter defect is not the deep invagination more anteriorly (right), but can just be seen at the extreme left. (sag. sect.)

x 400

Figure 60. A view under high magnification of the border between the herniated area and the incomplete neighbouring ectodermal cells. It is seen above in the circled area. x 950


PMG
B


EC
YK