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Introduction

In a number of previous papers we have reported on modifications in the morphogenesis of *Drosophila melanogaster Meig.* by x-irradiation, neutron bombardment, and under adverse environmental conditions. Additional data concerning the problems involved are found in the papers of workers in the same field, who have used the same or different methods of approach (Patterson, 1929; Geigy, 1931; Henshaw, 1933; Richards and Furrow, 1925; Jones, 1936; Russel, 1940; Hartung, 1942; Lawrence, 1937; Combs and Gravett, 1937; Mavor, 1927; Bardeen, 1910; et al).

A comparison of various methods used in the study of these questions show that the differences in the results obtained depend largely on the following factors: genetic constitution of the organisms used in the experiments, nature of the agent used to modify development, stage of development during which the agent was active and environmental conditions during and after the exposure.

Thus it has been shown (Patterson, 1929; Enzmann and Haskins, 1938; Combs and Gravett, 1937) that x-ray treatment of the eggs or of the newly hatched *Drosophila* larvae resulted in abnormalities which could be explained as effects of somatic mutations, somatic segregation, or of gross chromosomal aberrations. While mosaics in the compound eyes of the flies, or of twin spots of apparent different genetic constitution within the integument or affecting the bristles, seem to belong to this category. Other abnormalities find a more logical explanation in the derangement of normal embryological processes, such as in the faulty displacement of organ primordia, or of parts of them (f.i. in the case of an antenna growing out of the center of the compound eye), deletion of some cells of a given rudiment, thus disturbing the continuity of a growing complex followed by defective regulation (the duplication of the legs or wing or of entire body segments can be explained by this theory), or even the appearance of atavistic characters (as for instance the replacement of halteres by winglike structures).

Neutron bombardment produces essentially the same types of abnormalities as does x-ray treatment, but the effective doses are smaller with neutrons (Lawrence, 1937; Enzmann and Haskins, 1939). The proportion of gross abnormalities due to upsets in the normal formative processes—as opposed to genetic factors—is likewise greater under neutron bombardment than under x-ray treatment. Adverse environmental conditions (cf. Haskins and Enzmann, 1938) have produced non-hereditary modifications of a distinctive character: decreased body size, abnormal integument, defective eye structures, enormous delay of larval development.

It can be said, however, that each agent produces a definite type of abnormality; more often on and the same aberration may be induced by the separate action of more than one causative factor.

The present paper deals with modifications of the external anatomy of the fruitfly *Drosophila melanogaster* arising from exposure of the eggs or very young larvae to ultraviolet radiation.

Material and Methods

The material used in the present investigations was a stock of wild *Drosophila melanogaster* which has been bred in this laboratory for eight generations by brother-sister matings and thereafter in mass culture. Two mutations have appeared in the stock during the past year, one of them sex-linked, the other autosomal.

The methods of culturing the flies, collecting and counting the eggs and examining the treated flies has been described elsewhere (Haskins and Enzmann, 1937; Crozier and Enzmann, 1937). Circular discs of corn meal-agar-molasses, about 2.5-3 cm in diameter, were mounted on glass squares which fitted over the mouths of pint milk bottles. Before use these circular cookies were painted with parallel lines of India ink in order to facilitate the counting of the eggs. A fresh suspension of yeast cells in water was applied to the cookies which were then inverted over the openings of the bottles holding the flies. The egg laying females were always drawn into empty bottles in order to avoid having maggots crawl up from the culture medium and settle on the fresh yeast. Egg laying lasted on the average of one hour, after which the flies were returned to their cultures and the eggs were counted under a dissecting microscope. If young larvae were needed for experiments, the cookies holding the eggs were covered with shallow Petrie dishes, to prevent evaporation, and the eggs were left to hatch. The majority of the young larvae emerge 17-19 hours after egg deposition. The newly hatched larvae or eggs were exposed to graded doses of ultraviolet

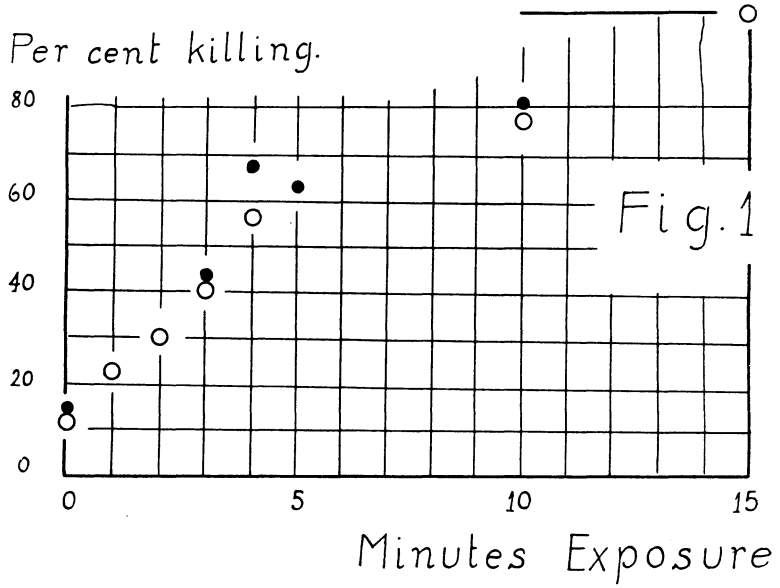

Fig. 1. Killing curve of Drosophila melanogaster by graded exposures to ultraviolet light. Open circles represent young larvae of the first instar, full circles newly laid eggs.

light from a quartz mercury vapor lamp. The target distance was 3 cm in all cases. The intensity of the energy source was such that an exposure of 4-5 minutes killed half of the newly emerged larvae. The killing curve is presented in fig. 1.

Discussion of the Results Obtained

Table 1 shows that ultraviolet treatment of young larvae of *Drosophila melanogaster* produces a relatively high rate of gross abnormalities of development resulting in various types of deformities of the body of the imago. The following preliminary classification has been adopted for descriptive purposes:

1) the right and left halves of one or more abdominal segments fail to meet and to fuse in the dorsal midline of the insect,
2) half of a body segment is absent and its partner on the opposite side of the body is left unconnected,
3) the halves of various body segments are fused with the wrong partners, i.e. the left half of a given segment is fused with the right half of a preceeding or a following segment,
4) one or more body segments are completely absent, resulting in a fly with a greatly foreshortened body,
5) the gaster is grossly distorted and twisted out of shape,
6) the external genitalia are misshapen,
7) some body segments are greatly retarted in growth, which results in pedicel-like constrictions of the adult gaster,

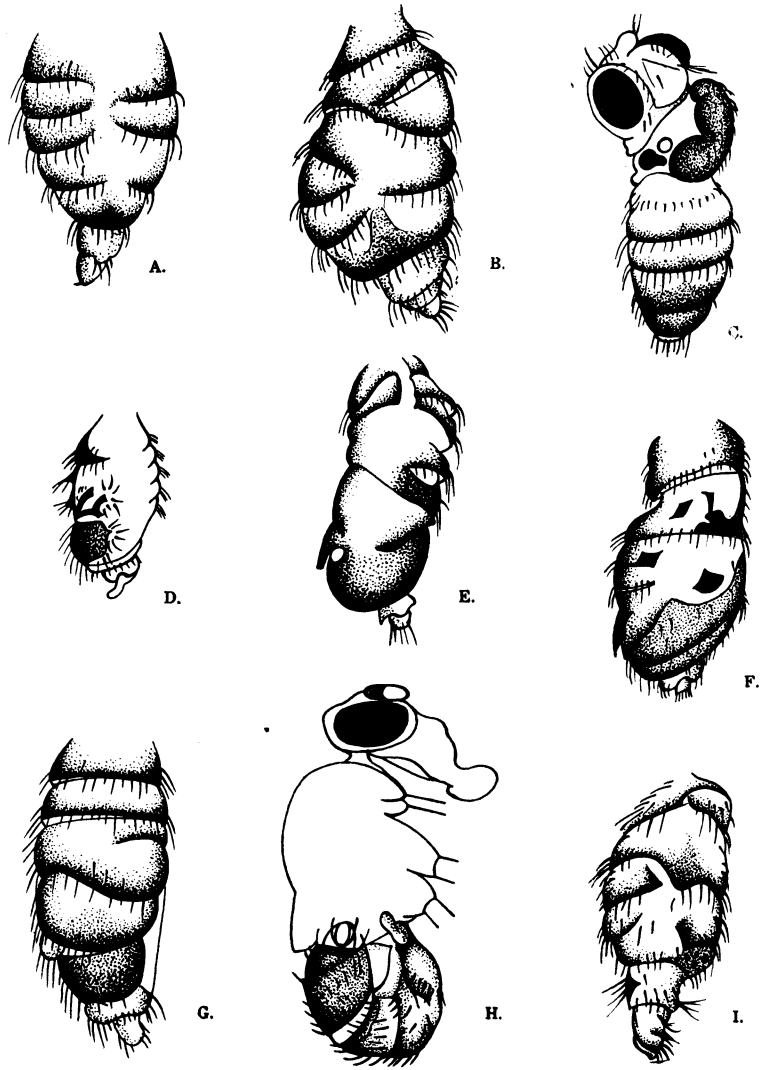

Figure 2. Semidiagrams of abnormalities in Drosophila melanogaster produced by exposure of newly laid eggs and young larvae to ultraviolet radiation.

a. female; failure of fusion of body segments in the mid-dorsal line.
b. female; several body segments are fused in a star-shaped pattern and the abdomen is grossly malformed.
c. male; the left half of the thorax is missing and there is a melanotic tumor in place of the absent left wing; the gaster is normal.
d. male; several abdominal segments are missing and the remaining ones are irregularly fused.
e. male; the abdomen is badly twisted out of shape to absence of some segments, fusion of others with the wrong partners and intercalated segments; the left side bears a black tumor.
f. male; irregular distribution of melanotic areas and pedicel-like constriction due to lack of growth of the second segment.
g. male; fusion of the wrong segments producing an abnormally shaped gaster.
h. male; the entire left half of the gaster is missing and the abdomen is badly twisted; the left wing is absent.
i. female; intercalated supernumerary half of a segment bearing freckle-like melanotic areas; the anal plate is reduplicated.

8) most of the body segments on one side of the body are absent which produces half a gaster,
9) segmental primordia have been split, leading to the formation of extra (intercalated) segments or parts of segments,
10) the anal plate is reduplicated,
11) the pigmentation of the abdomen is arranged in an irregular checkerboard pattern,
12) melanotic areas and melanotic tumors are present,
13) the wings are shortened, notched, crumbled or entirely absent on one or both sides of the body,
14) parts of the thoracic sclerites are absent,
15) legs are absent or duplicated.

Some of these abnormalities are shown in fig. 2.

The observed abnormalities have been listed roughly in the order of frequency of their occurrence. It was noted that often combinations of several abnormalities were present (a condition often found in human congenital abnormalities). It will be noted (fig. 2.) that the majority of the observed abnormalities involve the exoskeleton of the gaster and that there exists a consistent pattern not found in the injury pattern caused by penetrating radiations (x-rays, neutrons, etc).

The first series of experiments involved newly hatched larvae. The observed injury pattern suggested that it could have been due to the fact that most of the larvae are actively feeding at the time of the exposure to ultraviolet light and have their heads buried in the culture medium and shielded from the rays. This theory was tested by a second series of experiments in which newly laid eggs were exposed to U. V. radiation. The laying flies deposit most of their eggs in a characteristic position with the end bearing the micropyle and the two filiform appendages pointing upward, while the opposite pole is buried in the culture medium. A considerable number of eggs is, however, deposited so that their long axes are parallel to the surface of the culture medium. In any case, the orientation of the embryos in respect to the direction of the incident rays is different from that of young larvae and one would expect a different injury pattern to appear. The results of this second series of experiments were practically identical with those obtained from raying larvae, proving that orientation can not account for the characteristic radiation injuries.

Two theories suggest themselves as an explanation of the results obtained. It is known (cf. Williams, 1950) that the formation of the abdomen in *Cecropia* is controlled by an endocrine gland located in the thorax and that this gland is in turn influenced by the corpora allata. Radiation injury to one or the other of these two

Table I

Showing a) the number of young Drosophila larvae,
b) the number of eggs of Drosophila melanogaster which have been exposed to ultraviolet rediation and the per cent injuries produced by various dosages.

a) Larvae.

Exposure time in minutes	Number of rayed larvae	Number of abnormalities	Per cent of abnormalities
1	137	2	1.46
2	288	11	3.82
3	418	14	3.35
4	319	24	7.53
5	627	22	3.50
8	159	7	4.40
10	210	6	2.86

b) Eggs.

Exposure time in minutes	Number of rayed larvae	Number of abnormalities	Per cent of abnormalities
0	5042	0	0.00
1	540	0	0.00
2	720	10	1.39
3	644	17	2.64
4	44	3	6.81
5	485	40	8.20
8	165	6	3.64
10	2750	19	0.69
15	803	2	0.25

The smaller percentage of abnormal flies obtained with higher doses of ultraviolet light is due to the fact that gross abnormalities prevent normal emergence of the imagos.

organs might presumably upset the complete fusion of the imaginal discs of the abdomen during pupation.

A second theory seems to offer a more plausible explanation; this theory assumes a direct injury to the abdominal imaginal discs by the incident radiation. It seems that injury to the prothoracic gland or its isolation from the abdomen in the experiments reported by Williams acts on the "all or none" principle; either the abdomen develops in the typical fashion or it remains infantile (in the larval stage). In the present experiments pupation takes place, though in many cases one or more imaginal discs are eliminated entirely.

It is of interest to note, that the absence of a skeletal part seems to be compensated for in every case by a corresponding hypertrophy of the intersegmental membranes; the integument of the fly is always complete, regardless of how much of the body is missing.

Other abnormalities, such as duplications, failures of fusion, tumors, etc., find an explanation in the theories put forward to explain similar abnormalities in mammalian development.

The predominance of abdominal malformations in the present experiments raises still another question: according to Chen, 1929, and Geigy, 1931, the abdominal hypodermal discs become visible rather late during development. The times given (cf. Strasburger, 1935) are eight hours after the formation of the prepupa (compare fig. 3). The hypodermis is supposed to become continuous during the 60 hour stage.

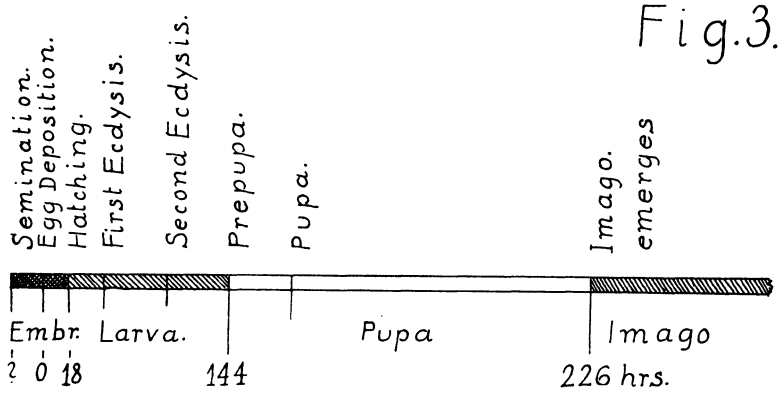

Fig. 3. Diagramatic representation of the life cycle of Drosophila melanogaster.

The present experiments suggest strongly that the abdominal discs as well as the discs of the copulatory organs are in existence much earlier and undergo active development long before they become discernable under the microscope.

No attempt has been made at the present time to study the internal organs of the deformed flies. It has been noted that abnormally developed females rarely become pregnant and that the few which do reproduce, have normal offspring in the first and second filial generations.

Summary

Exposure of eggs of *Drosophila melanogaster* or of its young larvae of the first instar to graded doses of ultraviolet light produces a high incidence of malformed imagos. The malformations fall into a characteristic injury pattern involving largely the exoskeleton

of the abdomen and the external genitalia. The results obtained are best explained by assuming a direct injury to the imaginal discs by the ultraviolet radiation.

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