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On A STUDY OF RADIATION ON THE BIOLOGY AND
POPULATION DYNAMICS OF THE CEREAL LEAF
BEEBLE, *Oulema melanopus* (L)

For the period 1 September 1968 - 31 January 1969

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A STUDY OF RADIATION ON THE BIOLOGY AND POPULATION DYNAMICS OF
THE CEREAL LEAF BEETLE, Oulema Melanopa (L)

INTRODUCTION

For the past four years we have been investigating the effects of X-ray radiation on the ecology and physiology of the egg, larval, pupal, and adult stages of the cereal leaf beetle. One of the most radiation sensitive stages is the embryo which was reported in detail in Report #3. However, it requires approximately seven to ten days to determine the relative radiation sensitivity, since "hatching" from the egg capsule is the criterion, and it takes a minimum of seven days for the embryo to reach this stage.

We have been looking for a more immediately radiation-sensitive system than an embryo and have been concentrating our efforts on the hemolymph system of the cereal leaf beetle. The cereal leaf beetle was adequate for making qualitative studies of the hemolymph (Report #3) since this usually required less than 5 λ samples, but for more accurate quantitative studies using automatic amino acid analysis techniques 50 to 100 λ samples were required. Because of their small size, 30 to 50 cereal leaf beetle larvae were required to provide this large a sample.

In order to procure more adequate quantities of insect blood we chose to work with the larvae of the wax moth, Galleria melonella and the honeybee, Apis mellefera. The wax moth hemolymph also contains a fine spectrum of hemocytes which was analyzed for radiation damage by electron microscopy and then correlated with biochemical changes in the hemolymph. The honeybee larvae were used primarily to study the biochemical changes following radiation since their hemolymph does not appear to contain many hemocytes.

HEMOCYTE STUDIES

METHODS

The hemolymph from 4th instar larvae of the wax moth was introduced directly into a vial containing a fixative of gluteraldehyde and osmium tetroxide; it immediately coagulated into strands. The hemolymph strands containing hemocytes were transferred to a centrifuge tube containing additional fixative and centrifuged at 3000 rpm for about two minutes, after which the pellet of hemocytes was transferred to another vial with fresh fixative and allowed to stand for 40 minutes. The tissue was then rinsed twice in phosphate buffer wash, dehydrated in a series of alcohol changes, and finally embedded in epoxy resin. Ultra thin sections of the hemocytes were cut, placed on grids, and examined with the electron microscope.

The formula for the hemocyte fixative and buffered wash is as follows:

SODIUM CACODYLATE BUFFERED GLUTARALDEHYDE AND OSMIUM

SOLUTION A - 2.5% Glutaraldehyde in 0.1 M Sodium Cacodylate

0.1 M Sodium Cacodylate Buffer

1.07 g Cacodylate powder in a 50 ml volumetric flask

Fill to 50 ml with distilled water

2.5% Glutaraldehyde

2.5 ml 25% Glutaraldehyde stock solution in a cylinder

Fill to 25 ml with 0.1 M Cacodylate

Refrigerate

SOLUTION B - 1% Osmic Acid in 0.1 M Sodium Cacodylate Buffer

0.1 M Sodium Cacodylate Buffer

Prepare as in SOLUTION A

1% Osmic Acid

0.5 g Osmic Acid in a glass vial. Break glass and drop vial into the Cacodylate

Make SOLUTION B the night before and refrigerate

Do not mix SOLUTIONS A and B more than one hour before use

PHOSPHATE BUFFER WASH

19 ml 0.2 M solution Monobasic Sodium Phosphate
1.38 g in 50 ml distilled water

81 ml 0.2 M solution Dibasic Sodium Phosphate
(anhydrous)
5.68 g in 200 ml distilled water

100 ml distilled water

PROCEDURE

Fix tissue in mixture of 1 (one) part SOLUTION A to 2 (two) parts SOLUTION B for at least 40 minutes. Wash the tissue in the Buffer Wash two times quickly

Approximately 30 4th instar larvae of the wax moth were selected and divided into two groups of 15 each. One group was placed in a plastic petri dish and exposed to a dose of 50,000 R of X-rays at a dose rate of 730 R/m; the other group of 15 was held as controls. A sample of hemolymph was withdrawn, within 60 minutes following irradiation, from several individuals of each group and prepared for examination by electron microscopy. A second sample of hemolymph was taken at 24 hours post-irradiation from some of the X-irradiated individuals which had been kept alive. Electron micrographs were then made of untreated hemocytes and X-rayed hemocytes at 1 and 24 hours after treatment.

RESULTS

Electron micrographs of untreated hemocytes can be seen in Fig. 1 which appear to be developmental stages of a single type of cell. The protein granules are most numerous in Fig. 1(a); decrease in number successively in Fig. 1(b-d); and are completely gone in Fig. 1(e). In all of these cells the rough endoplasmic reticulum (ER) is extensive and the golgi is well formed (G) indicating an active secretion process. The cell indicated by Fig. 2 is probably an adipocyte since it contains large globules of fat (F).

Hemocytes taken from wax moth larvae within 60 minutes after an exposure dose of 50,000 R can be seen in Fig. 3(a-d). The only obvious change appears to be a general degradation of the membranes especially the endoplasmic reticulum (ER). One can still see the regular pattern of the ribosomes (R), but only fragments of the ER to which they were attached prior to irradiation.

Hemocytes removed from irradiated wax moth larvae 24 hours post-irradiation show extensive damage and are in various stages of degradation, (see Fig. 4(a-f)). The hemocytes are swollen and the endoplasmic reticulum (ER), golgi (G) and nuclear membrane (NM) have disintegrated. Mitochondria (M) are also in various stages of disruption and breakdown. The cytoplasm contains large vacuoles with cellular debris and also large clear vacuoles which may be the result of accumulation of fluid from the hemolymph due to increased permeability of the cell membrane (CM). In many of the cells the nuclear membrane has degenerated and there is a mixing of nuclear and cytoplasmic contents, see Fig. 4(e).

Fig. 1(a-e) - Sections of wax moth hemocytes in various stages of development as evidenced by the number of protein granules (P). Other cell ultrastructures are the endoplasmic reticulum (ER), mitochondria (M), lysosome (L), Golgi (G), nucleus (N), and various vacuoles (V).

15,050X

Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1d

Fig. 1e

Fig. 2 - A cell known as an adipocyte with large vacuoles filled
with fat (F). 15,050X

Fig. 3(a-d) - Sections of hemocytes taken from a wax moth larvae 60 minutes after 50 KR of X-rays. Protein granules (P) unchanged; the endoplasmic reticulum (ER) and nuclear membrane (NM) are damaged; ribosomes (R) are numerous in the cytoplasm. All figures are magnified 21,000X.

Fig. 3a

Fig. 3b

Fig. 3c

Fig. 3d

Fig. 4(a-f) - Sections of wax moth hemocytes removed from larvae 24 hours after receiving 50 KR of X-rays. Vacuoles of various kinds may be seen in Fig. 4(a-e). The chromatin (C) and mitochondria (M) are mixed together as shown in Fig. 4(e). The nucleus (N) is constricted between vacuoles in Figs. 4(c) and (d)

15,050X

Fig. 4a

Fig. 4b

Fig. 4c

Fig. 4d

Fig. 4e

Fig. 4f

HEMOLYMPH ANALYSIS STUDIES

METHODS

In this study we have been analyzing the hemolymph of wax moth for free amino acid concentration following X-ray radiation to see if it differs significantly from hemolymph of untreated larvae (Report #4), pg. 11). It appeared, after a limited number of experiments, that there might be some significant difference; however, as more experiments were performed it became obvious that the amino acid content of hemolymph taken from wax moth larvae, within one hour after irradiation with X-rays, was not consistently different than hemolymph from untreated larvae. In trying to account for this inconsistency we hypothesized that (1) the wax moth larvae making up any test group were not necessarily the same age even though they were similar in size, and (2) the larvae were not genetically homogeneous since there had been no selection of adult moths for mating and deriving more pure lines. In an attempt to overcome the discrepancies which might result from genetic variation, several experiments were performed using honeybee larvae. All the larvae from a particular hive would be genetically homogeneous since they would develop from the brood of a single queen. Early prepupae were removed from the capped cells of the brood frame and placed on a piece of damp toweling in a petri dish. They were then divided into two groups, one for controls, the other for treatment with X-rays. Samples of hemolymph were extracted from the prepupae immediately and at one hour following irradiation with 50 KR of X-rays.

RESULTS

The analysis of hemolymph samples removed from the honeybee larvae immediately following irradiation showed no difference in amino acid concentration when compared with hemolymph from the control larvae. However, the analyses of hemolymph samples taken from the bee larvae one hour after the completion of the radiation treatment showed that all the amino acids had increased in concentration when compared to the controls (Table I). The concentration of the free amino acids in $\mu\text{M}/\text{ml}$ of hemolymph in non-irradiated and irradiated prepupae is shown in Fig. 5.

CONCLUSIONS

The free amino acid concentration of the hemolymph of honeybee prepupae seems to increase in time after irradiation. Although there is no clear-cut, significant difference in the concentration of the free amino acids between the control and irradiated samples, it seems that there is a definite trend toward an increase in free amino acids in hemolymph from irradiated prepupae (Fig. 5). We may also infer that the free amino acids would become more concentrated in the hemolymph with increased X-ray exposure and possibly with increased time after irradiation. Further experiments are under way to attempt to determine if these inferences are valid.

Table I - Comparison of Free Amino Acid Concentrations in Extracts of Hemolymph from Non-Irradiated and Irradiated Honeybee Prepupae

Irradiated Prepupae - Exposure 50,000 R
Exposure Rate 970/Rm
Tube Potential - 250 KVP
Hemolymph removed at 60 minutes post-irradiation

Free Amino Acid in Honeybee Hemolymph	Non-Irradiated $\mu\text{M/ml}$ Amino Acid			Irradiated $\mu\text{M/ml}$ Amino Acid			Average Controls			Average Irradiated Blood			Controls Std. Dev.	Irrad. Std. Dev.
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	1	2	3	1	2	3		
Aspartic Acid	0.104	0.056	0.064	0.152	0.060	0.049	0.075	0.087	0.021	0.046				
Glutamic Acid	0.130	0.140	0.239	0.168	0.248	0.392	0.170	0.256	0.049	0.075				
Proline	37.360	30.480	32.999	44.336	36.580	35.593	33.613	38.836	2.842	3.910				
Glycine	5.046	4.754	6.441	4.916	5.544	7.121	5.414	5.850	0.736	0.928				
Alanine	2.208	3.014	3.373	2.394	3.668	4.097	2.867	3.386	0.486	0.723				
Valine	2.768	2.768	2.789	3.260	3.196	2.995	2.775	3.150	0.010	0.113				
Methionine	0.118	0.104	0.129	0.174	0.134	0.149	0.117	0.152	0.010	0.016				
Isoleucine	1.038	1.032	1.247	1.194	1.214	1.375	1.106	1.261	0.099	0.081				
Leucine	0.636	0.394	0.319	0.598	0.594	0.496	0.450	0.549	0.135	0.066				
Tyrosine	0.560	0.450	0.588	0.568	0.520	0.671	0.533	0.586	0.050	0.063				
Phenylalanine	---	---	0.332	0.408	0.328	0.431	0.332	0.389	---	0.044				
Lysine	1.958	1.138	1.276	3.150	1.464	1.460	1.471	2.025	0.377	0.796				
Histidine	1.100	1.100	1.048	1.276	1.032	1.192	1.083	1.167	0.025	0.101				
Arginine	2.642	2.352	2.235	2.942	2.520	2.727	2.410	2.730	0.171	0.172

POPULATION STUDIES

During the spring of 1968 a series of field tests was conducted at The Ohio Agricultural Research and Development Center under the direction of Dr. Robert Treece. The objective of these experiments was to test the feasibility of the "sterile male" technique as a possible population regulating mechanism in the cereal leaf beetle. Preliminary work (Report #3) indicated that X-ray radiation of males might significantly reduce the hatchability of eggs laid by female beetles previously mated with them.

Cereal leaf beetles overwinter as adults usually in an old field or wooded area. As soon as the spring temperature reaches 75° to 80° F for two or three days the adult beetles become active and can be collected in large numbers. It is possible to make these collections before the adults have had an opportunity to mate; however, when field-collected adults are used in experiments such as those described in this study one cannot be sure that all of the females are virgin. Since the supply of laboratory-reared beetles was limited and in order to conduct any of the "sterile male" experiments it was necessary to use adult cereal leaf beetles collected in March, 1968, at or near New Carlisle, Indiana. They were stored in one-half pint ice cream containers until time for treatment when they were removed and sexed. Approximately 950 male beetles were given an exposure dose of 5000 R of X-rays at a dose rate of 292 R/min. The irradiated and non-irradiated beetles were then taken to the field sites for release in the outdoor cages as shown below:

Table II

Treat- ment	Experimental Matings		Repli- cates
1	48 (untreated) ♂	X 48 (untreated) ♀	5
2	48 (5000 R) ♂	X 48 (untreated) ♀	5
3	24 (5000 R) ♂ + 24 (untreated)	X 48 (untreated) ♀	5
4	48 (5000 R) ♂ + 48 (untreated)	X 48 (untreated) ♀	5
5	48 (5000 R) ♂ + 24 (untreated)	X 48 (untreated) ♀	5

The adult beetles were released in the cages on May 10, 1968, and 51 days later all the larvae were counted. The results of the matings are shown in Table III.

Table III

Treatment	Replicates - No. of Larvae/Cage					
	Cage A	Cage B	Cage C	Cage D	Cage E	Mean
1	486	386	1432	859	1187	870
2	496	176	176	8	5	172
3	259	424	890	734	569	575
4	477	109	462	523	521	418
5	345	687	908	777	982	740

On the basis of our laboratory experiments we would expect no larvae to be present in the matings of Treatment 2. We must infer that some of the field-collected females had mated and consequently laid fertile eggs, especially in Cage A. However, it is obvious that Treatment 2 resulted in the lowest number of larvae produced. Treatment 3 which had treated and untreated males present in a ratio of 1:1 caused a reduction in larvae of about one-third when compared with Treatment 1.

This experiment will be repeated this spring (1969) in order to test the effectiveness of the sterile male technique using virgin females.

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May 1, 1969

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May 5, 1969