The Differential Staining of Heterochromatin and Euchromatin of the Salivary Gland Chromosome with Neutral Violet¹

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G. Yasuzumi and others (1948) have revealed with histochemical methods the difference in chemical composition between euchromatin and heterochromatin of the salivary gland chromosome to be as follows: The euchromatin shows positive reaction to arginine, whereas the heterochromatin shows negative reaction. The same result may be obtained by Neubauer-Rohde's method and also by Voisnet, Fürth and Lieben's method. The isoelectric point is found at pH 3.2 in euchromatin and at pH 1.35–1.90 in heterochromatin. T. Caspersson (1940) has found, by means of the ultraviolet absorption spectra, the presence of a small quantity of protein of the globuline type in the euchromatic region of the chromosome and a protein of the histone type in the heterochromatic region.

In the present study we have attempted to make out by the staining with neutral violet the difference in the chemical composition between euchromatin and heterochromatin of the crushed salivary gland chromosome which was previously treated with supersonic vibration.

Materials and Methods

Mechanical effect of supersonic vibration on the chromosome:—Wild-type *Drosophila virilis* cultured in our laboratory was employed. The larva put in a hard-glass test-tube containing the insect Ringer's solution plus 0.7% gelatin was placed over supersonic vibration of 1,700 v and 140 m.a. The effect of the vibration on the chromosome by this kind of treatment is considered merely mechanical (Oyama and others 1941). After 2 minutes, the salivary glands of the larva were dissected in 45% acetic acid solution, and the chromosomes were fixed for 3–5 minutes in the usual manner.

The cover slip was removed, and the crushed chromosomes were stained with 0.5% neutral violet solution. After 20 minutes, the preparation was rinsed in distilled water and carried through the usual series of increasing concentration of alcohol, and finally through two

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Fig. 1. Micrographs of salivary gland chromosomes of *Drosophila virilis* stained with 0.5% neutral violet solution. A, control; B, mechanical action of supersonic vibration; C, oxidizing action of supersonic vibration. $\times 1,200$.

changes of absolute alcohol, the neutral red molecules absorbed by lipoids were removed.

Oxidizing action of supersonic vibration.—The larva of *Drosophila* kept in a hard glass test-tube which contained only insect Ringer's solution was placed over the supersonic vibration under 1,700 v and 130 m.a for 2 minutes. This kind of treatment causes oxidizing change of the chromosome. The staining technique is the same as describe above.

Observation

The euchromatin bands and heterochromatin regions were observed after the treatment by the supersonic vibration described above. The following results were obtained:—

	Euchromatic band	Heterochromatic region
Control (Fig. 1 A)	Purplish red	Purplish red
Treatment A: under mechanical action of supersonic vibration (Fig. 1 B)	Orange red	Orange red
Treatment B: under oxidizing action of supersonic vibration (Fig. 1 C)	Purplish blue	Purplish red

The oxidizing effect of the supersonic vibration on the neutral violet solution was examined immediately. The original purplish-red color of the neutral violet had changed into purplish blue by oxidation by the supersonic vibration under conditions of 1,700 v and 130 m.a for 7–10 minutes. The color, however, disappeared in 45 minutes, the cause of this color change being unknown.

The diffusion experiment provides us with a simple method for distinguishing dimensions of molecules of semi-colloid or colloid. For this purpose a 10% gelatin was used. Hot solution of the gelatin was poured in a test-tube and left to congeal. The neutral violet solution was added on this gelatin layer, and the tube was left standing under constant temperature. The purplish blue particles gradually spread down into the jelly, while the orange red particles did this only very slightly. This difference apparently was due to the fact that the orange-red particles was larger in size than that of purplish-blue particles. Thus the orange-red coloring of chromosomes previously treated with mechanical action of the supersonic vibration (Fig. 1 B) seems to be due to the penetration of the larger particles of the stain into the crevices of chromosome formed by the destructive activity of the supersonic vibration. The change of the neutral violet color in the euchromatic bands in contrast to the unchanged heterochromatin regions (Fig. 1 C) suggests that the chemical composition of the euchromatin bands is more

easily oxidized than that of the heterochromatin regions by the action of the supersonic vibration.

Summary

By means of the oxidizing activity of supersonic vibration, the euchromatic bands can be made to take purplish blue when stained with neutral violet, while the heterochromatin region remains purplish-red. This shows the difference in chemical composition between euchromatin and heterochromatin.

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