EXPERIMENTAL PUFFS IN SALIVARY GLAND CHROMOSOMES OF DROSOPHILA HYDEI*

By
H. D. BEERENDES, F. M. A. VAN BREUGEL and Th. K. H. HOLT

(Received September 27, 1964)

Introduction

Tissue-specific and age-specific localized morphological changes in certain types of chromosomes have been interpreted as an expression of functional changes in individual gene loci (BEERMAN 1952). Recently a number of cytological investigations concerning in particular chromosomal puffs of Dipteran giant chromosomes, have given strong support to this hypothesis (BEERMAN in press).

Since puffs present in giant chromosomes may be considered as expressions of specific gene activities, it has become important to test various changes in physiological conditions, and to assay various substances and types of treatment, for their influence on the behaviour of individual puffs as well as the puffing pattern in general.

Some suggestions were obtained about factors involved in the activation of genes. However, the chemical nature of these effects is still a matter of conjecture. Most of the experiments revealed some influence of the treatment that was used, on the activity of certain chromosome regions. Remarkable is the fact that by most of the treatments regions could be activated, which were not known to be present as puffs at any time during the normal development. In this connection it should be pointed out that the period where observations on puffs can be made, is only a fraction of the total time of development.

As puffs are sites on the chromosomes active in RNA synthesis (PELLING 1964) decisive progress could be made by resolving what kind of RNA is produced in normal as well as in experimental puffs. It may be assumed that most puffs contain messenger RNA, necessary for normal cell metabolism. What kind of message, then, does an experimental puff represent? Only in a very few cases something is known about the relation of a puff to its effect in the cellular metabolism (BEERMAN 1961).

Transplantation experiments, the administration of hormones in vitro and in vivo, and the effect of salt solutions in vitro, revealed that

* Dedicated to Prof. H. BAUER on the occasion of his sixtieth birthday.
the composition of the cell milieu can be regarded as an important factor in the activation of specific chromosome regions (Kroeger 1960, Becker 1962, Clever 1961, 1963). Recent investigations by Kroeger (1963, 1964) on the administration of heavy metal ions to nuclei isolated from cytoplasmic constituents, indicate that the nuclear sap may be an intermediate in the activation of puffs.

This paper reports an investigation on modifications of the puffing pattern, induced by three types of treatment, viz. temperature shocks, and changes in potassium or sodium concentrations, in larval salivary glands of *Drosophila hydei*. Attention was focussed on two questions: 1. whether a given treatment induced different reactions depending on developmental age, and 2. whether different changes were produced by different treatments. As a prerequisite, a study of puffs in normal development has been undertaken. These observations revealed, as expected, stage-specific and tissue specific puffs. The appearance of some stage-specific puffs could not be related to known changes in the hormonal balance of the hemolymph (Berendes 1963b).

**Material and Methods**

A wild type stock of *Drosophila hydei* Sturtevant was used, originated from São Paulo (courtesy Dr. C. Pavan) and kept in the laboratory since 1956. The age determination of the larvae that were used, is based on a standard culture method at 25°C described by Berendes (in preparation). The time of the second larval moult is 92–95 hours after oviposition. The time of puparium formation (prepupal moult) is 160–164 hours after oviposition. All puffing pattern analyses were made on fresh orcein-acetic acid squash preparations. The localisation of the puffs is based on a salivary gland chromosome map (Berendes 1963a). A total number of 148 puffs have been recorded for the five long chromosome arms, and in each of the experiments the total pattern was investigated. From 110 of these puffs "activity diagrams" were made, i.e. diagrams indicating the size of a given puff over the period of its presence on the chromosome. A quantitative evaluation of "activity", or size, based on morphological criteria, was obtained by using a classification into five arbitrarily chosen degrees of puffing, as follows: Regions that are known to be puffed at some period during development, but appear neutral at the moment of investigation are designated as class O. A puff has reached its maximum activity (class 4) when its diameter is 2.0 to 2.5 times the diameter of the neighbouring non-puffed region. Intermediate puffs are assigned to class 1, 2 or 3, depending on their relative size. This scale is independent of the maximum activity that any of the observed puffs in fact can attain.

Temperature shocks were applied in vivo and in vitro, treatments with various salt solutions in vitro only.

**Results**

**Temperature shocks**

Temperature shocks were given to whole dissected glands in Drosophila Ringer solution. This solution (essentially following Becker 1959) was composed of 3.25 g NaCl, 0.07 g KCl, 0.10 g NaHCO₃, 0.06 g CaCl₂ and 0.005 g Na₂HPO₄ in 500 ml water, with a pH of 7.3. In each of the experiments one of the glands of the larvae was fixed and stained just after dissection at room temperature, and the second