

DROSOPHILA SALIVARY GLANDS IN VITRO

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INTRODUCTION

The salivary glands of larval dipterans with their giant polytene chromosomes have been used extensively in cytogenetic studies, analysis of chromosome structure, and in the analysis of gene activity during differentiation (1, 4-7, 21, 29, 30, 35, 39). Specific local modifications at given gene loci, which are related to differentiation, are sites of RNA synthesis, and have been shown to be dependent upon the following parameters: extracellular environment, cytoplasmic environment, ions in the nuclear sap, as well as the state of reactivity inherent in the cell and chromosomes (3, 10-13, 23-26, 31, 36, 37).

Investigations on the factors involved in chromosome puffing have been facilitated by in vitro techniques in which salivary glands have been maintained in Ephrussi-Beadle saline for 1-2 hr (3, 36). The uptake of radioactive precursors has also been studied in explanted glands in Ephrussi-Beadle saline (16, 17, 32-34, 37). The maintenance of salivary glands during 24 hr of explantation to tissue culture media has been demonstrated in *Sciara coprophila* by Cannon (9, 10), who observed DNA synthesis and puff formation and condensation (DNA puffs) in chromosomes. The purpose of the present research is to compare various insect tissue culture media to see whether

morphological integrity and DNA synthesis can be obtained during extended explantation of salivary glands of *Drosophila melanogaster*.

MATERIALS AND METHODS

Swedish B8+, a wild-type strain of *Drosophila melanogaster*, was used. The flies were grown either at 20° or 23-25°C on standard medium, and third instar larvae which had crawled up the sides of the bottle were selected. The larvae were rinsed in 95% alcohol and transferred to sterile culture medium. The salivary glands were dissected free, the remainder of the larvae was removed, and the glands were rinsed with sterile medium. We explanted the salivary glands, using the lying-drop method, to approximately 0.5 ml/gland of the specified medium.

A comparison was made of the maintenance of six glands, from larvae grown at 20°C, after 24 hr of explantation in the following media: H-5 medium with 10% newborn calf serum (19); Schneider's medium (38); Grace's medium without insect hemolymph (18); Cannon's medium (9); and Jones and Cunningham medium with the insect hemolymph replaced with 10% newborn calf serum (20).

For studying DNA synthesis, 5 μ c per ml thymidine-³H (Schwarz Bioresarch, Orangeburg, N. Y.; specific activity 6.5 c/mmole) was added to the explantation medium. The incorporation of thymidine-³H in glands of larvae grown at 25°C was studied: (1) after 24 hr of explantation to the medium plus isotope; (2) after 1-hr exposure to the medium plus



FIGURE 1 Aceto-orcein squash preparation; Jones and Cunningham medium with serum. Chromosome banding is very precise; nucleolus and intranucleolar thread are clearly distinguishable. Arrow points to intranucleolar thread. $\times 1800$.

FIGURE 2 Aceto-orcein squash preparation; unexplanted gland as control. Arrow points to intranucleolar thread. $\times 1800$.

isotope at the beginning of explantation; and (3) during 1-hr exposure to the isotope after 24 hr of explantation to Jones and Cunningham medium with serum. No attempt was made to check the continuous availability of the isotope during 24 hr. For comparison, the incorporation of thymidine- ^3H in salivary glands explanted and incubated with the isotope in Ephrussi-Beadle saline for 24 hr was also studied.

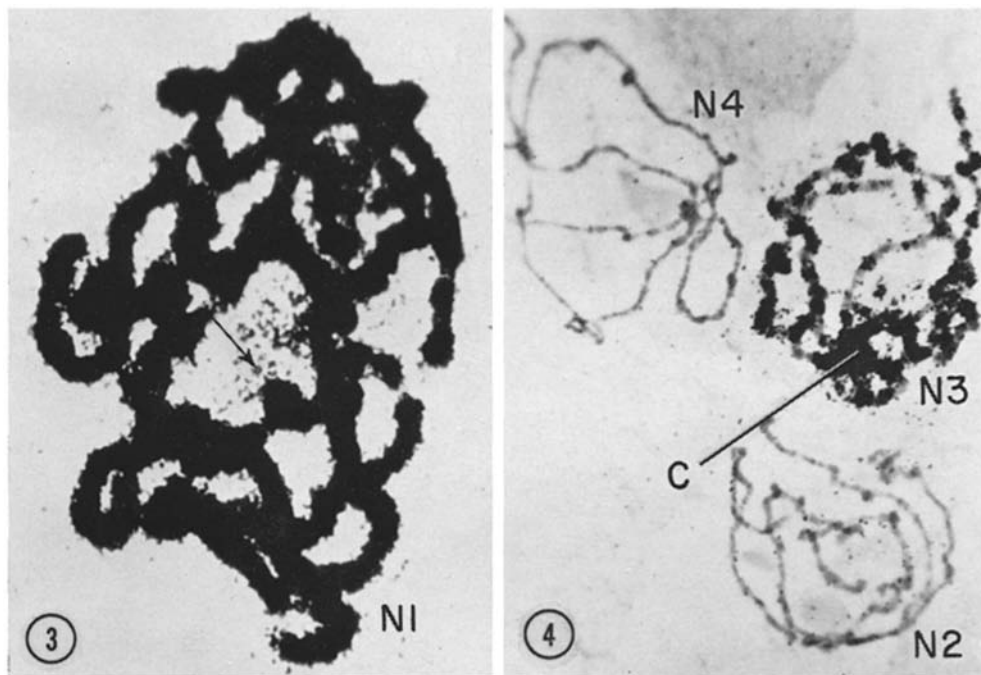
Permanent aceto-orcein squashes were prepared by the method of Oster and Balaban (28). Squash preparations for radioautography were stained and dehydrated. Then, as in the method of Pelling (31), the slides were rehydrated through a graded series of alcohols with the water replaced with a 10% formalin solution. Feulgen-stained sections of whole glands were also prepared for radioautography (2). The procedure for application, exposure, and development of the photographic emulsion Kodak NTB 3 was that of Taylor (40). The emulsion was exposed for 2-3 wk in black plastic boxes with added Drierite.

RESULTS

The criteria of viability of explanted glands included the morphology of the explanted gland and the pattern of incorporation of thymidine- ^3H .

Morphological Variation in Salivary Glands Explanted to Different Media

The maintenance of structural integrity was evaluated by the precision of chromosomal banding, the appearance of puffs at distinct chromosomal loci, and the appearance of the nucleolus and intranucleolar threadlike structure. Salivary glands, or individual cells within a gland which had been damaged during dissection exhibited dense staining of chromosomes, clumping of chromosomes, and pycnosis within 6 hr after explantation to any medium.



FIGURES 3-6 Radioautographs, aceto-orcein squash preparation, Jones and Cunningham medium with serum, plus thymidine- ^3H , 24 hr. All the nuclei are derived from the two salivary glands of one individual, and are on the same slide.

FIGURE 3 Larval salivary gland cell nucleus ($N1$), completely labeled. Arrow points to intranucleolar thread. $\times 900$.

FIGURE 4 Three larval salivary gland cell nuclei. Nucleus 2 unlabeled ($N2$). Nucleus 3 partially labeled ($N3$). Heavily labeled chromocenter (C). Nucleus 4 unlabeled ($N4$). $\times 300$.

The various media differed with respect to their efficacy in maintaining structural integrity. In H-5 medium, after 24 hr of explantation of the glands, the chromosomes appeared stringy and vacuolated, and chromosomal banding and nucleoli were not observed in proximal cells. The maintenance of the structural characteristics appeared better in Schneider's medium than in H-5 medium; although the chromosomes were attenuated, the differentiation between bands and interbands was distinct and nucleoli were observed. Chromosomal banding, nucleoli, and intranucleolar threadlike structures were visible after 24 hr of explantation to Cannon's medium, Grace's medium, and Jones and Cunningham medium with serum. The maintenance of puffs, the nucleolus, and a delicate threadlike intranucleolar structure was routinely observed after 24 hr of explantation to Jones and Cunningham medium with serum (Fig. 1). The appearance of

the chromosomes in control glands is presented for comparison (Fig. 2).

Patterns of Incorporation of Thymidine-³H

After 24 hr of explantation of salivary glands to Jones and Cunningham medium with serum plus 5 μ c/ml thymidine-³H, there were: (1) nuclei in which the chromosomes were totally labeled (N1, Fig. 3); (2) nuclei in which the chromosomes were partially labeled (N3, Fig. 4; N5, Fig. 5; N6, Fig. 6); and (3) nuclei in which the labeling above the background scatter did not exceed the background scatter (N2, Fig. 4; N4, Fig. 4).

The pattern of incorporation of thymidine-³H in the polytene chromosomes of explanted salivary glands exhibited the following characteristics: the chromocenter was labeled when the nucleus was labeled; the banded regions of the chromosomes were labeled at discrete bands or regions; and the number of bands labeled varied from cell to

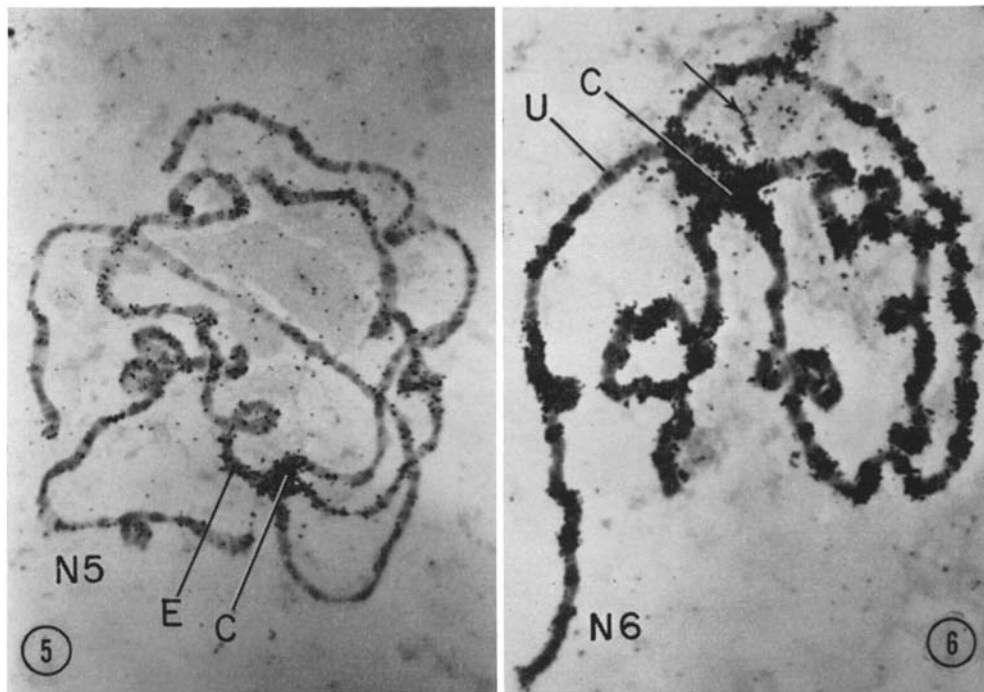


FIGURE 5 One larval salivary gland cell nucleus. Nucleus 5, (N5) labeling visible in scattered regions of euchromatic parts of the chromosomes (E); labeling heavy over the chromocenter (C). $\times 450$.

FIGURE 6 One larval salivary gland cell nucleus. Nucleus 6 (N6), heavy labeling in euchromatic region of the chromosomes, with only scattered regions unlabeled (U). Chromocenter (C) labeled. Arrow points to intranucleolar thread. $\times 800$.

cell. The incorporation of thymidine-³H within the nucleolus was observed in cells in which many of the banded regions of the chromosomes were also labeled (*N1*, Fig. 3; *N6*, Fig. 6), and the intranucleolar DNA synthesis was not uniquely associated with one discontinuous labeling pattern.

The same general patterns of incorporation of thymidine-³H were found in salivary glands explanted to Ephrussi-Beadle saline for 24 hr. However, the maintenance of the banded character of the chromosomes was not so precise as that in Jones and Cunningham medium with serum. Incorporation of the isotope was observed in radioautographs which were stained by the Feulgen technique prior to application and exposure of the photographic emulsion.

In order to assess the extent of labeling during culture, we determined the frequency of labeled nuclei: (a) after 24 hr of explantation and incubation; (b) after 1 hr of explantation and incubation; and (c) during 1 hr of incubation after 24 hr of explantation (See Table I).

TABLE I
Labeling Patterns*

A multivariate analysis of variance was performed on the data by Mr. Bernard Carol and Mr. Alex Miller of the Computer Center, New York Medical College. The partially labeled and nonlabeled proportions were used as the two dependent variables in the study. On an over-all basis, these two variables differed among the groups, with a P value less than 5%. The test used was the regular 3 sample F test involving Wilks W statistic.

	Time		Completely labeled	Partially labeled	Un-labeled
	Explantation	Incubation			
	hr	hr	%	%	%
a)	24	24	6.3	37.6	56.1
			5.	41.6	53.4
			9.	36.8	54.2
b)	1	1	5.7	25.7	68.6
			2.3	25.8	71.9
			4.4	16.1	79.5
c)	24	1	0	9.4	90.6
			0	10.3	89.7
			0	24.5	75.5

* Each listing represents counts of the nuclei of the two salivary glands of one individual.

The presence of labeled nuclei in a 1-hr pulse after 24 hr of explantation, and the increased frequency of labeled nuclei after 24 hr when compared with 1 hr of explantation and incubation, suggest that DNA synthesis continues during the longer explantation interval.

DISCUSSION

The results of the explantation studies show that salivary glands of third instar larvae of *Drosophila melanogaster* maintain cellular and structural integrity in various insect tissue culture media. The ability of salivary glands to incorporate tritiated thymidine during 24 hr of explantation to Jones and Cunningham medium with serum was also demonstrated. The composition of the five insect tissue media studied differed with respect to ions, osmotic pressure, sugars, and amino acids (9, 18-20). The three media, Cannon's medium, Grace's medium, and Jones and Cunningham medium with serum, in which structural integrity was maintained, have low Na⁺/K⁺ ratios and high Mg⁺⁺ concentrations, values which resemble those of the composition of *Drosophila* hemolymph (8).

The results of the studies on the incorporation in explanted *Drosophila* salivary glands are in agreement with those of other in vivo and in vitro studies as well as studies on other Diptera (14-17, 22, 27, 32-34, 41). The relationship between the observed labeling patterns and the temporal sequence of replication is not yet clear (cf. Plaut et al., 1966). The extended viability of explanted glands may provide a system which can yield further information on replication in salivary gland chromosomes of *Drosophila*.

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