Developmental Studies in *Drosophila*. X. Developmental Electrophoretic Pattern of Salivary Gland Unspecific Proteins in *D. pallidipennis*[1]

A microdisc electrophoretic analysis of *Drosophila pseudoobscura* salivary glands revealed important qualitative and quantitative variations in unspecific proteins during larval and early pupal development of that insect[2-4]. Since a comparable study is not available for any other *Drosophila* species or even any other Diptera, I chose to carry out an identical investigation with *Drosophila pallidipennis*, a species phylogenetically remote from *D. pseudoobscura*.

Materials and methods. A *Drosophila pallidipennis* stock from Bucaramanga, Colombia (strain 17 of PASTEUR and KASTRITSIS[5], or University of Texas at Austin stock No. H 191.48) was used. The synchronization of the flies, the sample technique and the microdisc electrophoretic technique have been described in details by PASTEUR and KASTRITSIS[2].

Two larval stages were studied, i.e. 135 h and 160 h after hatching. Under the experimental conditions, the larvae leave the nutritive medium to crawl along the sides of the containers between 120 and 130 h after hatching from the egg; these larvae are those of the 125 h stage. The larvae of the 160 h stage can be considered as being at or near the point of everting their spiracles; spiracle eversion (SE) occurs at 160 h on the average.

The early pupal developmental stages were timed in reference to spiracle eversion (directly observed under a dissecting microscope). They included the stages of spiracle eversion (SE), 4 h, 10–16 h and 20 h after SE.

Results and discussion. The Figure represents the developmental variations in unspecific proteins (stained with Coomassie brilliant blue) which occurs during the development of the salivary glands. For purposes of comparison, the drawings of the unspecific proteins contained in hemolymph, fat body and whole-fly were included.

Several protein bands displayed quantitative variations during development, but such variations will not be considered further (although they have been tentatively represented on the drawing by different symbols) because of lack of densitometric data.

From the Figure, it can be observed that a total of 27 unspecific protein bands could be detected at some stage of the development of the salivary glands. Among these, 13 display stage(s) specificity: *10, 13, 15, 21, 30, 33, 50, 54, 58, 66, 76, 86* and *93*. Only those that seem of particular interest will be discussed.

The protein band *86* is present at the 125 and 160 h stages, i.e. at stages when the salivary glands are known to be actively synthesizing the glue proteins which will be released at puparium formation. A protein band presenting a similar developmental behavior was also detected in *D. pseudoobscura*[2,3]. The glue synthesized by the salivary

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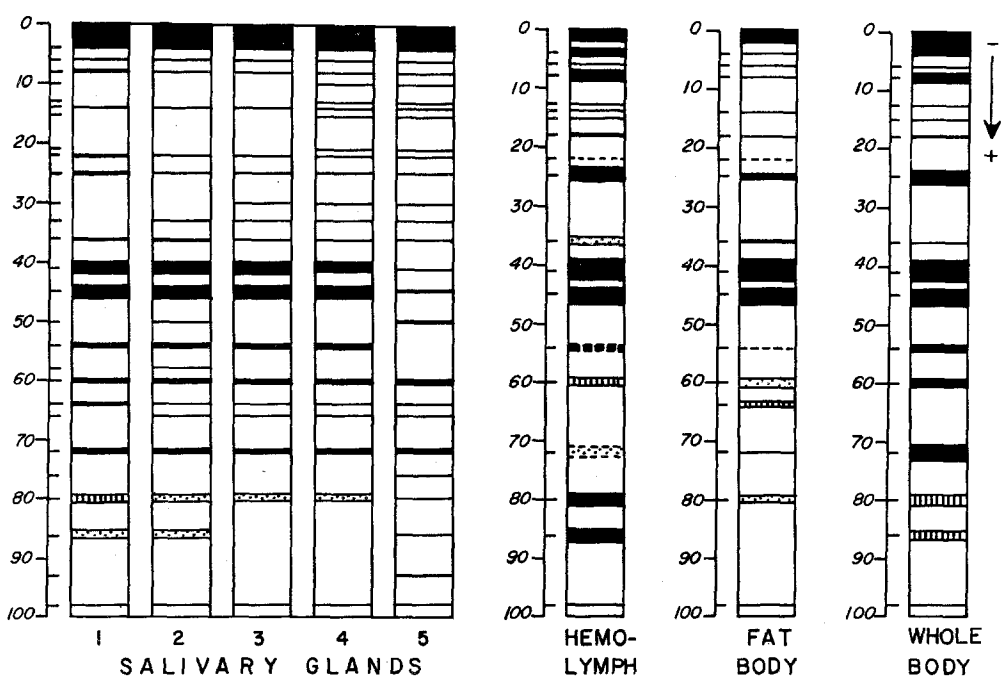

Electrophoretic pattern of unspecific proteins of *Drosophila pallidipennis*. Hemolymph, fat body and whole-body electrophoretic pattern drawings represent a composite of the pattern obtained at the different developmental stages studied; the interrupted lines designate the protein bands showing variations during development. The numbers under the drawings for the salivary glands designate the different developmental stages: 1, 125 h; 2, 160 h; 3, spiracle eversion (SE); 4, 4 h after SE; 5, 20 h after SE. The slanted numbers on the left side of the drawings designate an arbitrary scale according to which the different protein bands have been designated. ■, intensely stained; ▨, moderately stained and ▦, faintly stained protein bands.

glands is known to be rich in mucoproteins which are large molecules generally unable to enter an acrylamide gel of the concentration used here (7%). On the other hand, the glue also contains proteins of smaller size[6]. The *D. pallidipennis* protein band *86* as well as the equivalent *D. pseudoobscura* protein band (band *55*) probably belong to this category.

The protein bands *50* and *58* which appear at the 160 h stage could be under ecdysone control. But while band *58* is specific of this stage and may have a function related to glue secretion, band *50* is also present at the 20 h after SE stage and might be related to molting (puparial molt at 160 h and pupal molt around 20 h after SE). Identical behaviors were observed for none of the *D. pseudoobscura* protein bands.

A very interesting observation is that new protein bands appear at the 20 h after SE stage, i.e. a few hours before the complete histolysis of the salivary glands. 2 protein bands were never present before this stage, i.e. bands *76* and *93*. That 2 completely new proteins also appear at an identical developmental stage in *D. pseudoobscura* salivary glands[3,7], bands *82'* and *85'*, is stricking. In both cases these 2 bands were not detected in other tissues; therefore they are probably tissue specific. Further investigation is undoubtedly needed in order to determine whether these proteins are synthesized de novo at this stage or come from proteins of high molecular weight which have been partially degraded. This could be of particular importance for possible correlation with puffing patterns, the study of which has shown the formation of new puffs very late in the development of the salivary glands[8,9].

The study of the developmental pattern of unspecific proteins of the salivary glands of *Drosophila pallidipennis* has thus confirmed several observations made with *Drosophila pseudoobscura*, i.e. the disappearance of certain protein components at the time of spiracle eversion and the appearance of new such components a few hours before the complete histoloysis of the salivary glands. These observations suggest that such phenomena may take place throughout the genus *Drosophila*[10].

Résumé. Les protéines présentes dans les glandes salivaires de *Drosophila pallidipennis* ont été analysées grâce à une technique de microélectrophorèse discale. Les variations observées pendant le développement de la larve et de la jeune pupe ont été comparées avec celles de *Drosophila pseudoobscura*. Il semble que certaines variations soient le résultat de phénomènes communs à toutes les espèces du genre *Drosophila*.

NICOLE PASTEUR[11]

Department of Cell Biology, The University of Texas, Southwestern Medical School, Dallas (Texas 75235, USA), 23 March 1972.

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[11] Present address: Laboratoire de Génétique expérimentale des Populations, Université des Sciences et Techniques du Languedoc, Place Eugène Bataillon, F-34 Montpellier (France).

Constant Oxygen Enhancement Ratio During the Mitotic Cell Cycle in X-rayed *Drosophila melanogaster* Embryos

With a variety of cell types, a strong stage-dependent variation of the radiosensitivity during mitosis or meiosis is observed. This was demonstrated by WÜRGLER for the mitotic stages of the early cleavage divisions in *Drosophila melanogaster* (WÜRGLER, ULRICH and SCHNEIDER-MINDER[1]). He used an egg collection technique (WÜRGLER, ULRICH and SPRING[2]) which allows the obtaining of samples with ample numbers of eggs within 3 min. These samples were exposed to X-rays at various times after collection. For convenience, the cells in samples, which are older than 15 min and which in fact are early embryos, are still called 'eggs'.

A cyclic variation of the radiosensitivity with age (see also Figure 1) was observed. The criterion used to measure the radiosensitivity was the percentage of embryonic lethality (= number of eggs from which no larvae hatched/number of eggs irradiated). Cytological analysis showed that the radioresistant cells are in inter-/pro-phase, the most sensitive cells in ana-/telophase. Recently LEUTHOLD[3] irradiated cleavage stages obtained from stocks with variable chromosome numbers. He found that radiosensitivity is positively correlated with the amount of chromosomal material irradiated per cleavage nucleus. This indicates that embryonic lethality is the consequence of radiation-induced chromosome lesions. MATTER[4] postulated that part of the stage-dependent changes in sensitivity results from a variation in the concentration of free SH groups within the chromosomes. The basis of the remaining variation is not yet clear.

For some cell types it is known that the oxygen consumption changes from stage to stage during the cell cycle (for review see MAZIA[5]). It was shown by KIHLMAN[6] that active respiration decreases the oxygen concentration within the cell nucleus compared to the oxygen concentration present outside the cell. Stage-dependent variations in the respiratory activity could therefore lead to variable oxygen concentrations in the radiosensitive structures during the cell cycle. Therefore in experiments in which exposure to X-rays is performed in a normal air environment at least part of the sensitivity differences between stages might result from variation in the oxygen content within the radiosensitive structures. Assuming this hypothesis to be true for *Drosophila* eggs, for which the radiosensitive structures during cleavage are chromosomes, we predict that the relative radiosensitivities of different mitotic stages should differ, depending on whether radiation is given under oxic or anoxic conditions. Consequently

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