Some Aspects on Effects of *Drosophila* Tissue Extracts on the Puffing Pattern of Incubated *Drosophila* Salivary Glands[1)]

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(With 2 Text-figures and 2 Tables)

The polytene chromosomes of dipterous larval tissues show generally local enlargements known as puffs. Puffing patterns of polytene chromosomes have been reported to undergo remarkable changes during developmental stages in relation to molting process (Berendes, 1966; Becker, 1959; Beermann, 1952): in *Dorsophila*, the hormone, that is excreted from ring glands of larvae, induces molting and the subsequent change of puffing pattern (Becker, 1962). It has been shown that the tissue specificity of the puffing patterns is related to the difference of puffing patterns in an individual and at the same developmental stage (Berendes, 1966). Now, the puffs are interpreted as indications of gene activity.

Recently, changes of puffing patterns of salivary chromosomes have been studied under short time incubation with media by several investigators (Federoff and Milkman, 1963; Ritossa, 1964, and Clever, 1965). Clever (1962) reported that, in *Chironomus*, ionic strength of inorganic salts in medium was an important factor for maintenance of the salivary gland *in vitro*, and that puffing patterns were affected by the presence of ecdysone in the saline solution.

The present author has interested in the change of the puffing patterns of *Drosophila* salivary chromosomes under short time incubation. This paper deals with some preliminary notes on the puffing pattern changes under the influence of tissue extracts of eggs and young first instar larvae.

Before going further the author wishes to express his gratitude to Professor Sajiro Makino, Hokkaido University, for his keen interest in this study, and to Dr. Eizi Momma for his kind guidance and encouragement throughout this work.

Material and Method : The late third instar larvae and early prepupae of *D. melanogaster* (Sapporo strain established in 1959) were used as donors of the salivary glands for experiment. The glands were incubated with the use of concavity slides covered with cover slips and sealed with vaseline. The amount of incubation media was about 0.08 to 0.10 ml and the pH of the media was adjusted to 7.0 with 1M KOH solution.

Salt and sugar solutions described as H-6 medium by Horikawa *et al.* (1966) for *Drosophila* embryo tissue cultures were used for incubation. Three kinds of stock solu-

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tions, A, B and C, as given in Table 1, were prepared. For experiment, three kinds of the stock solutions, 1 ml from each, were mixed and the stock mixture thus prepared was diluted with distilled water. The proportions of the stock mixture and distilled water were 3:3, 3:5, 3:7, 3:9, 3:11 and 3:13. For exmaination of puffing pattern changes, one of the sister glands was incubated in the medium as control and the other one in medium containing tissue extract as test series. Tissue extracts were obtained as follows: the eggs laid about six hours before experiments were washed and homogenized in 0.5 ml of the medium. About 50 first instar larvae hatched by 8 hours before experiments were treated by the procedure as above.

Table 1. Compositions of stock solutions (mg/100 ml.)

Stock solution A		Stock solution B	
NaCl	7,000	$CaCl_2 \cdot 2H_2O$	20
$NaH_2PO_4 \cdot 2H_2O$	200	$MgCl_2 \cdot 6H_2O$	100
$NaHCO_3$	350	Stock solution C	
KCl	200	Glucose	5,500
		Sucrose	5,500

Microscopic slides were prepared according to the routine acetic orcein method, and observed with phase optics. Localization of puffs were examined in reference to Bridges' chromosome maps (Bridges, 1935).

Results

In two-hour-incubation samples with different incubation media, the maintenance of the structure of the salivary chromosomes was examined. The mixture 3:3 produced a complete damage to the chromosomes. In the mixtures, 3:5 and 3:7, the chromosome displayed abnormal banding patterns, in approximately one half of the nuclei studied, the number of bands being less than that observed in control specimens. In the mixtures, 3:9 and 3:11, the chromosome structure involving banding patterns was well maintained, so that detail observations were permitted to certain extent on these chromosomes. In these media, no sign of degeneration was observed in the chromosomes on 8-hour incubation. In the mixture, 3:13, the chromosome structure was not maintained so well as in the above two media. On the above bases, the medium, 3:11, was used for the observation of puffing pattern changes in the following.

The results of the changes of puffing patterns in seven pairs of the salivary glands are presented in the 1st to the 7th column of Table 2. In order to examine effects of the extract from the first instar larva, the salivary glands from the 1st, the 2nd and the 3rd series were incubated for one hour. For examination of the egg extract, the glands from the 4th and the 5th series were incubated for one hour, and those from the 6th and the 7th series for two hours.

The puffing patterns observed in the control specimens were compared with the patterns and developmental stages reproted by Becker (1962). The puffing patterns so far observed by the author differed from those reported by Becker (1962).

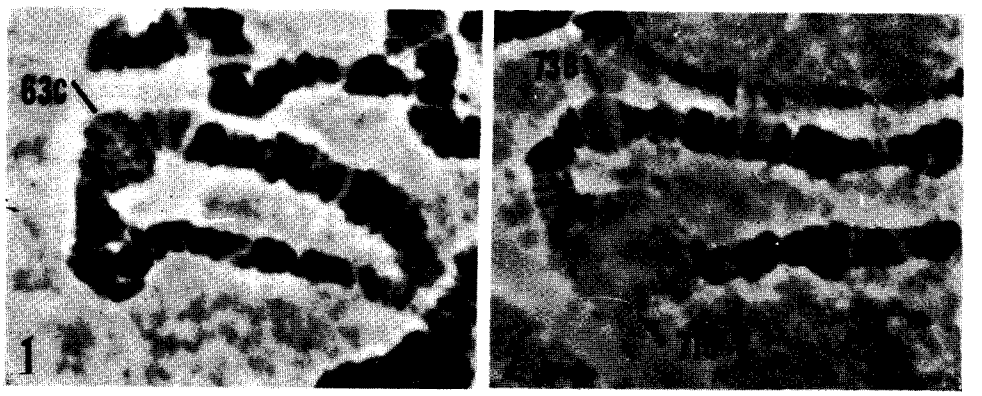
Table 2. Puffing patterns in glands of seven larvae (I to VII), one gland of each larva was incubated with tissue extracts as test gland (t), the other one incubated in the medium as control (c). The fractions refer to the ratios of nuclei in which puffs were found to the nuclei observed. Glands I-III were incubated for one hour for examination of extract from first instar larvae. Glands, IV-VII were incubated for examination from egg extract for one hour (IV and V) and two hours (VI and VII)

	I		II		III		IV		V		VI		VII	
	t	c	t	c	t	c	t	c	t	c	t	c	t	c
X 1BC	—	—	—	—	1/8	—	—	1/12	—	—	—	—	—	—
1CD	—	—	1/11	—	—	—	—	—	—	—	—	—	—	—
2B	—	—	7/11	9/11	—	—	1/15	—	—	—	—	—	—	—
2E	—	—	1/11	—	—	—	—	—	—	—	—	—	—	—
4D	—	—	1/11	—	—	—	—	—	—	—	—	—	—	—
8C	—	—	—	—	—	—	1/15	—	—	—	—	—	—	—
20B	1/11	—	—	—	—	—	—	—	—	—	—	—	—	—
2L 21B	—	—	—	—	—	—	—	—	—	—	1/8	—	—	—
30EF	—	—	1/12	—	—	—	—	—	—	—	—	—	—	—
31F	—	—	1/12	—	—	—	—	—	1/12	—	—	—	—	—
35AB	1/11	—	—	—	—	—	—	—	—	—	—	—	—	—
35B	—	—	—	—	—	—	—	—	1/12	—	—	—	—	—
2R 42ABC	—	—	—	—	—	—	—	—	1/13	—	—	—	—	—
46DEF	—	—	—	—	—	—	—	—	1/13	—	—	—	—	—
47ABC	11/11	6/7	12/12	8/11	—	—	1/15	—	1/13	—	—	—	2/15	—
49B	—	—	—	—	—	—	—	—	1/13	—	—	—	—	—
50CD	11/11	6/7	12/12	11/11	6/8	7/8	—	—	—	—	7/9	—	1/15	—
54D	1/11	—	—	—	—	—	—	—	—	—	—	—	—	—
55BC	—	—	1/12	—	—	1/8	—	—	—	—	—	—	—	—
56D	1/11	—	—	—	—	1/8	—	—	—	—	—	—	—	—
59D	—	—	—	—	—	—	—	—	1/13	—	—	—	—	—
60B	—	—	—	—	—	—	4/15	—	—	—	—	—	—	—
3L 61A	—	—	—	—	—	—	—	—	—	—	1/10	—	—	—
61E	—	—	1/13	—	—	—	—	—	—	—	—	—	—	—
62A	—	—	—	—	—	—	1/16	—	—	—	—	—	—	—
63B	—	—	—	1/11	—	—	—	—	—	—	—	—	—	—
63C	—	—	—	—	—	—	6/16	—	—	—	—	—	—	—
63E	—	—	—	2/11	—	—	—	—	—	—	—	—	—	—
66B	—	—	1/13	—	—	—	—	—	—	—	—	—	—	—
66D	—	—	—	—	—	—	—	—	1/13	—	—	—	—	—
68B	—	—	—	—	—	—	—	—	1/13	—	—	—	—	—
71CDE	—	—	—	—	—	—	14/16	7/12	12/13	13/15	—	—	1/15	—
73B	—	—	—	—	—	—	8/16	—	—	—	—	—	—	—
74EF	—	—	12/13	11/11	8/8	—	—	—	—	—	—	—	—	—
75B	11/12	7/7	12/13	11/11	8/8	—	—	—	—	—	—	—	—	—
78D	—	—	—	1/11	—	—	—	—	—	—	—	—	—	—
78EF	—	—	—	—	—	—	—	—	—	—	—	—	1/15	—

	I		II		III		IV		V		VI		VII	
	t	c	t	c	t	c	t	c	t	c	t	c	t	c
3R 82C	—	—	—	—	1/8	—	—	—	—	—	—	—	—	—
82F	—	—	—	—	1/8	—	—	—	—	—	—	—	—	—
84E	—	—	—	—	—	—	—	—	1/13	—	—	—	—	—
88E	—	—	—	—	—	—	1/16	—	—	—	—	—	—	—
91F	—	—	—	—	—	—	—	—	1/13	—	—	—	—	—
93CD	—	—	1/12	—	—	—	—	—	—	—	—	—	—	—
94CD	1/10	—	—	—	—	—	—	—	—	—	—	—	—	—
95B	—	—	—	—	—	—	—	—	1/13	—	—	—	—	—
95EF	—	—	1/12	—	—	—	—	—	—	—	—	—	—	—
96A	1/10	—	—	—	—	—	—	—	—	—	—	—	—	—
97D	—	—	—	—	—	—	1/16	—	1/13	—	—	—	—	—
98A	—	—	1/12	—	—	—	—	—	—	—	—	—	—	—
98F	—	—	1/12	—	—	—	—	—	1/13	—	—	—	—	—
99EF	—	—	—	—	—	—	1/16	—	—	—	—	—	—	—

Taking as an example a gland shown in II-c of Table 2, the developmental stage corresponded to PS-8 shown by Becker and a puff 63B was seen in only a single nucleus.

Much more puffs were observed in various loci in nuclei of the test glands than in those of the control glands (Table 2). The puffing patterns of the chromosomes in a gland differed from nucleus to nucleus in the same gland. Some puffs were found in several nuclei of two test glands: 2R–60B, 3L–63C and 3L–73B occurred in the gland of the 4th series and 2R–50CD in that of the 6th series, while puff 50CD was observed in both test and control glands of the 1st, 2nd and 3rd series.



Figs. 1-2. Puffs observed in the III-L chromosome arms. The puffs 63C (Fig. 1) and 73B (Fig. 2) were found in the chromosomes of glands incubated with egg extracts. The puff 71C-E (Fig. 2) was found in both control and test specimens.

The salivary glands incubated in the medium containing the tissue extract of young first instar larvae showed infrequent puffs in only test glands. In the 3rd series, puffs 3L–74EF and 75B were found in only the test gland, whereas they were observed in both control and test glands of the 1st and the 2nd series. In the test gland of the 4th series incubated with the egg extract, three puffs, such as 2R–60B, 3L–63C and 3L–73B, were observed in some nuclei (Table 2).

Discussion

The effect of ionic strength on the chromosome and puffing patterns was described by Clever (1965): sodium and potassium chloride solutions of physiological concentrations did not signfiicantly affect the puffing patterns, while incubations in solutions of higher concentrations led to irreversible changes of the chromosomal structure. The present experiments revealed that when the concentration of salts and sugars was high, the chromosomes were destroyed completely, though the effect of ionic strength of single salts was not examined.

It has been shown that the puffing patterns change in connection with changes of cell function or larval development, as well as with extracellular factors. This concept has been supported by injection of the molting hormone (Clever and Karlson, 1961), and by incubation of the salivary gland in the Ringer solution containing amino acid (Federoff and Milkman, 1964) or in the medium containing ecdysone (Clever, 1965). The results of the present experiments indicated that the puffs observed in the test glands were more numerous than those observed in the control glands. They were observed only in the test glands: this seems to be a chromosomal reaction to the tissue extract. Clever (1965) reported many infrequent puffs in the salivary chromosomes incubated in media. In the present experiments, the puffing pattern reactions to the extract varied from nucleus to nucleus in the same gland; many puffs were seen only in a nucleus, while a few in several nuclei.

Based on the presence of many puffs in the test glands, it is evident that the salivary chromosome has a potentiality to react to the extracellular factors in the medium within the scope of the present examinations. The puffs characteristic to extract of eggs and first instar larvae were not observed clearly in nuclei of the test gland. Three puffs, 2R-60B, 3L-63C and 3L-73B, were found to occur in several nuclei in the test gland with egg extract, but not in control specimens. It seems apparant that these puffs represent a gene activity characteristic of eggs.

Summary

In order to examine the maintenance of the salivary chromosomes, media of different constitutents and at various concentrations were examined in incubation of the salivary gland of *Drosophila melanogaster*. When the glands were incubated in media mixed with tissue extracts from eggs and young larvae, salivary chromosome showed many puffs in various loci in comparison with the control specimens

incubated in the medium without extracts. Three puffs, 2R–60B, 3L–63C and 3L–73B, were found to occur in several nuclei in only the glands incubated with egg extracts. It seems apparant that these puffs represent a gene activity charactersitic of eggs.

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