Hägele, K. and W.-E. Kalisch. Ruhr-Universität Bochum, Germany. ^{3}H-thymidine labeling intensity over a prominent band group prior to and during puffing.

^{3}H-thymidine labeling experiments on polytene chromosomes of D. melanogaster have been made in order to check whether or not an alteration in the morphology of a chromosome section is correlated with a change in the amount of silver grains over it. In late third instar larvae (115 h after egg deposition) and white prepupae (0-2 h after puparium formation) region 61A-64C of chromosome arm 3L was analyzed autoradiographically at the discontinuously labeled end phases of the replication cycle. Especially section 63E1-5 was studied because the prominent band group of this region forms a large puff in prepupae.

Table 1. Silver grain labeling intensities over puffed and non-puffed 63E1-5 sections compared to reference sections.

	Ratios of silver grain numbers over sections		
	63E1-5/62C1-2	62A1-2/62C1-2	63A1-3/62C1-2
Puff 63E1-5 present	1.26	0.18	0.84
Puff 63E1-5 absent	0.64	0.22	0.85

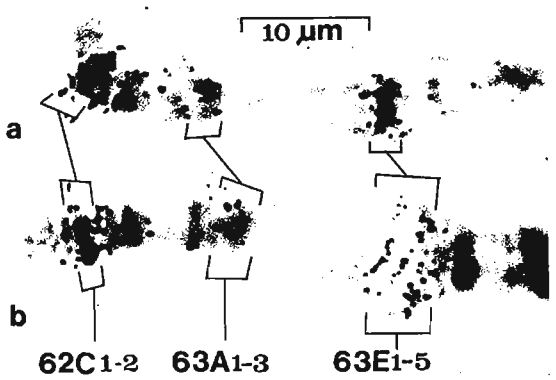

Fig. 1 a and b. Autoradiographs at the late replication phase of region 62C-63E in a late third instar larva (a) and a prepupa (b). Labeling intensities over 62C1-2 and 63A1-3 correspond with each other in (a) and (b). The puffed 63E1-5 section shows approximately twice as much silver grains as the 63E1-5 bands in (a). ^{3}H-thymidine incubation of the excised salivary glands 10 min (50 µCi/ml; spec. activity 24.0 Ci/m mol), exposure time 11 days, Giemsa stained. Autoradiographic methods and background determination have already been published (Hägele and Kalisch 1974; Kalisch and Hägele 1973, 1977).

The ratios of silver grain numbers over the puffed and non-puffed 63E1-5 sections and the reference sections 62A1-2 and 63A1-3 to those over section 62C1-2 were determined (Table 1). In 53 chromosomes, silver grain ratios between the reference sections correspond with each other during late third instar larvae and prepupae. However, the ratios of the 63E sections show that the labeling intensity over the puffed 63E1-5 section is twice as high as over the non-puffed 63E bands. Fig. 1 gives an example for labeling distributions over the 62B-64A region in a late third instar larva and a prepupa.

Our data do not agree with those of Zhimulev and Belyaeva (1975), which claim that the amount of silver grains over the 63E1-5 section does not depend on whether the puff is present or not. Experimental methods used in this study, however, are quite different from ours. After hatching, larvae were incubated up to the 0 h and 1 h prepupa stage in a medium containing the radioactive precursors. In our experiments, pulse labeling with ^{3}H-thymidine was only allowed to take place before (late third instar larva) or during (prepupae) puff formation of 63E. Furthermore, Zhimulev and Belyaeva (1975) have used a "correction factor" for silver grain calculations which is thought to correct region geometry and self-absorption of β-particles (Holmquist 1972). We have omitted this correction because the factor does not deal with the increase of a region's volume in the special case of puffing of a condensed band.

With regard to our results it could be argued that the molecules, necessary for DNA synthesis, more easily reach the DNA in the puffed state than in the condensed situation because of the less dense packing of the DNA protein complex in the puff (Berendes 1966). This would imply that replication in the puffed 63E1-5 section could proceed faster and that, therefore, the labeling intensity after ^{3}H-thymidine application is higher than in the condensed 63E section. However, replication duration of 63E remains unchanged whether this region is puffed or not (Hägele and Kalisch, in press). Thus, an increased labeling intensity of the puffed 63E section, based on a faster replication, can be excluded. It seems more likely that in a

labeled puff section the changed conditions in comparison to the non-puffed state have an increasing effect on the silver grain numbers. In the transcriptionally active, puffed situation there is a greater area of chromosome contact with autoradiographic emulsion, a decreased clustering of the precursors incorporated and, presumably, a lowered self-absorption of β-particles on the basis of the changed DNA histone (nucleosome) configuration.

References: Berendes, H.D. 1966, Chromosoma 20:32; Hägele, K. and W.-E. Kalisch 1974, Chromosoma 47:403; _____ and _____, Chromosoma (in press); Holmquist, G. 1972, Chromosoma 36: 413; Kalisch, W.-E. and K. Hägele 1973, Chromosoma 44:265; _____ and _____ 1977, DIS 52:127; Zhimulev, I.F. and E.S. Belyaeva 1975, Chromosoma 49:219.

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Hydropyrimidine hydrase in D. melanogaster.

The black mutation (2-48.5) of Drosophila is the result of a partial deficiency in beta-alanine synthesis (Hodgetts and Choi 1974); however, the specific enzymatic lesion is still unknown. Beta-alanine can be synthesized via a number of pathways including direct decarboxylation of aspartate, or by synthesis and degradation of uracil. In Musca, the major biosynthetic pathway proceeds through uracil (Ross and Monroe 1972), and in Drosophila, Jacobs (1974) found that, like beta-alanine, exogenous uracil, dihydrouracil and beta-ureidopropionate promoted normal coloration in black adults; and label from both orotate and uracil was incorporated more strongly into black than wild type cuticles. These data would suggest that black causes a partial metabolic lesion in the pathway via uracil, possibly prior to orotate. However, studies of several gene-

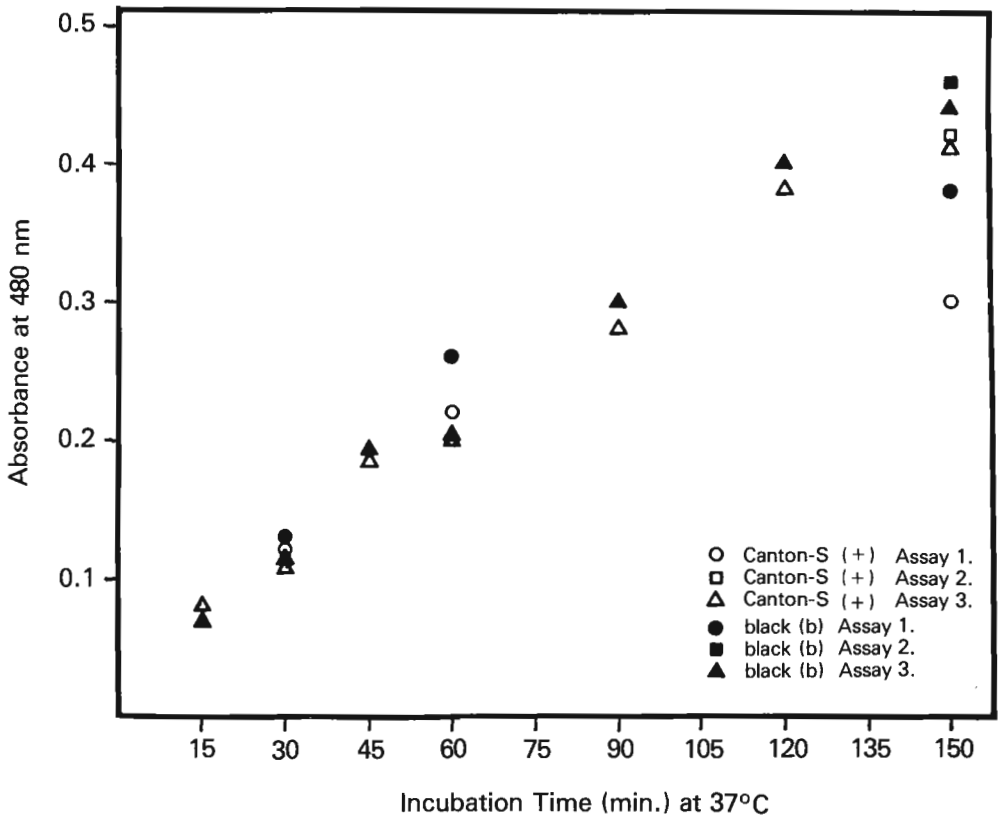

FIGURE 1. Hydropyrimidine Hydrase Activity in Canton-S and black **Drosophila**. Assays were performed using crude **Drosophila** supernatant of 100 flies/ml. Protein determinations were performed for assay no. 3 and no significant difference was found between black and Canton-S supernatants. Flies were usually no older than 9 hours.