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LOCALIZATION OF PROSPECTIVE AREAS IN THE BLASTODERM OF THE PEA BEETLE CALLOSOBRUCHUS MACULATUS FABR., AS DETERMINED BY ULTRAVIOLET (2537 A) IRRADIATION INJURY

ALFRED BRAUER

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Alfred Brauer

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LOCALIZATION OF PROSPECTIVE AREAS IN THE BLASTODERM OF THE PEA BEETLE CALLOSOBRUCHUS MACULATUS FABR., AS DETERMINED BY ULTRAVIOLET (2537 A) IRRADIATION INJURY

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INTRODUCTION

The nature of organization of the egg of the pea beetle, Callosobruchus maculatus Fabr., has been studied experimentally by means of hair-loop constriction, electrothermocautery, and susceptibility to chemicals (Brauer and Taylor, '36; Brauer, '38, '42). In the main the studies have shown that, 1) as the egg is deposited it is indeterminate and labile in its formative capacity except as to anteroposterior orientation, but during a first period (from deposition to 6½ hours thereafter at a temperature of 29° C.) a physiological center becomes established in the anteroventral cortical cytoplasm upon which integrated development becomes dependent. 2) A second period ensues, (6½ to 12 hours) during which cleavage cells enter the cortical cytoplasm and with it form a blastoderm. Areas of the blastoderm appear to have specific formative values since eggs treated with oxidation inhibiting agents, or with parts of cortex removed by thermocautery or mechanical isolation, always develop an axiate though modified embryo. 3) A third period, (12½ to 16 hours) marks the formation of an axiate ventral embryonic plate in which neurulation is foreshadowed by mediolateral susceptibility to inhibiting agents, and modification by partial or complete bifida is the rule. Blastoderms react most readily to chemical modifiers during first and third periods.

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The studies suggested: a) that the cortical cytoplasm of the egg specifically, and the egg as a whole generally, rather than cleavage cells, determine and regulate development into an integrated embryo, since the induced modifications of the first period were primarily the result of interference with functions of the cortical cytoplasm rather than to injury of cleavage cells and, b) that notwithstanding the high degree of determination attained in the embryo as a whole in the second period, definite areas in the blastoderm have not yet arrived at their definitive locations, and that cell movements and localizations are still in progress.

If conditions were reversed, so that interference with, or injury to the earliest cleavage cells could be effected without material injury to the egg cytoplasm, the results would tend to establish previous contentions more strongly, or to disprove them. Also by means of such injury to cells of early and later blastoderms, the cells could be traced through changing locations to their fate in tissues and organs.

Radiation techniques appeared to be the most likely methods for accomplishing these purposes. Ultraviolet 2537 A seemed particularly applicable since it is highly specific for nucleic acid, and can be directed at specific small areas. The degree of treatment may be regulated by varying either the amount or the intensity of energy, and problems of measuring and monitoring are not forbidding. Precedent use of ultraviolet radiation for similar problems is the work of Geige ('31), who employed it (wave lengths and intensities not mentioned) in tracing injury in Drosophila from embryo to adult, and by Aboïm ('45) also in Drosophila, for injuring the embryonic "pole cells" and through them producing partial to complete castration of adult flies. This latter investigator employed the complete range of wave lengths of the ultraviolet spectrum. Seidel ('35) used ultraviolet injury as a means of marking,

and thereby mapping the blastoderm of a Libellulid (Odonata) in much the same manner that it is used in this paper.

MATERIALS AND METHODS

Culture of the beetle, the eggs, and methods of handling them have all been described (Brauer '25, '38). The only variation used here was that early developmental rates were established for temperatures ranging from 14 to 29° C., so that eggs could be set aside to attain a particular stage at a convenient time. Twenty-nine degrees C. is standard incubation temperature for stages mentioned herein.

For irradiation of entire eggs, specimens of known age and developmental stage were left on blackeyed peas on which they were deposited. Peas were fixed firmly in Fuller's earth contained in petri dishes and oriented so that the ventral, formative side of the egg was uppermost. For partial, or spot irradiation, eggs were removed from the peas and placed (usually ventral side up) on depression slides of black bakelite, with a central depression 0.5 mm. deep and 1 cm. in diameter with a polished bottom. One half the depression was covered by a shield of X-ray film which had holes drilled near the free edge. The eggs were oriented beneath the holes in the shield so that a particular area of 1/10 to 1/14 of the ventral germinative surface was exposed. A film of soft wax was sometimes used to hold eggs in position for irradiation through the flat dorsal surface, which was done when it was desired to penetrate ooplasm before hitting the germinative side. After irradiation, eggs were incubated at standard temperature to a desired point of development, usually 48 to 96 hours. Emergence occurs at 100 to 120 hours.

To insure systematic exposure of specific areas, a typical egg was drawn in ventral view, its convex surface projected as a flat map, and this divided

into octants. Octants were numbered as were points of intersection of transverse lines with the meridian anteroposterior line (fig. 1).

While octants were chosen over smaller divisions, this by no means implies that the target area was as much as an eighth of the surface area. Including scattered radiation around the incident beam, it was usually less than 1/10.

The ultraviolet source was a 17½-inch tubular G. E. low pressure mercury vapor lamp with special envelope, giving about 80% its total, and 90% its ultraviolet radiation in 2537 A. Intensity was measured with a G. E. ultraviolet standard photometer at varying distances from the source and from these readings intensities for intermediate distances were plotted. This was done at the beginning and repeated near the conclusion of experimentation. Although differences in readings were small, an intensity of 17.5 ergs/sec./mm.^{2} is average for the two sets of readings at the distance at which all exposures were made.

For spot or target irradiation however, ultraviolet rays passing through a perforation are limited to a point source in the tube directly above the target. Therefore in order to obtain sufficient amounts of energy to produce noticeable effects it was necessary to prolong exposure (1:20 to 3:30 hrs.). Exposures of more than one hour were usually made in a cold room at 14° C. which is slightly above physiological zero for development.

Establishment of doses and reaction of eggs by stage

Doses to be used for marking and tracing cells and blastodermal areas had to be such that, (a) irradiated cells could be recognized within tissues at later developmental stages, (b) that in a considerable proportion of each series irradiated, injured areas be of such degree and dimension as to be identifiable in stained, unsectioned embryos, and (c) that lethal but not

Figure 1. Map of projected convex ventral surface of egg showing target areas and points of exposures. Shaded triangles are inserted when convex egg surface is flattened.

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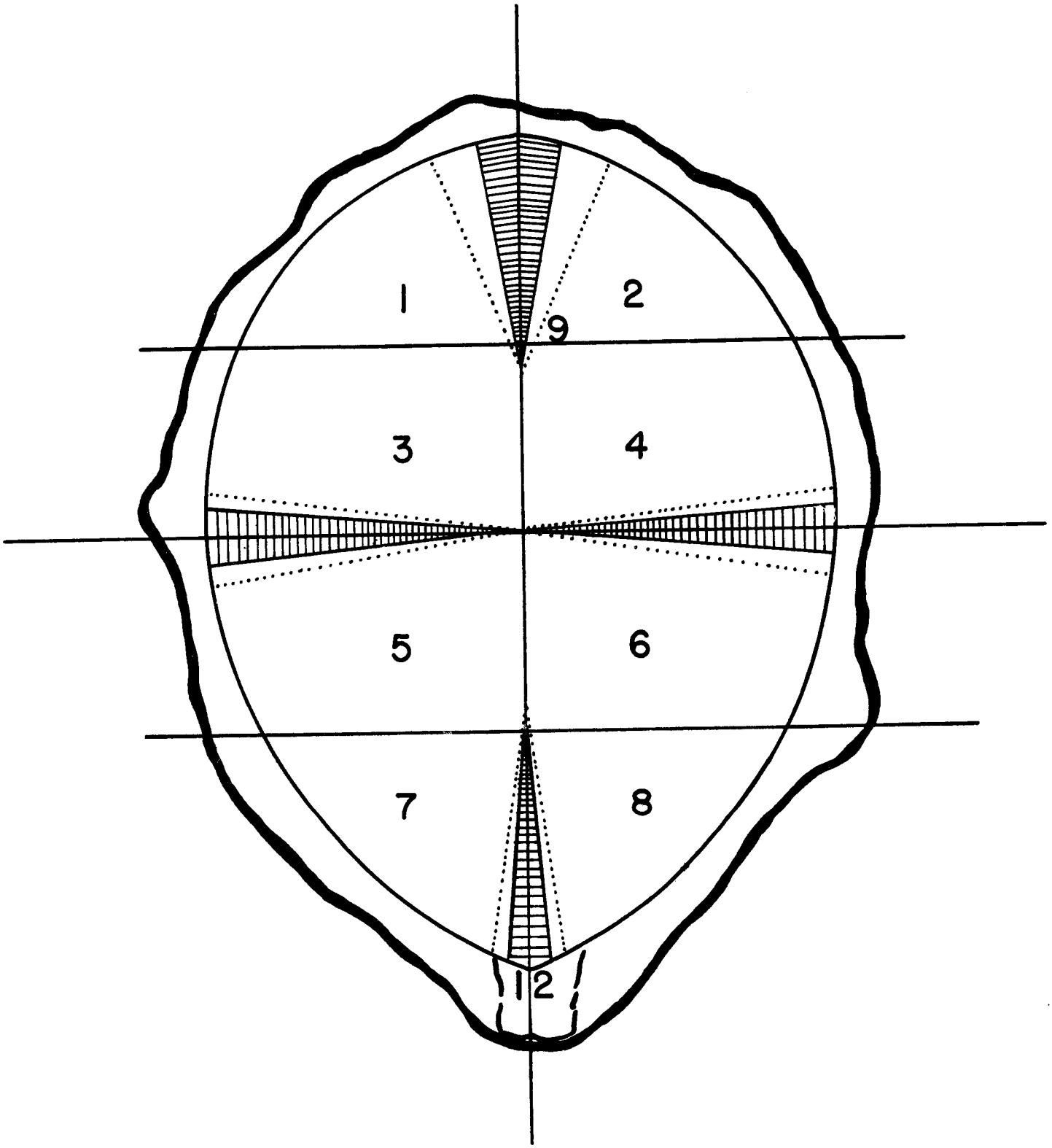
MAP OF PROJECTED VENTRAL EGG SURFACE



Shaded areas are supplied when this surface is projected

FIGURE 1

necessarily deforming doses be established for comparison with lethality charts made of this egg for oxidation inhibiting agents and with such a chart made for ultraviolet irradiation of Drosophila eggs by Geige ('31).

To establish these points, 3 to 5½-hour, 6½ to 9-hour, and 9 to 13-hour old eggs were irradiated entire for 15 to 40 minutes (6,600 to 17,000 ergs per egg). Among those receiving the larger doses a considerable number of deformed embryos appeared, and a few exhibited incoherent development in which the embryonic plate was broken up into separated areas with each having considerable organization such as segmented, axial development.

The range of exposures determined maximum doses, but minimum doses which would give recognizable irradiated cells in section were not found immediately. After considerable time it was discovered that cells exhibited varying manifestations to smaller doses and that they could be recognized with considerable certainty, especially in target irradiated embryos in which there was marked contrast between injured and uninjured areas.

The lethal-dosage chart (table 1) is constructed from eggs exposed for, but not used in microdeterminations, and from others in which dosage had to be lessened to determine the minimum. Great significance was not attached to lethal dosages at the time, hence the small number of eggs in each category. The significance of the chart is that it shows a smaller percentage of larvae emergy from second period than from first period eggs after a given dose, and a still smaller number emerge from third period than from second period eggs.

Irradiation by stage

Blastokinesis (indeterminate period). This occupies the first 6½ hours after oviposition, up to the time that the first cells become established in the cortical cytoplasm.

TABLE 1

AGE - SUSCEPTIBILITY RELATIONSHIPS OR RELATION OF DOSES TO VIABILITY

Age of Eggs		Number of Eggs	Time of exposure	Total ergs per egg	No. larvae emerged from egg	Percent	No. adults emerged	Percent
Stage	Hrs.							
Cleavage	3-3½	20	8 min.	4,800	12	60	8	40
	3-3½	16	15 min.	9,000	8	50	7	43
	3-3½	20	20 min.	12,000	9	45	6	30
	5-5½	23	8 min.	4,800	11	47.8	7	34
	5-5½	14	15 min.	9,000	5	33.3	2	14.3
	5-5½	22	20 min.	12,000	8	36.6	4	18
Blastoderm	8-8½	15	15 min.	9,000	2	13.3	0	
	8-8½	16	20 min.	12,000	2	12.2	1	6.2
Early Emb. plate	11-13	20	8 min.	4,800	0	00	0	00
	11-13	21	15 min.	9,000	0	00	0	00
	11-13	15	20 min.	12,000	0	00	0	00

The purpose of irradiation in this stage is twofold, (a) to determine whether or not severe injury to cleavage cells inhibits organized development and, (b) to afford a means of tracing injured cleavage cells into the germ layers, and embryonic tissues if development is integrated.

For the first objective eggs were left on the peas and irradiated entire for 20 to 40 minutes while for the second, they were removed from the peas, placed on depression slides and partially shielded. Shielding consisted of bringing the edges of two cover glasses imperfectly together over the slide depression and orienting eggs under the gap to expose transverse quadrants of their ventral surface. Treatment was for approximately twice the former dose (45 to 80 minutes).

Irradiation of entire eggs for 20 to 40 minutes results in gross malformation of many embryos and less obvious but nevertheless lethal effects in others which may be characterized as follows:

a) Normally formed embryos with modifications in head, thorax or abdomen and in irregularities of ventral nerve cord such as displacement of ganglia.

b) Dwarfed but entire embryos many of which reach the emergent larval stage and perform movements of emergence but are unable to leave the egg or enter the pea.

c) Prominent abnormalities of the nerve cord with fusion, displacement, or separation of ganglia.

d) Partially bifid embryos, probably resulting from extreme ganglionic separation. It is confined to distances of two or three somite lengths.

Irradiation of the egg by transverse quadrants was expected to produce its most severe effects when central quadrants were exposed, since cleavage cells are concentrated in the egg center in the early part of the period. This was borne out as follows:

a) Eggs in which the anterior quadrant was exposed prior to 3 hours of age exhibit little or no effect. Treated similarly after 3 hours, injured areas may appear in maxillar-prothoracic region. However, in some, head development is impeded so as to make them dwarfed. In section, injured cells were found in head tissues only when irradiation occurred after 3 hours, and were more prominent when irradiated after 4 hours. Such cells were found among both ectodermal and mesodermal derivatives, stomodaeal and oesophageal epithelium and muscle fiber nuclei. Muscular differentiation takes place but may be delayed.

b) Exposure of each of the central quadrants results in defects listed for this group under entire irradiation. There is little difference between these embryos and those in which the entire egg is irradiated. Microscopically, injured cells appear in all body regions and in all tissues. In many young embryos they appear in ectoderm and mesoderm as they do in tissues formed by these. They are always prominent in neuroblasts of the nerve cord.

c) Early irradiation of the posterior quadrant has little effect. In later stages, ($4\frac{1}{2}$-$5\frac{1}{2}$ hours) it gives severe deformities such as failure of the entire abdomen and posterior thorax to develop. Such embryos also have inhibited anterior development which is not the direct result of irradiation.

A consistent feature of the series was organized axial development even by the most grossly distorted and incoherent embryos.

<u>Early blastoderm</u> (determinate phase). Eggs in this stage range from some having only a few scattered cells embedded in the cortical cytoplasm to others having a concentration of cells in the center of the convex ventral surface, but in which pole cells have not yet made their appearance. When the concentration of cells becomes still greater, (10-12 hours) and when pole cells are established, the stage is designated "embryonic plate" (figs. 4,5,6).

Irradiation of areas 1 and 2, and of point 9 (fig. 1), in three-fourths of the eggs treated produced little or no marked change in the structure of the embryonic heads at an exposure time of 150 to 200 minutes. The remaining fourth have deficiencies in the dorsal chitinous cephalic plate, in the anterior center of the head, the labrum, and have distortion of mandibular rudiments. The apparently undamaged embryos, when sectioned, show but little developmental retardation of head musculature or of head capsule. Irradiation of the same areas from the dorsal side has the same general effect but somewhat more severe mandibular distortion as well as injury to the surrounding area.

Irradiation of points between 9 and 10, but not including 10, produce injuries shown in toto or in section in ventral head parts namely, mandibles, stomodaeum, maxillae, mesodermal derivatives of head, cerebral and suboesophageal ganglia are sometimes slightly injured. Deficiency of the anterior entodermal rudiment or its derivative, the mesenteronic lining, has not been observed.

Injured cells in lateral areas 3 and 4 are incorporated in the more anterior pleura, maxillary bases, and in mesodermal derivatives of pleurae and maxillae.

Injury to point 10 and surrounding area extending into areas 2,3,4, and 5, becomes localized as follows:

a) head maxillae

b) ganglia 1-2, 3,4,5,6

c) mesodermal derivatives of medial portion of the embryonic plate.

There is considerable evidence that irradiation of point 10 affects procephalic development outside the target area indirectly since a number of embryos so exposed have drastically reduced and deformed heads without

irradiation injury appearing in cells of the malformed head capsule. The nervous, metencephalic portion developing directly from the irradiated area, on the other hand, has severe histological injury (fig. 7).

Irradiation of areas 7, and 8, near the midline, produce partial to complete developmental failure of the abdomen. This region is largely responsible for the posterior extension of the embryonic plate into a caudal plate flexing dorsocephalad. Its fate will be treated in the following section.

In general, irradiation during the early blastodermal stage produces direct effects extending farther than the target area. This is true especially if the target center falls on or near the median line, and is due to the fact that cells in the irradiated site at the time of irradiation have not yet reached their ultimate destination.

Early embryonic plate. This stage is normally attained in 10 hours at 29° C., but for this work eggs were kept at 16° C. for 20 to 24 hours prior to and during irradiation.

A concentration of cells is found in the center of the ventral surface from which they diminish uniformly in all directions. Pole cells are present at the posterior extremity of the blastoderm and some extension of blastoderm anteriorly and posteriorly from the developmental center, point 10, has taken place but the rapid elongation at the extremities of the plate has not begun (fig. 3).

Cells of areas 1 and 2 become located in the dorsal part of the head. After irradiation of the areas, injured cells are found there in the head capsule and among the hypodermal cells from which the capsule is derived. The resulting injury takes the form of delayed chitinization rather than of a deformity of structure. In the musculature of the procephalic portion

TABLE 2

EXPOSURES OF EARLY BLASTODERM

Central point of target	Number of series	Number of eggs	Number conforming to described type	All Others*
Pt. 9	4	32	15	17
" 10	6	62	23	39
" 11	2	24	16	8
" 12	2	16	11	5
Ar. 1,2	3	25	9	16
" 3,4	3	22	14	8
" 5,6	5	46	29	17
" 7,8	2	22	14	8
Totals	27	249	131	118

* Discarded for showing no effects, being too severely injured for study, or being off target during exposure.

Figure 2. Prospective areas of early blastoderm incubated at 29° C. for 6 to 8 hours.

EARLY BLASTODERM
6-8 Hours incubation

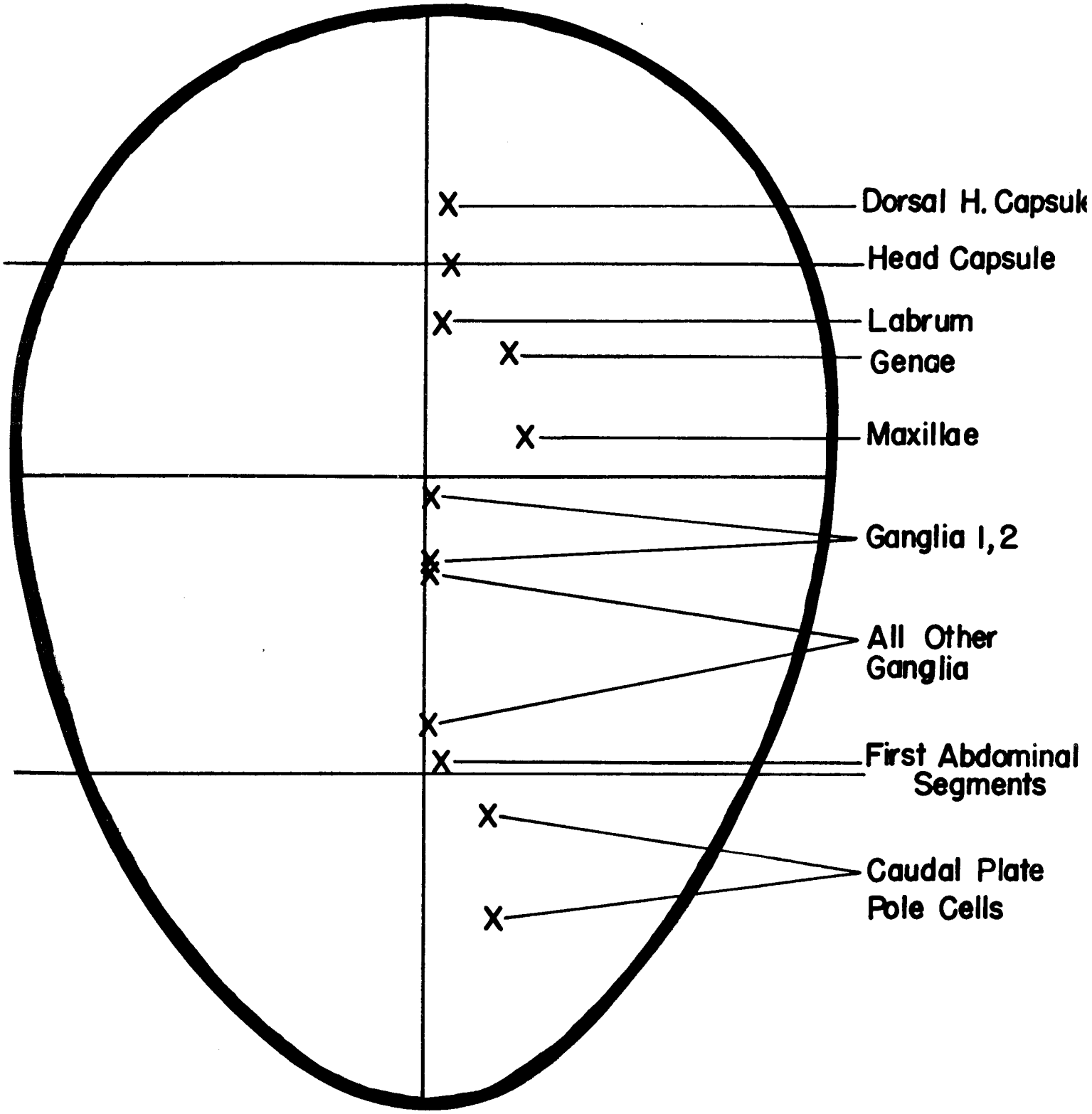


FIGURE 2

Figure 3. Prospective areas of early embryonic plate. Incubation time 10 to 14 hours.

EARLY EMBRYONIC PLATE
10-14 Hours incubation

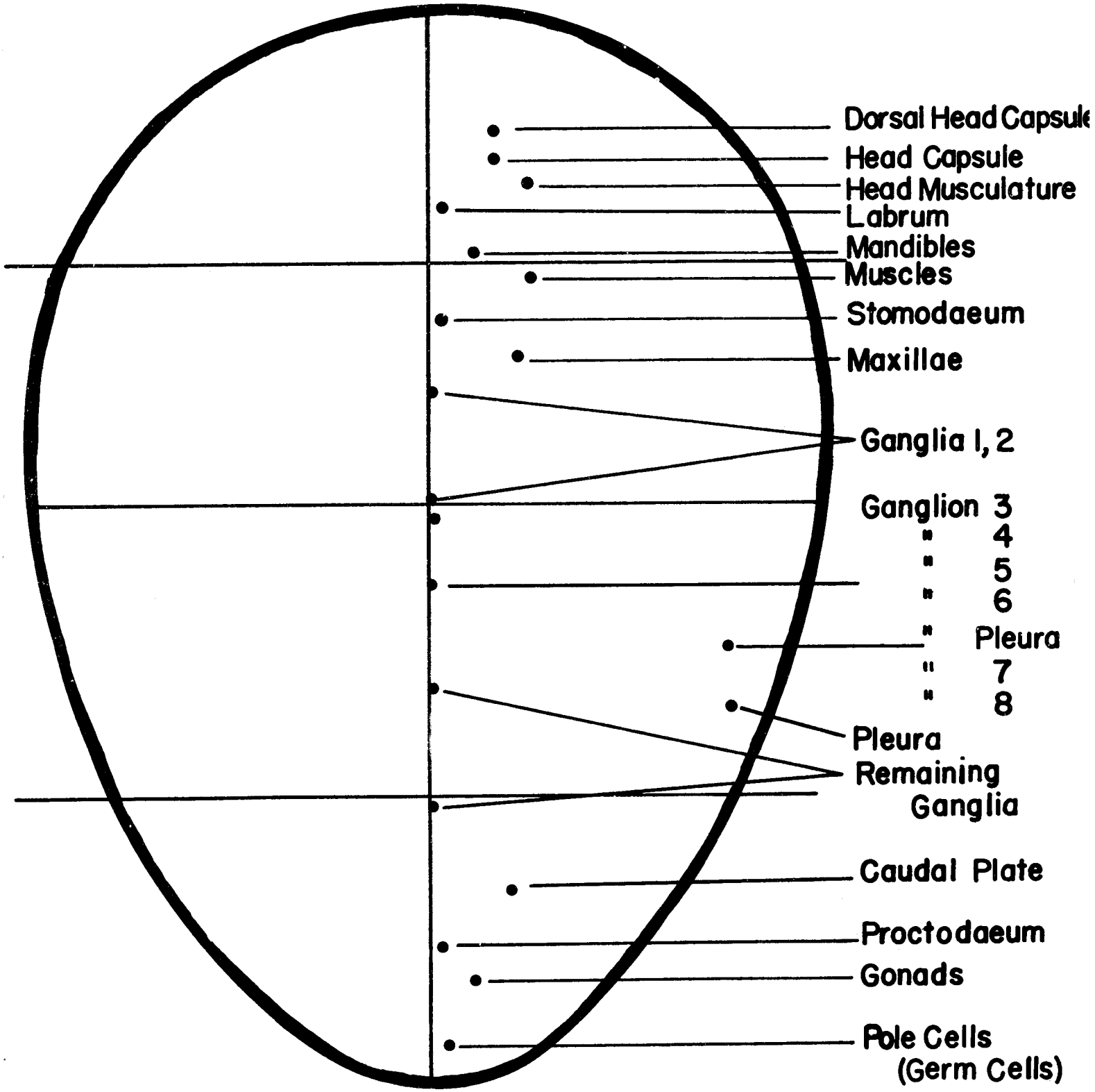


FIGURE 3

there is also an appearance of injured cells, and its differentiation coincides with chitinization of the capsule. In other words there is no asynchrony of differentiation in the components. The nearest approach to head deformity obtained by irradiating areas 1 or 2 laterally, was found in 4 embryos in which a flattening of the head surface appeared on the irradiated side. Actually, this appears to be due to a retraction of head structures from the place of injury. In only two embryos is there a partial failure of head differentiation on the injured side (fig. 9).

Defective development of labrum and antennary lobes were observed in embryos irradiated near the midline. Irradiation here extending into areas 1, 2, from point 9, affects the mandibles, the positions of one or both of these being shifted so that they are asymmetrical. Such defects have been associated with failure of some young larvae to complete emergence, although behavior at emergence appeared normal. They were unable to gnaw into the peas after their heads penetrated the egg chorion and were found dead in a half-emerged position. More often mandibular failure was obtained by irradiating the midline with the target center slightly posterior to point 9.

Areas 3-4 laterally, are associated with dorsal head covering or <u>cephalic plate</u>, since their irradiation delays chitinization and muscularization of this structure. Medially the areas become incorporated into maxillae. Stomodaeal invagination was affected but never completely inhibited by their irradiation. Injured cells were incorporated in its margin and in oesophageal lining.

Changes have occurred along the midline from the earlier blastoderm to its present somewhat axiate form in the localization of primary neuroblasts in the midline. Areas destined to become first and second ganglia

are now slightly anterior to point 10. Irradiation of these areas strikingly affected the entire anterior development of the embryo in the following manner: neuroblasts underwent degenerative changes so that the ganglia failed to separate from the original ganglionic mass; ganglia 3 and 4 sometimes were distorted, possibly by scattered irradiation around the incident beam; and, most striking was the reduction of the entire morphological head, anterior to and not within the irradiated area. This last characteristic has been exhibited by all eggs irradiated slightly anterior to point 10 of this stage and has been reduplicated in numerous control repetitions (figs. 10, 11). Posterior to point 10 and anterior to point 11, along the midline, formative areas of all thoracic ganglia are located. Material around and slightly posterior to point 11 gives rise to all remaining ganglia.

Irradiation of areas 5 and 6, which touch the lateral margin of the definitive blastoderm, affects development of the lateral body wall, and with it the pleural thickenings, the hypodermis, musculature, tracheae, and their formative hypodermis. In general, the blastoderm forms a thin body wall in the injured region.

Irradiation of targets along the midline between points 11-12, and spots somewhat lateral to the midline either prevents the formation of, or produces abnormalities in the caudal plate—an extension of the embryonic plate around the posterior extremity of the oöplasm. It forms rapidly between 16 and 24 hours until it almost touches the cephalic portion of the embryonic plate which is flexing around the anterior oöplasm at the same time. The caudal plate normally flexes dorsally to the right in about 70% of embryos, while in those irradiated it flexes right or left, away from the point of injury, or around it if located within the growing region. Irradiation data indicate that this plate, upon contraction, forms the posterior abdominal extremity,

proctodaeum, colon, ileum, and Malpighian tubles. It also forms the rudimentary genitalia and the primordial germ cells become embedded in its wall. In the irradiated specimens some or all of these structures may be lacking or defective. The abdomen may appear almost complete excepting that a small knob-like prominence remains attached to it, an indication that the caudal plate has failed to contract properly (fig. 12). The dorsal body wall may fail to close and the posterior mesenteron rudiment may be absent entirely or unilaterally, since it appears to form from bilateral components at each side of the proctodaeal invagination.

Irradiation at point 12, or pole cell site, strikingly affects development of the germinal epithelium and the gonads. Relatively light irradiation, 9000 ergs per mm.^{2} produces germ cells with enlarged chromosomes or pyknotic chromatin (fig. 20).

Cellular injury by irradiation. Recognition of injured cells is difficult only where degree of injury is slight or where there are few interspersed among normal cells. Injured areas can be recognized readily. Such cells or tissues formed by them have been followed through several stages of development in sections of graded embryos.

Cleavage cells (preblastoderm). In eggs irradiated entire shortly after deposition, all cleavage cells are injured and many are enlarged. Nuclei of some have diameters 12 to 15% greater than normal and enlarged prophase chromosomes which appear ragged or riddled. Nuclei of others are of nearly normal size, have a tendency to stain acidophilically and have peripherally clumped chromatin. Cells of both types reach the egg periphery and take part in forming the blastoderm while others remain behind, as in the normal egg, to become yolk cells.

Blastodermal cells. Within the blastoderm injured cells carry the defects

TABLE 3

TABLE OF EXPOSURES IN LATE BLASTODERM TO EARLY EMBRYONIC PLATE STAGES

Central Point of Target	Number of series	Number of eggs	Number of eggs conforming to type	All others
Pt. 9	4	40	32	8
" 10	4	46	18	28
" 11	3	28	13	14
" 12	2	16	12	4
Area 1,2	3	31	18	13
Area 3,4	2	16	10	6
Area 5,6	4	38	12	26
Area 7,8	3	20	15	5
Total	25	235	130	104

shown previously. Mitoses observed in a limited number of sections of these stages are decidedly irregular, with chromosomes dividing atypically, forming daughter cells with smaller nuclei having peripherally clumped chromatin. Metaphase chromosomes are decidedly irregular and ragged. Spindle fibers are prominent and carry conspicuous granules (figs. 13, 14, 15, and 16).

Mesoderm or mesoblast nuclei are of the two types described previously, namely large and pyknotic or smaller with massed chromatin. The two types are apparently due to degree of injury to blastodermal or preblastodermal cleavage cells initially. Muscle fibers may differentiate from the first type but apparently not from the second. (fig. 17, 18, and 19).

Germ cells are injured and become clearly distinguishable after the irradiation of point 12, of the early embryonic plate. The cells eventually become localized in the abdomen between the primitive gut and the body wall. It is noteworthy that the cells develop symptoms of irradiation after exposures too brief to injure tissues which develop from the adjacent plate cells. All groups of such germ cells observed conformed only to one type, namely enlarged cell bodies, and nuclei with pyknotic prophase chromosomes. No mitoses were observed in them in 96-hour larvae.

Nerve ganglia and their neuroblasts are most susceptible to irradiation injury, the ganglia readily becoming distorted and malformed. Ectoderm cells of the first type (enlarged nuclei) form ganglia which appear to differentiate commissures and tracts, but with evidences of injury remaining in the cells. In such ganglia there is often bilateral asymmetry in the size of components and deviation from midline or horizontal plane. Small, dense ganglionic masses which fail to differentiate ganglionic cells are formed by those neuroblasts with densely staining nuclei. Such masses are asymmetrical and frequently unpaired.

DISCUSSION

Susceptibility to ultraviolet (2537 A) irradiation on the basis of lethality as shown in this study increases directly by period up to 14 hours. These stages are cleavage, during which the egg is indeterminate; blastoderm formation, during which embryonic potencies become established along the longitudinal axis; and axiate blastoderm or embryonic plate in which the first suggestions of neurulation appear. This increasing susceptibility appears in agreement with a study by Geige ('31) as to susceptibility of Drosophila eggs to ultraviolet irradiation, in which age of eggs was expressed in hours only, without mention of developmental stage, and in which adult emergence was the criterion of susceptibility. On first view this appears in disagreement with the observations on susceptibility of the egg to chemical, oxidation inhibiting agents (Brauer '38, '42) in which it was found that susceptibility was especially high in the first period, rather high in the third, but relatively low in the second. The criterion of susceptibility was degree of development attained after treatment rather than lethality.

Considering the action of ultraviolet rays the difference in susceptibility by period is logical. During the first period the proliferating cleavage cells are deep in the oöplasm, and other number relatively small (16 to 120). In order to strike them the rays must penetrate the chitinous egg chorion, some of the syncytial cytoplasm, and a considerable portion of the yolk (lecithin). Both chitin and cytoplasm can be expected to absorb some of the energy, and the yolk an additional amount. The nuclear target within the egg is relatively small. In the second and third periods the cleavage cells are peripheral in location and occupy much more of the surface area. The rays now have only to pass through the chorion to be effective.

In addition a greater percentage of the total radiation can strike cell nuclei, since a much greater percentage of the surface is nucleate. In the third period the principal structural change is that blastodermal cells become concentrated in the center and along the midventral line of the blastoderm. These cells receive the full incident radiation. In development, after irradiation, they become incorporated into the axial embryonic structures, especially the nervous system where they form large defective areas.

Through the selective action of ultraviolet rays on nucleic acid, which is contained in the cleavage cell nuclei of the early egg, the cortical cytoplasm of the egg remains relatively uneffected. The cleavage cells, although injured, become enclosed in it and organized into an axiate embryo. This contrasts with the development of eggs after treatment with respiration inhibiting agents (Brauer '38, '42) after which the cleavage cells are relatively uneffected in the first embryonic period. They proliferate freely, move toward the egg cortex, and arriving there may or may not become established in it depending on the degree of injury to it. If not established, the cells can only proliferate without forming an axiate embryo, but if established, axiation results.

The principal difference between the two types of injury as regards embryonic development is that injury to cleavage cells by ultraviolet irradiation produces anatomical modification and abnormality of structures within the embryo, whereas, injury to cortical cytoplasm by respiration inhibiting agents produces abnormality of the embryo as a whole, namely axial organization and development, differential inhibition of cephalic development, lateral growth and otherwise abnormal relative growth rates between parts.

A possible causal relationship of development merits mention, namely,

that of head development. This has been discussed as to morphophysiological relationship in a previous paper (Brauer '46). In the present work, irradiations made on a target between points 9-10, in the third period, affect development of cerebrosuboesophageal ganglia, and when their development is impaired a corresponding lack of development also occurs in the head capsule. This exposure was often repeated for the elimination of possible experimental error, so that it is certain that injury to the capsular head is due to this particular injury. Unfortunately, the method of experimentation does not afford a means of proof of the implication.

Particular tissues in which irradiation effects are especially pronounced are germinal, and nervous tissues. In the former, follicles of injured germ cells appear in relief against surrounding uninjured tissue in the abdominal wall when the posterior egg extremity is irradiated in late blastoderm or early embryonic plate stages. By means of the injury they have been identified for the first time in this embryonic larva. Since it is possible to irradiate the primordial germ cells in their isolated polar position with almost no exposure of adjoining blastoderm, only the germinal tissue is injured. Aboïm, ('41) employed this method for tracing germinal tissue and gonad development in Drosophila and Crowdus ('48) followed irradiated pole cells from egg to pupal gonads in Callosobruchus.

In nervous tissue, damage by irradiation appears to extend farther than in others and seems to prevent complete differentiation. Mass movements and projection of the embryonic plate can account for the extension of damage but cannot entirely explain its qualitative differences in tissues. To account for this it is necessary to look for intrinsic differences, but it must be remembered that no qualitative differences are present in the presumptive and prospective neuroblasts irradiated. A distinctive feature of

nervous tissue which could account for the difference might be found in the Nissl bodies which are known to contain nucleic acids. It could be possible that irradiation of prospective neuroblasts disturbs the mechanism of differentiation of these distinctive features.

As a means of delimiting prospective areas and cellular displacements in the insect egg, ultraviolet irradiation serves well but is not ideal, since it imposes abnormalities upon the tissues and thereby makes their ultimate fate uncertain. However in this respect it is superior to cautery and some operative techniques in that it does not destroy or remove cells outright but leaves them so that their development may be followed.

Because of individual organic variability, variations in irradiated eggs under the best of controlled technical conditions certainly occur. In this study attention was centered on those individuals considered average or typical for any given exposure. Because of the number of eggs so irradiated and the relative number of typical abnormalities obtained and studied in toto and in section, interpretations are believed to be correct. They are also in approximate agreement with those of Seidel ('35) for Drosophila, excepting his conclusion that presumptive areas first occupy a larger area of the egg surface prior to their concentration at the "formative center," unless "presumptive area" is intended to convey the meaning of nonspecificity.

SUMMARY

1. Eggs of the pea beetle Callosobruchus maculatus were treated with ultraviolet 2537 A at an intensity of 17.5 ergs/mm^2/sec. to determine: (a) general susceptibility by stage to ultraviolet irradiation; (b) value of cleavage cells in embryonic organization; (c) localization of formative values of points and areas within the early blastoderm and in the embryonic plate.

2. Susceptibility to irradiation increases directly by developmental stage of early development. These stages are, cleavage, blastoderm formation, axial development.

3. In the first stage, irradiation short of destruction of the first cleavage cells, results in deformed but organized and axiate embryos. This is interpreted to mean that embryonic organization does not rest in the cells per se, but in the egg as a whole and in the cytoplasmic cortex in particular.

4. Target irradiation of the early blastoderm indicates that formative values of embryonic areas are principally located along and immediately to each side of a ventral median line. Supra- and suboesophageal ganglia arise from cells directly in the center of the ventral surface and all other ganglia from cells just posterior to this center. The capsular head, not including ganglia, originates from cells immediately anterior to this "center of development."

5. In the embryonic plate, formative values are extended anteriorly and posteriorly along the median line. Supra- and suboesophageal ganglia are more widely spaced posteriorly along the ventromedial line. More specific values become attached to points lateral to it.

6. Injury to cleavage, blastodermal, and various tissue cells indicates that cellular degeneration proceeds as follows: a) enlargement and pyknosis of chromosomal threads with enlargement of nucleus and cell. Chromosomes appear ragged, mitotic figures have prominent spindles with granules located on the fibers; b) continued pyknosis with peripheral clumping of chromatin. Portions of, or entire nuclei tend to stain acidophilically, nuclei and cells divide atypically and spindles are not found c) vacuolation of nucleus occurs with a breakdown of nuclear membrane and cell.

7. If susceptibility is registered differentially in the early tissues,

its effects appear most pronounced in neuroblasts and in the formation of ganglia, and least in muscular differentiation.

8. Possible causal relationship between parts of the embryo is shown in that severe injury to supra- and suboesophageal forming area registers a corresponding lack of development in the head capsule even though the latter area is uneffected by incident of scattered irradiation.

Figure 4, 5, 6. Early blastoderm, early, and later embryonic plates respectively.

Figure 7. Early blastoderm egg irradiated in target area 3. Note effects on anterior ganglia with resulting reduction of capsular head.

Figure 8. Early blastoderm irradiated in area 5. Central ganglia, and pleural folds of right side are affected.

DWG. 7365

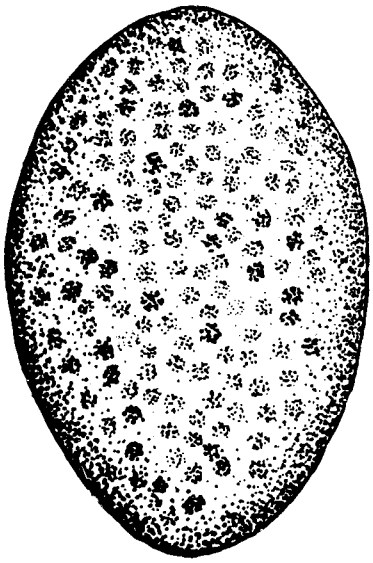


FIG. 4

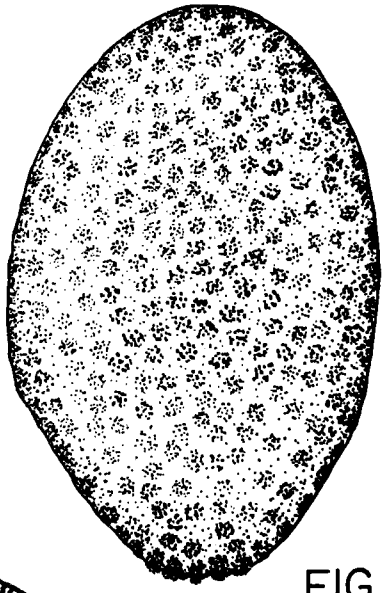


FIG. 5

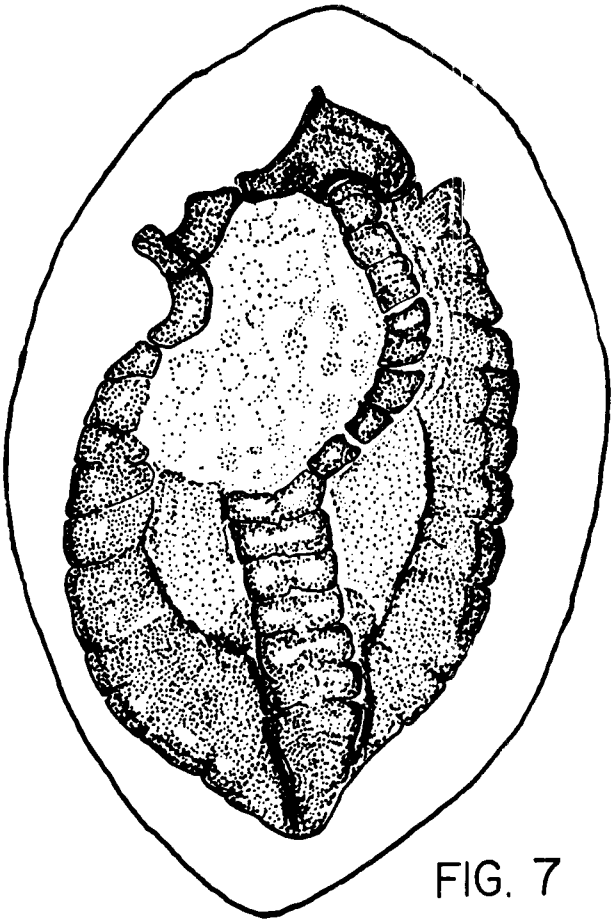


FIG. 7

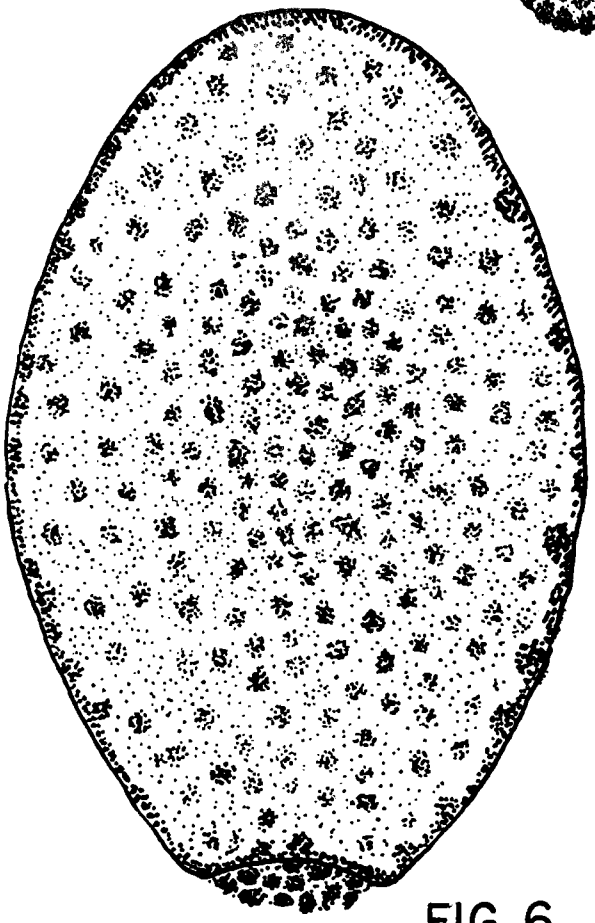


FIG. 6

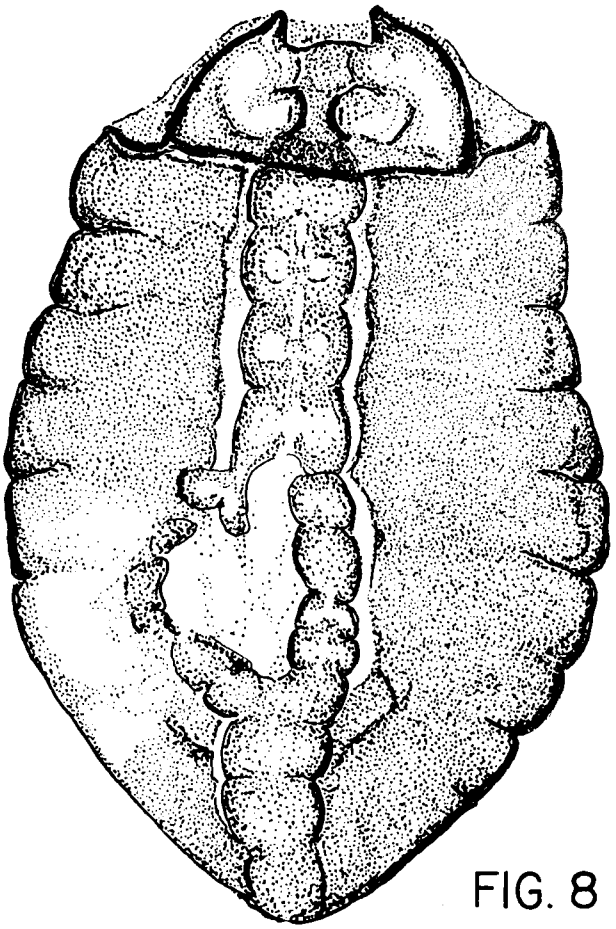


FIG. 8

Figure 9. Embryonic plate irradiated in area 2. Effect is registered on left side of capsular head.

Figure 10. Embryonic plate irradiated at point 10. Anterior ganglia are poorly differentiated with the resulting reduction of capsular head.

Figure 11. Embryonic plate irradiated between points 9 and 10. Anterior ganglia fail to differentiate and result in very marked reduction of head. Abdominal portion of embryo has failed to keep pace with thoracic segments.

Figure 12. Embryonic plate irradiated in area 8. Abdomen has failed to develop properly. Irradiated side also shows lack of posterior mesenteron rudiment.

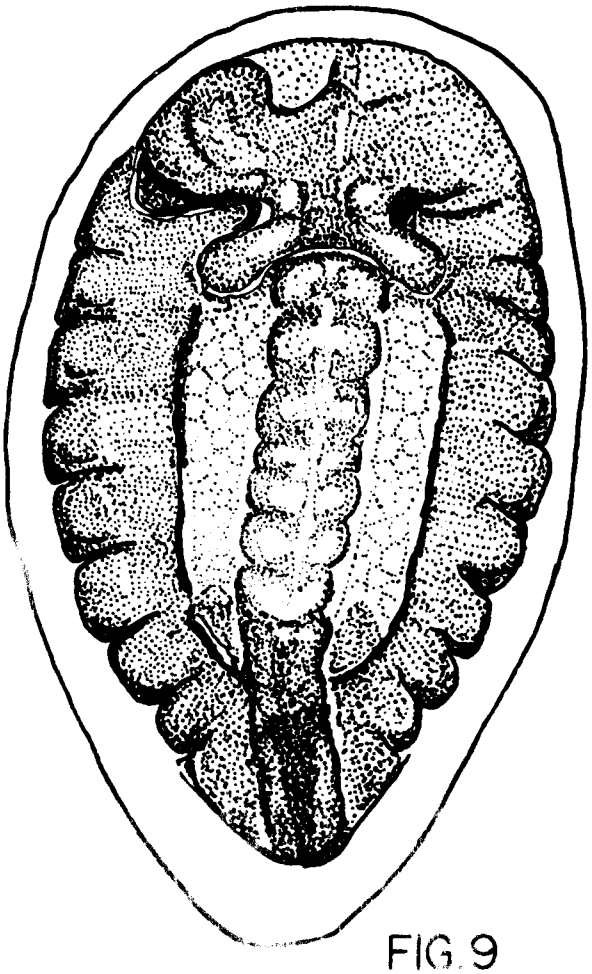


FIG. 9

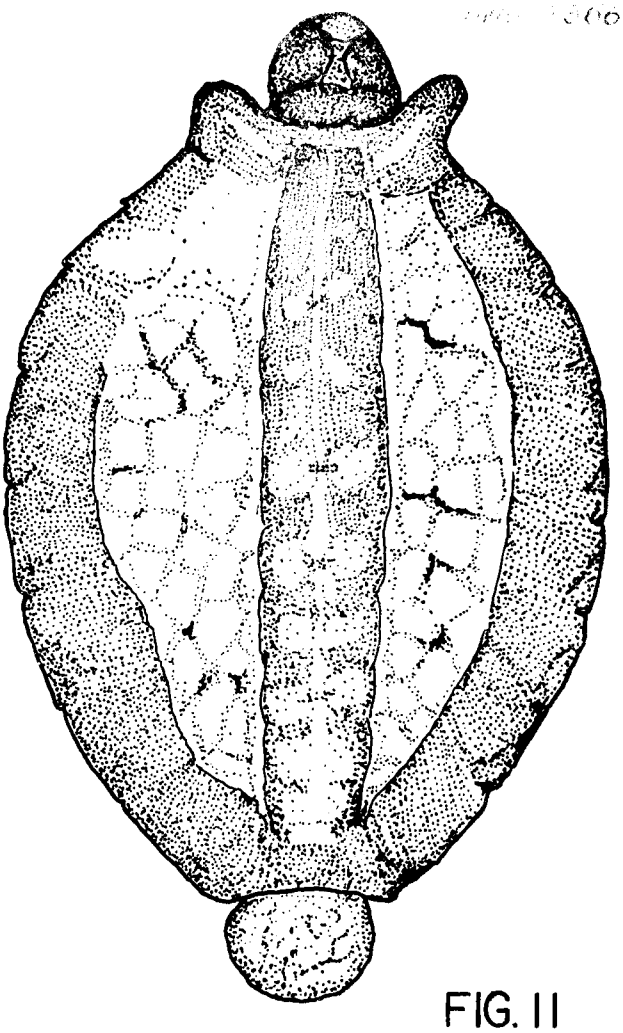


FIG. 11

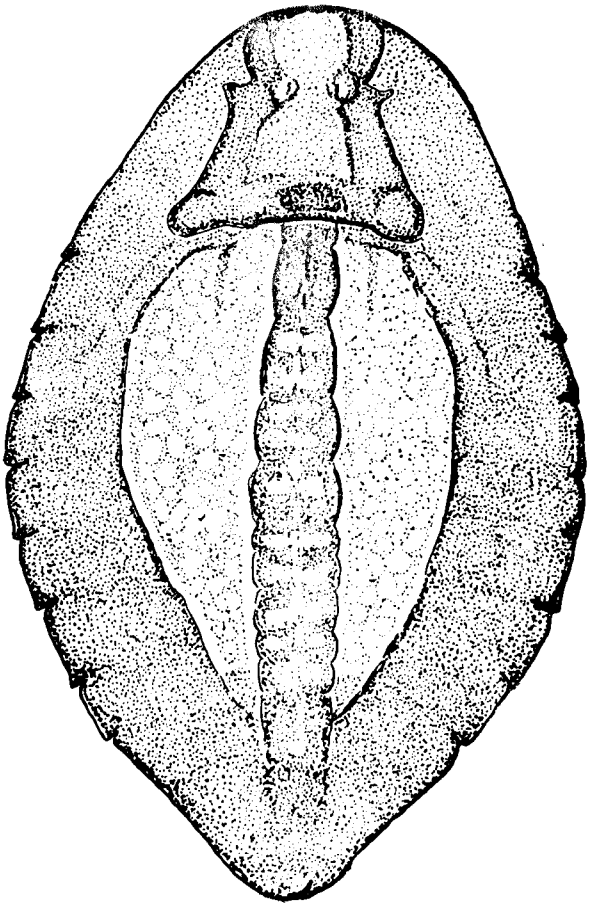


FIG. 10

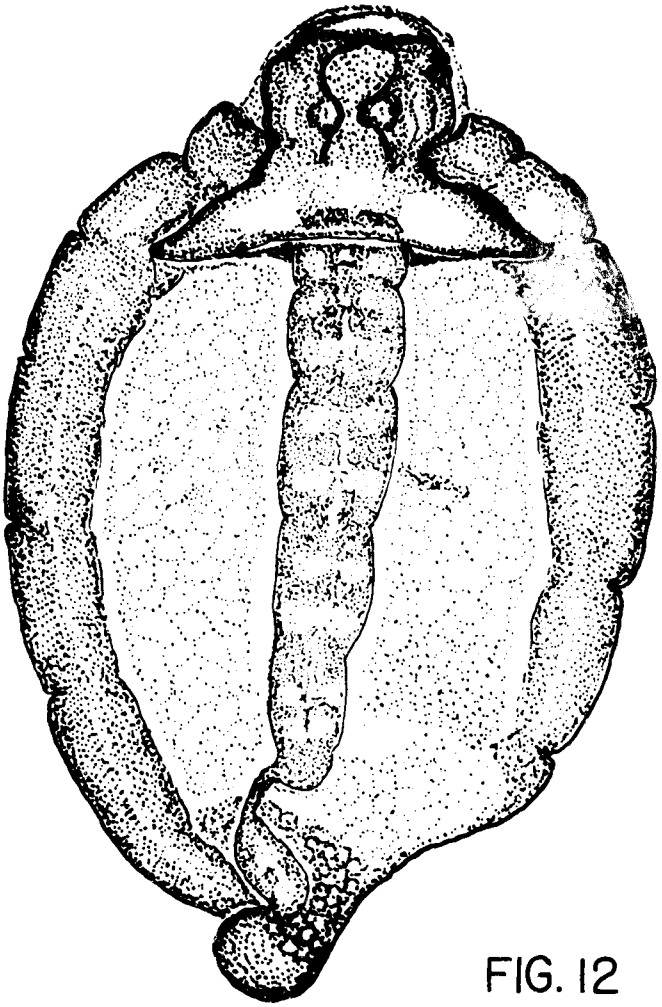


FIG. 12

Figure 13, a,b. Irradiated and normal cleavage cells respectively.

Figure 14. Irradiated cleavage cell in cortical cytoplasm.

Figure 15, a,b. Irradiated and normal cleavage cells in division.

DWG. 7367

FIG. 13a

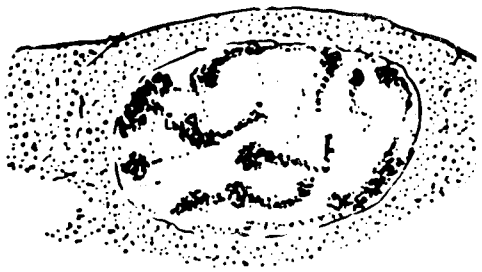


FIG. 14

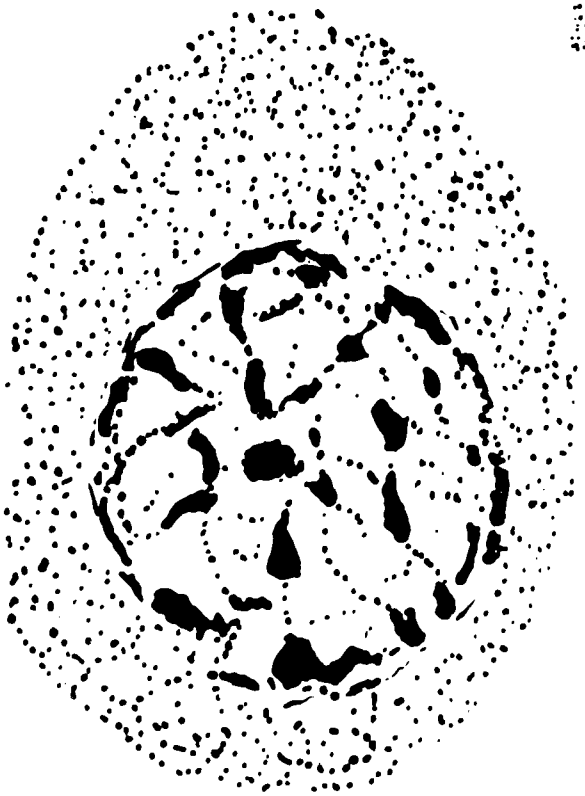


FIG. 13b

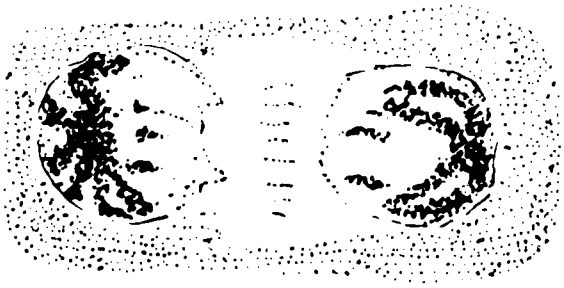


FIG. 15a

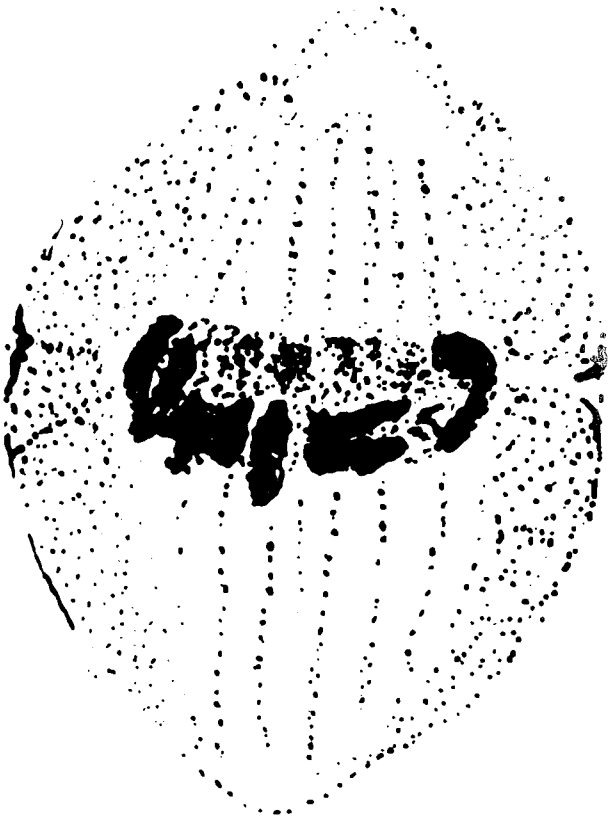


FIG. 15b

Figure 16, a,b. Irradiated and normal ectodermal cells respectively of a 36-hour embryo.

Figure 17. Mesodermal cell showing severe irradiation effects.

Figure 18, a,b. Affected and normal mesodermal cells respectively in division.

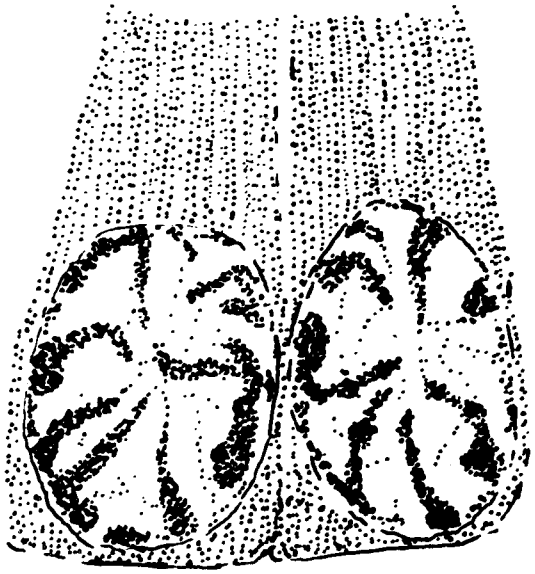


FIG. 16a

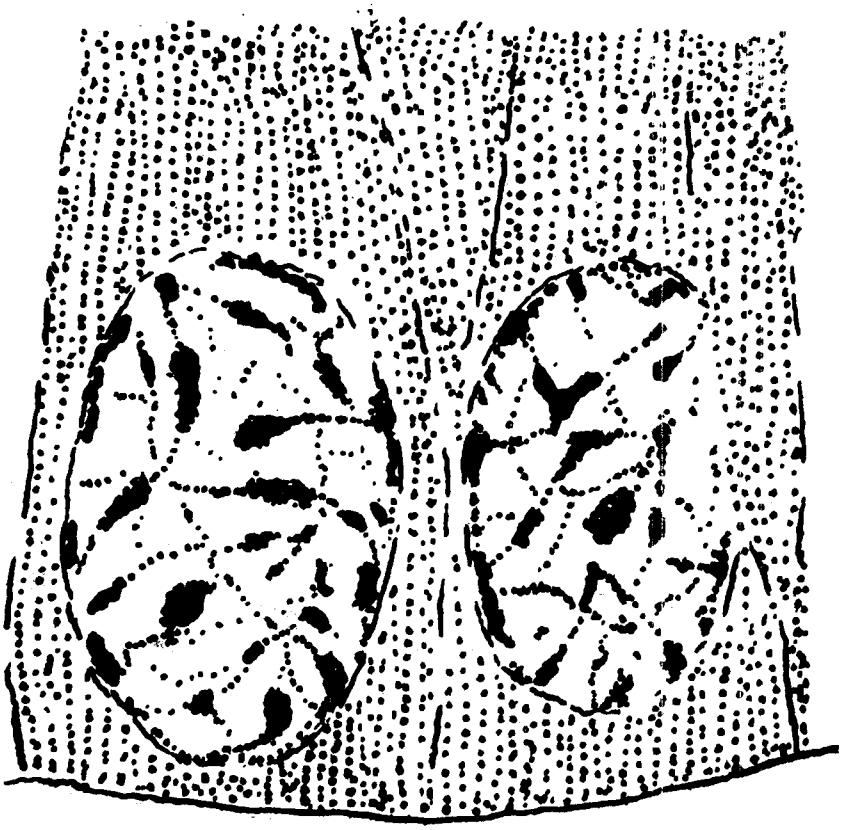


FIG. 16b

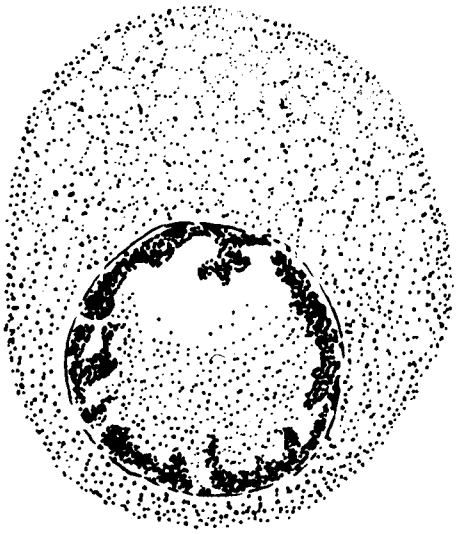


FIG. 17

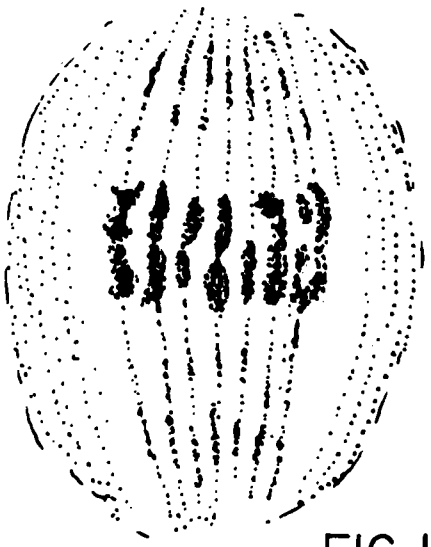


FIG. 18a

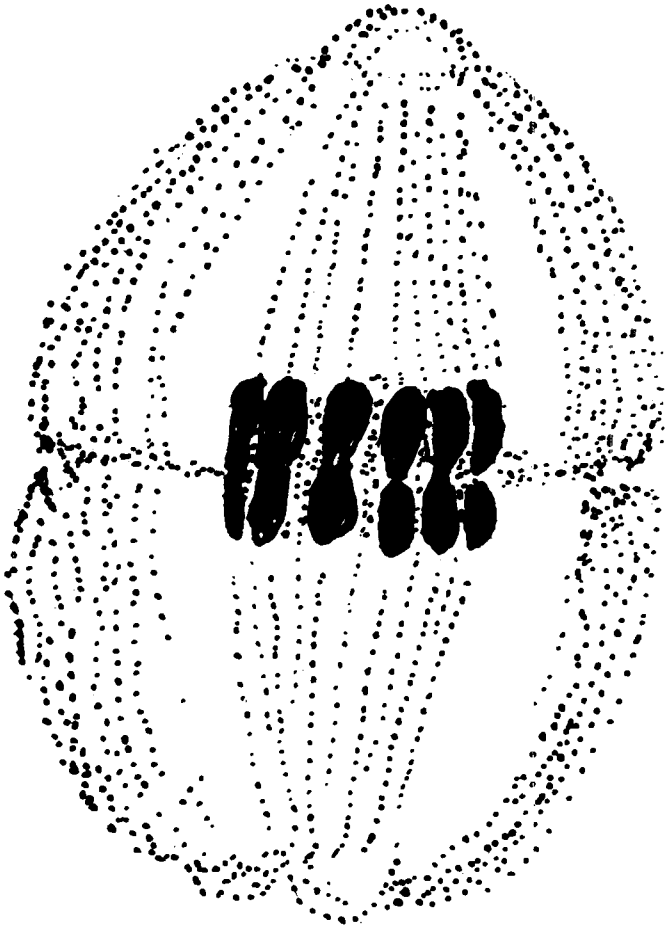


FIG. 18b

Figure 19. Severely affected mesodermal cell prior to disintegration.

Figure 20, a,b. Affected and normal germ cells respectively, irradiated as pole cells in embryonic plate stage.

DWG. 7369

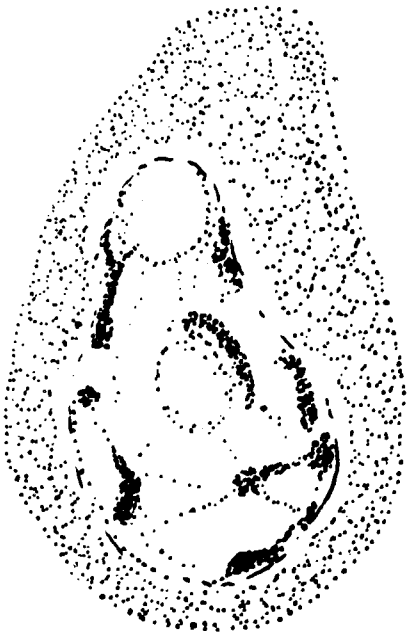


FIG. 19

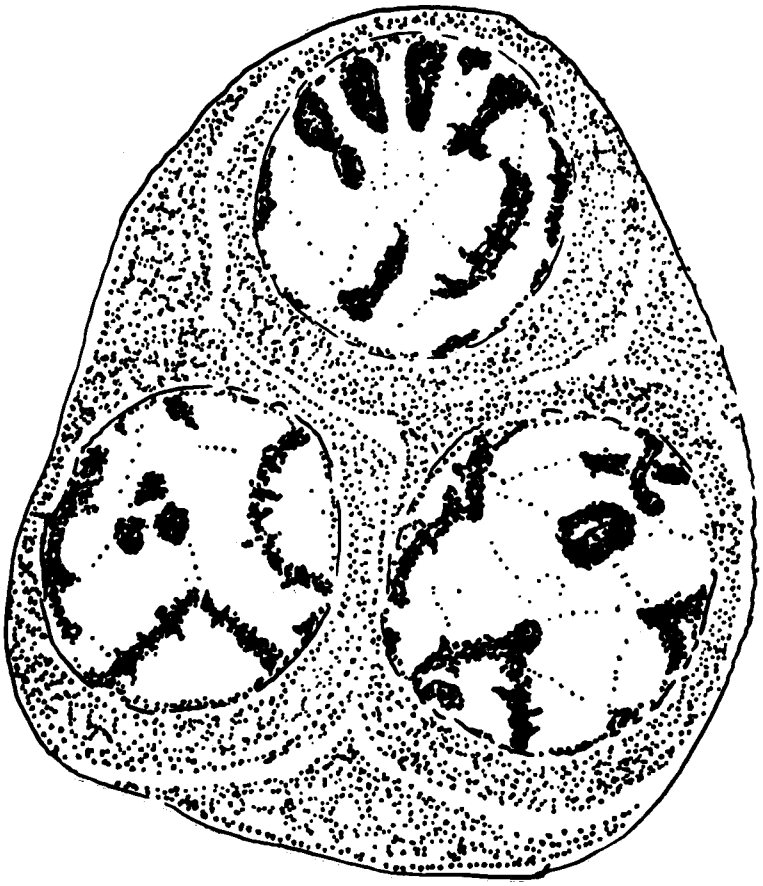


FIG. 20a

FIG. 20b

FOOTNOTES

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