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# EFFECT OF TRYPSIN ON THE CHROMOCENTRIC MASS OF THE SALIVARY GLAND CHROMOSOMES OF *DROSOPHILA MELANOGASTER, D. HYDEI, D. VIRILIS* AND *D. MIRANDA*

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Chromocentre of the polytene chromosome is a heterochromatin mass and is late replicating. It is very difficult to study the pattern of replication and transcription of the genetic material of this area without unmasking the protein-nucleic acid association of the chromocentre. In the present set of experiments attempt was made to digest some proteins of the chromocentre by trypsinization. Trypsin treated chromocenters of the four species of *Drosophila* studied showed different degrees of morphological changes.

## INTRODUCTION

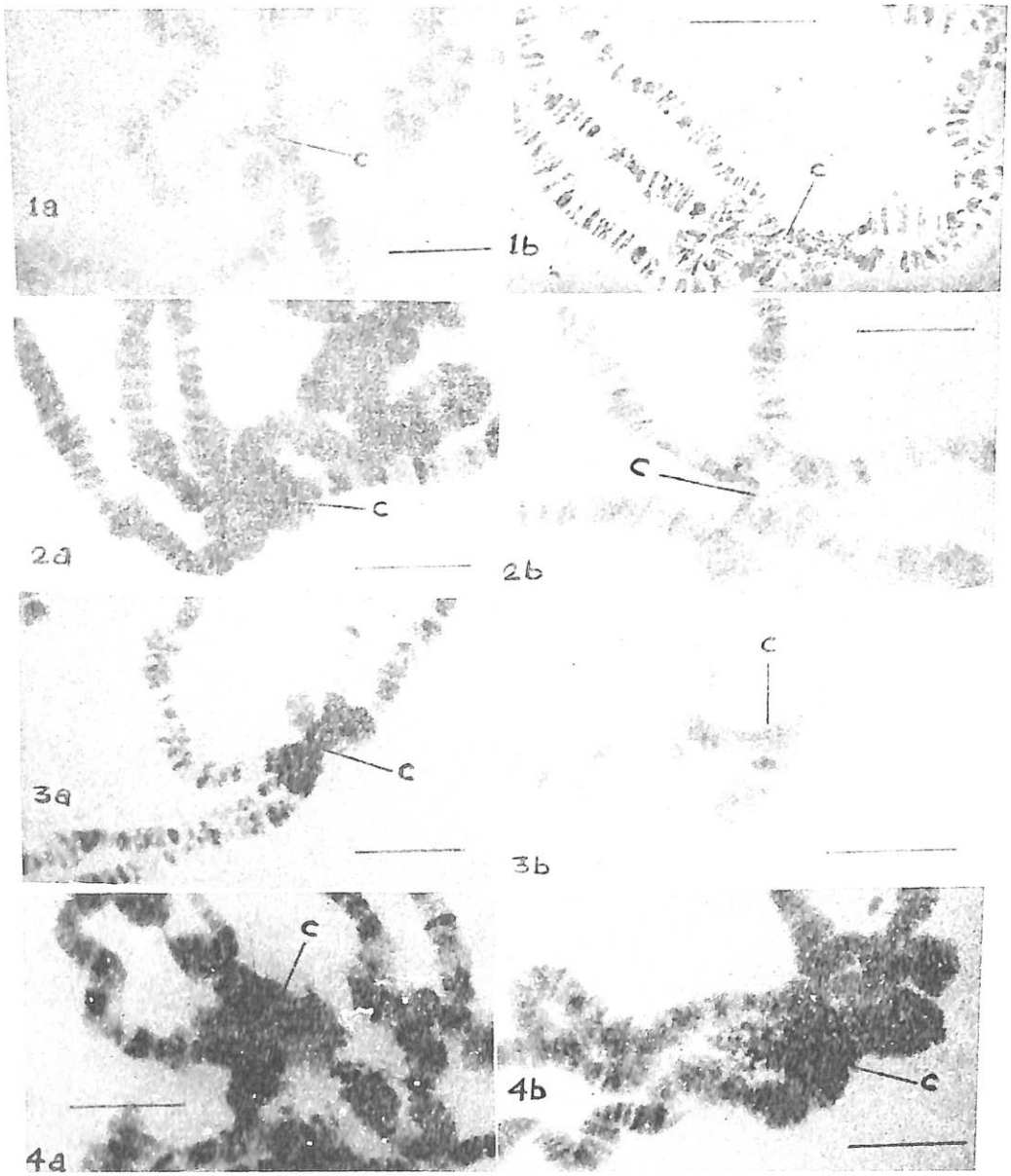
The proteolytic enzyme trypsin is highly active towards the basic proteins and protamines (Catarow & Schepartz, 1967). Chromosomes treated with trypsin have been studied by several investigators to elucidate the architecture of chromosomes with special emphasis on their strandedness and replication. Radio active thymidine incorporated trypsinized mitotic chromosomes of *Vicia faba* was studied by Hirahara *et al.* (1973). They observed fairly expanded nuclei after trypsin digestion. More or less similar observation was reported earlier by Trosco & Wolff (1965). Brown & Stubblefield (1974) noticed striking effect of trypsin on the mammalian cell free system. Chakrabarti (1975), Chakrabarti & Mukherjee (1978), Chakrabarti (1989), reported the effect of trypsin on the functional morphology and puffing activities of the polytene chromosomes of *Drosophila melanogaster.*

The present set of experiments was designed to visualize the morphology of chromocentric mass after trypsin digestion. The purpose of these experiments was to see the differentiation of the $\alpha$ and $\beta$ heterochromatin areas of the chromocentre in four different species of *Drosophila* among which the morphology and condensation of the heterochromatic materials in the chromocenters were variable.

## MATERIAL AND METHODS

The wild type stocks of *Drosophila melanogaster, D. hydei, D. virilis* and *D. miranda* were used. The cultures were raised in separate half pint milk bottles containing culture medium, prepared by agar agar, cornmeal, molassess, yeast, nipagin and propionic acid and maintained at $24^0 \pm 1^0$C in B.O.D. Males and females of the different species were separately placed in the egg laying chambers and the eggs were collected in the petridishes containing agar food medium.

Primary synchronization was achieved by collecting eggs over a period of every half an hour. Second synchronization was obtained by transferring just hatched larvae to a




Figs. 1-4. 1a. Untreated chromosome of *Drosophila melanogaster*; 2b. 0.10 mg/ml trypsin treated polytene chromosome of *D. melanogaster*, showing the chromocentre; 2a. Untreated chromosome of *D. hydei;* 2b. 0.10 mg/ml trypsin treated polytene chromosome of *D. hydei;* 3a. Untreated chromosome of *D. virilis;* 3b. 0.10 mg/ml trypsin treated polytene chromosome of *D. virilis;* 4a. Untreated chromosome of *D. miranda;* 4b. 0.10 mg/ml trypsin treated polytene chromosome of *D. miranda.*

(C in all figures represent Chromocentre; Bar represents 10 $\mu$m in all figures)

fresh culture medium. The larvae were allowed to grow at $24^0 \pm 1^0$C and were sacrificed after 96 to 100 hours.

The salivary glands were dissected out in buffered ringer at pH 7.2 (Berendes, 1972). One of the two glands of a pair was incubated in trypsin solution 0.1 mg/ml of ringer buffer at pH 7.2 for 15 minutes (besides this concentration, two other concentrations, 0.05 mg/ml and 0.15 mg/ml were also used but 0.1 mg/ml was found much suitable for these experiments). The contralateral gland was incubated in ringer solution for the same period as control.

After incubation, the glands were fixed in aceto-alcohol (1 part glacial acetic acid and 3 part absolute alcohol V/V) on a clean greasefree slide for 1-2 minutes and stained with a mixure of aceto-orcein and aceto-carmine (1 : 2) for 5-6 minutes. The glands were then washed in 50% acetic acid and squashed on a drop of lacto-aceto-orcein. The slides were then sealed. The slides thus prepared were observed under Carl Zeiss phase and light microscope using 100 X oil immersion objective and photographs were taken.

## RESULTS

### *Drosophila melanogaster*

a) *Control* : Untreated chromosomes showed fairly large and dark stained chromocenters (Fig. 1a).

b) *Trypsin treated* : Trypsin treated chromocenters of *D. melanogaster* clearly resolved into $\alpha$ and $\beta$ heterochromatin areas. In addition, some extra bands or band-like structures also appeared in the extended chromocentre (Fig. 1b).

### *Drosophila hydei*

a) *Control* : The chromocentre in this species is formed by the association of the centromeres of all chromosomal elements including the small 6th autosome. Untreated chromocenters appeared very distinct and deeply stained (Fig. 2a).

b) *Treated* : Trypsin treated chromocenters of *D. hydei*, showed conspicuous morphological change. They became greatly reduced, occasionally with thread like structures near the chromocentric mass (Fig. 2b).

### *Drosophila virilis*

a) *Control* : The chromocentre of *D. virilis* includes the centromeres of all five large chromosomes and almost the entire 6th autosome. Untreated chromocenters were big and deeply stained (Fig. 3a).

b) *Treated* : Due to trypsin action the compactness of the chromocentre was lost and this appeared as decondensed mass (Fig. 3b).

### *Drosophila miranda*

a) *Control* : The chromocentre in this species is formed by the fusion of the centromeres of all the autosomes and sex chromosomes. Untreated chromocenters were very distinct and compact (Fig. 4a).

b) *Treated* : The trypsinized chromocenters showed decondensation with granular chromatin materials inside the chromocentric mass (Fig. 4b).

## DISCUSSION

In most *Drosophila* species, excepting *D. buskii*, the polytene chromosome arms are associated at their centromere region to form a chromocentre. This region is especially difficult to analyse. Heitz (1934) reported that the chromocentre is formed of compact $\alpha$ heterochromatin which is especially prominent in *D. virilis*. According to Gall *et al.* (1971), the diploid karyotype of *D. virilis* contains 45% satellite sequences, located in the $\alpha$ heterochromatin that does not replicate in the highly polytene salivary gland cells of 3rd instar larvae. On the other hand, Brown & Stubblefield (1974) found a remarkable effect of trypsin on the rate of DNA synthesis. They reported that the DNA synthesis in a broken cellular preparation of Chinese hamster cells enhanced approximately 10 folds by a brief trypsin treatment.

It was reported earlier that the trypsin can induce puffing activities in some bands of the X-chromosome of *D. melanogaster* polytene chromosome (Chakrabarti, 1975; Chakrabarti & Mukherjee, 1978).

The current findings confirmed that trypsin has remarkable effect on the heterochromatin mass of the polytene organisation. Strikingly enough, the same concentration of trypsin used in all cases for a fixed time has produced differential effect on the chromocentric mass. The more the mass was compact the less the effect of the chemical (enzyme) was on the chromocentre.

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