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The Study of the Effects of Paradichlorbenzene on *Drosophila melanogaster*

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THE STUDY OF THE EFFECTS OF PARADICHLORBENZENE ON "DROSOPHILA MELANOGASTER"

By

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Table of Contents

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-INTRODUCTION-

The object of this investigation was to produce polyploid cells in the salivary glands of Drosophila melanogaster by treatment with paradichlorbenzene. Polyploidy is the condition in which the number of chromosomes in the nucleus is an exact multiple of the somatic or diploid number greater than two. This phenomena has often been referred to as produced by incomplete nuclear division.

Many observations have been made of induced polyploidy in plants, but very few cases have been recorded in the case of animals. Several cases were observed at the turn of the century, but were not exactly interpreted as such. Wilson (1925) gives the following account of some of these early investigations. Boveri (1886) observed monasters in the testis cells of the cray-fish. However he did not describe these observations until 1901 and 1905. Morgan (1900) obtained similar results by the treatment of eggs with strychnine. R. Hertwig (1896) treated sea urchin eggs with strychnine and observed "half spindles " and "fan nuclei". Zeigler (1898), Boveri and Painter observed the monaster in sea urchin eggs after subjecting them to mechanical injury or agitation. Zeigler's method consisted in constricting the eggs with cotton fibers. Monocentric mitoses has been artificially induced in cells by various other chemicals. Examples of these chemicals are ether (Wilson), phenol urethane (Painter), hypertonic sea water (Morgan, Wilson) and carbon

dioxide (Herbst).

More recently natural polyploidy has been observed in Drosophila as the result of special methods of mating. Dobzhansky (1941) presents the results of some of this work.

> "A reduplication of the chromosome complement in an organism like Drosophila may give rise to tetraploid females (4X, 4A) and tetraploid males (2X, 4A). The reduction division in tetraploid males has not been studied, but on theoretical grounds it is believed likely to give rise mostly to 1X, 2A spermatozoa. Such spermatozoa uniting with the eggs of a normal diploid female (1X, 1A) would produce intersexes (2X, 3A). A tetraploid female produces diploid 2X, 2A eggs, which on fertilization by the sperm of a normal diploid male (1X, 1A and 1Y, 1A) give triploid females (3X, 3A) and intersexes (2X, 3A). A triploid female crossed to a diploid male produces triploid and diploid females, diploid males, intersexes, superfemales, and supermales."

After subjecting snowy tree cricket adults to X-ray treatment, Beach (1938) reports a reduction in the number of eggs laid, inhibition in cell division and the production of polyploidy. X-ray treatment also showed a tendency of the chromosomes to fragment. After two or three days of treatment, abnormal spermatogonia were found.

Simonet and Guinochet (1939) treated Linum Vsitatissimum with various amounts of paradichlorbenzene for periods of one to five days. Their procedure consisted of placing the flax seeds in petri dishes on damp filter paper. Paradichlorbenzene was placed on watch glasses in the petri dishes which were then covered. They found that at the end of the first day the flax cells contained thirty chromosomes which is the diploid number. After two days of treatment sixty chromosomes

were present, while at the end of the third day ninety chromosomes could be seen. The results of this type of induced polyploidy were the same as those obtained by Blakeslee (1937) with colchicine.

Carey (1943) produced polyploidy in onion root tips with paradichlorbenzene by methods quite similar to those of Simonet and Guinochet. The onion seeds were placed in closed petri dishes on damp filter paper and amounts of paradichlorbenzene varying from one-tenth gram to one and one-half grams were placed on watch glasses. The seeds were treated from 10 A.M. to 2 P.M. for one day. It took a day for the seeds to recover. Polyploid cells were found in the seeds treated with one-fourth gram, one-half gram and one and one-half grams. External examination revealed the tips to be shortened and stunted. Miss Carey also studied the effects of paradichlorbenzene on Drosophila. The period of treatment ranged from twelve hours to three days. No evidence of polyploidy was seen in the salivary gland slides. The flies appeared puny and lifeless when treated with the larger concentrations of paradichlorbenzene.

The most recent and perhaps the most conclusive experimentation in the production of polyploidy in animals is that of Braungart and Ott (1942). These men, working at the Catholic University of America, have induced polyploidy in brain cells of Drosophila melanogaster with colchicine. One method used was to immerse the larvae in .50% solution of colchicine for periods of one to two and one-half hours. Another was to pierce the body wall of the larvae and then to subject them to .50% and 1.0% solutions of colchicine for various lengths

of time up to one hour. The most satisfactory method employed was to replace some extracted body fluid with .0013% solution of colchicine by the use of a hypodermic needle. From these methods the following results were obtained:

1. "The salivary gland cells were little affected by colchicine treatment.
2. "The larval period in most cases was prolonged.
3. "The absence of a spindle and the lack of a typical equatorial plate was observed.
4. "The chromosomes were clumped in the colchicine affected cells.
5. "No true anaphase was observed.
6. "Definite polyploid areas in the brain tissue showing the increased number of chromosomes were often visible."

MATERIALS AND METHODS

The mutant type, eosin eye, variety of Drosophila melanogaster was used exclusively for this investigation. These flies have been raised over a period of several years by Dr. E. S. McDonough. The flies have been placed in new cultures of either banana or corn meal agar and mated every three weeks.

In the early experiments a banana culture was used. About one-third of a banana was mashed in the bottom of each bottle. The bottles were sterilized for twenty minutes at fifteen pounds pressure and then some yeast was placed in the culture. A corn meal agar culture was used in later experiments instead of banana culture. This corn meal culture is a modification of Bridges' (1937) culture and of that found in Principles of Genetics, by Sinnott and Dunn (1939). The culture was prepared as follows:

1. 1.0 grams of moldex was dissolved in 5.0 milliliters of 95% alcohol.
2. 15 grams of agar-agar were cut into 500 milliliters of distilled water and allowed to soak for ten minutes.
3. 110 grams of corn meal were soaked in 250 milliliters of distilled water.
4. 125 milliliters of molasses were measured out.
5. The moldex was added to the agar solution.
6. The agar was boiled over a low flame until dissolved.
7. The molasses was added to the agar solution with constant stirring, and then brought to a boil.
8. The corn meal suspension was then added while stirring.

9. This was then brought to a boil while stirring.
10. The culture was then placed in half-pint milk bottles and sterilized for twenty minutes at fifteen pounds pressure.
11. A cake of yeast was placed in 250 milliliters of distilled water and a few milliliters of the solution was then placed in each culture bottle.

The chemical used in attempting to produce polyploid cells was paradichlorbenzene, which is obtained on the market as a mothicide. In the experiments it was weighed into sterilized vials, which were suspended in the culture bottles by means of string attached to the outside of the bottle.

A few temporary slides were made of the salivary glands. The larvae were placed in a drop of Ringer's solution on a slide, since it was found that the glands could be dissected out much easier if they were placed in this solution. With the use of binoculars and fine dissecting needles, the glands were dissected out with some difficulty. The isolated glands were stained with aceto-carmine. A cover glass was placed on the slide and gentle pressure applied until a satisfactory smear was obtained. The cover glasses were sealed with vaseline.

In the first experiments the paradichlorbenzene was suspended in the bottles for various lengths of time after the larvae appeared. This procedure failed to give the desired results. It was then decided upon to treat the first batch of eggs laid by the female as soon as possible, preferrably

within eight hours, after laying. According to Paulson (1937) the salivary glands begin to appear about eight hours after fertilization and reach there maximum development about fourteen hours after fertilization. This procedure necessarily required virgin females. Therefore pupae were isolated in separate sterilized vials. Since it was impossible to judge just when the eggs were laid after mating, it was necessary to examine the culture to be sure that eggs had been laid. In order to facilitate this examination of the culture, powdered charcoal was added to the media before sterilization. It was then possible to locate the white eggs against the black background with the use of binoculars.

OBSERVATIONS

In the first experiment four cultures were started. Various amounts of paradichlorbenzene were placed in vials which were suspended in the bottles just before larvae were about to appear. No larvae appeared and the adults were dead at the end of the second day of treatment. (Table I) From these results it was concluded that the adults could not withstand treatment for more than forty-eight hours.

Five cultures were then started and .5 gram of paradichorbenzene was placed in each bottle. In this experiment the object was to treat the cultures for different lengths of time as shown in Table II. In the culture treated for three hours a slide was prepared of the salivary glands but polyploidy was not present. When these flies hatched they were very active and abundant, and appeared somewhat larger. There were no slides made of the larvae treated for five and one-half hours. Very few of the flies hatched. No slides were obtained of the larvae treated for twenty and one-third hours. From this culture a large number of very active flies hatched but no change in external characteristics was noticed. In the cultures treated for forty-five hours and sixty-eight and one-third hours no larvae appeared.

Flies were taken from cultures I and II in the preceding experiment and mated in freshly prepared bottles. .5 gram of paradichlorbenzene was placed in each bottle. (Table III) All the adults survived during treatment and were removed as soon as larvae appeared. Slides were prepared from the

salivary glands of the larvae from each bottle. No evidence of polyploidy could be seen. Examination of the external characteristics of the flies after hatching showed them to be normal in every respect.

In the fourth experiment the object was to treat the eggs as soon as possible after they were laid. In order that the presence of the eggs could be ascertained, charcoal was mixed in the corn meal culture. Test tubes were substituted for the ordinary culture bottles in order that the cultures could be examined with binoculars. Freshly hatched, virgin females were transferred to the test tubes and mated. About one gram of paradichlorbenzene was suspended in each test tube after the eggs were believed to be laid. The periods of treatment varied from nineteen and two-thirds hours to forty-eight hours. No larvae appeared and the adults died in about two or three days.

The method used in the preceding experiment proved unsatisfactory, so media containing charcoal was placed on the caps of ordinary culture bottles and the bottles then inverted over the caps. With this method the caps could be removed and the media examined more satisfactorily under the binoculars. Virgin females were mated in six freshly prepared bottles. Periodic examinations of the cultures for several days showed no eggs present. The adults died two days after mating. In this experiment, as well as the preceding one, it was found that the charcoal had a tendency to form a crust around the media, thus preventing the flies from obtaining food. It

appeared as though the flies died of starvation before any eggs could be laid. This accounts for the fact that no eggs were seen.

Table I

Culture	Weight of para-dichlorbenzene (gms)	time of treatment (hours)	Parents	Larvae
1	.0994	47	died	none appeared
2	.040	47	died	none appeared
3	.0133	47	died	none appeared
4	.020	47	died	none appeared

Results of experiment I showing that the time of treatment was too long.

Table II

Culture	Weight of para-dichlorbenzene (gms)	time of treatment (hours)	Parents	Larvae	Adults
1	.50	3	lived	appeared	many hatched quite active
2	.50	$5\frac{1}{2}$	lived	appeared	few hatched less active
3	.50	201/3	lived	appeared	many hatched quite active
4	.50	44 1/3	died	none appeared	none
5	.50	68 1/3	died	none appeared	none

Results of experiment II showing that the larvae did not withstand treatment longer than forty-four hours.

Table III

Culture	Weight of paradichlorbenzene (gms)	time of treatment (hours)	Parents	Larvae	Adults
1	.50	20½	lived	appeared	normal
2	.50	23	lived	appeared	normal
3	.50	44½	lived	appeared	normal

Results of experiment III indicate that the paradichbrbenzene had no affect on the larvae and adults for the length of time indicated.

Summary

The larvae and adults were able to withstand treatment with paradichlorbenzene for about only forty-four hours. The amount of chemical used was not significant since the size of the evaporating surface was the same for all experiments.

The paradichlorbenzene did not appear to affect the development rate of the larvae.

The flies appeared normal in every respect after treatment.

Results obtained indicate that further study would be quite justified. A greater variety of more refined experimental methods could be used.

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