Developmental Studies in *Drosophila*. IV. Quantitative Protein Changes in Organs and Whole-Fly Homogenates During Development of *D. pseudoobscura*[1]

During the course of an investigation of the changes in electrophoretic pattern of some enzymes and other proteins during the development of several tissues and whole individuals of *D. pseudoobscura*[2], we observed important qualitative variations in certain protein bands. At the same time it became evident that the data should be analyzed quantitatively. Such an analysis was performed and the results obtained are reported here. Also included are new data on further developmental stages of all organs studied[2] and on several stages of development of the body wall.

Material and methods. A standard strain of *D. pseudoobscura* from Mather, California, was used. The synchronization method, the sampling technique, as well as the micro-disc electrophoretic technique used for this study have been described in detail elsewhere[2].

The microgels (1.5 mm in diameter and about 1 cm in length), stained with Coomassie brilliant blue, were scanned with a densitometer (Densicord, model 542, Photovolt Corporation, New York, New York). The scanning was always performed after the 'dark-point' adjustment of the instrument on the most intensely stained band of an arbitrarily chosen specific gel.

Results and discussion. Figure 1 represents the scanning data obtained from the different developmental stages of fat body, hemolymph, and salivary glands; Figure 2 those obtained from whole-fly and body-wall homogenates.

On the figures, the numbers on the ordinate represent optical densities while those on the abscissa number above each peak) represent the bands on a hypothetical gel containing all protein bands of all organs and developmental stages studied (Figure 3).

Among the 10 protein bands of the fat body which showed some degree of quantitative variation at one stage or another, 4 could be followed by the densitometer (30, 45, 50 and 74); bands 45 and 50 seem of particular interest. These 2 bands are of low optical density (between 50 and 60) in early larval stages suddenly increasing at the 120 h stage to a maximum (between 90 and 100), and remaining so thereafter. It is known, that *Drosophila* fat body stores proteins from mid-third instar on[3], i.e. between the 96 h and 120 h stages. Moreover, several authors[4–6] have proved that some of these stored proteins are hemolymph proteins. From Figure 1 it can be seen that these 2 protein bands (45 and 50) are present in the hemolymph in all developmental stages and do not undergo any change in optical density. An interesting hypothesis would be that bands 45 and 50 of the fat body represent stored hemolymph proteins.

Among the 9 protein bands which show quantitative variations in the hemolymph, 5 could be registered by the densitometer i.e., 30, 55–58, 85–90 (55 and 58 as well as 85 and 90 do not appear as separated bands on the scanning). At this moment no explanation can be proposed for these changes; however, it is known that 70% of hemolymph proteins are secreted by the fat body[7–9]. Since all these bands (except band 90) are also present in the electrophoregrams of the fat body it is conceivable that they are secreted into the hemolymph by this organ.

The protein pattern of the developing salivary glands shows that the major protein bands increase progressively in optical density from the 96 h to the spiracle eversion stage. This is most evident for bands 45, 50, 63, 65 and 68.

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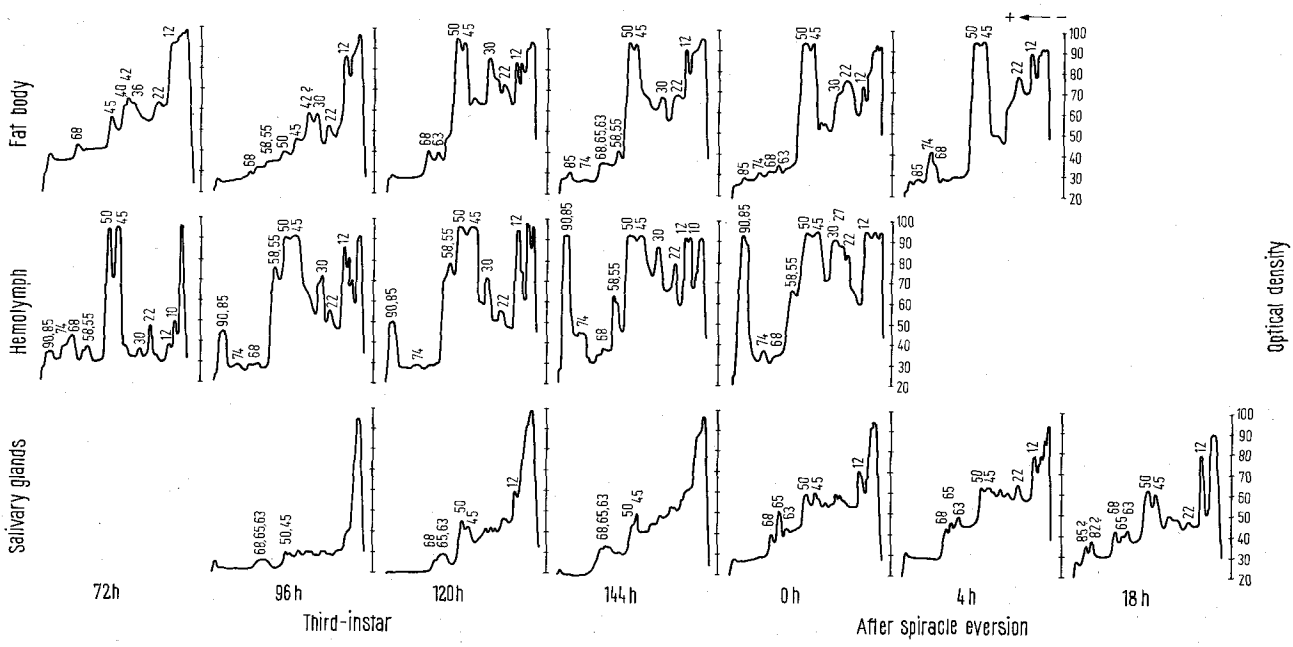

Fig. 1. Densitometric curves of protein patterns obtained through microelectrophoretic analysis of fat bodies, hemolymph and salivary glands of *D. pseudoobscura* at different developmental stages. Ordinate, optical density (O. D. of 20 corresponds to no band); abscissa, position of band in gel.

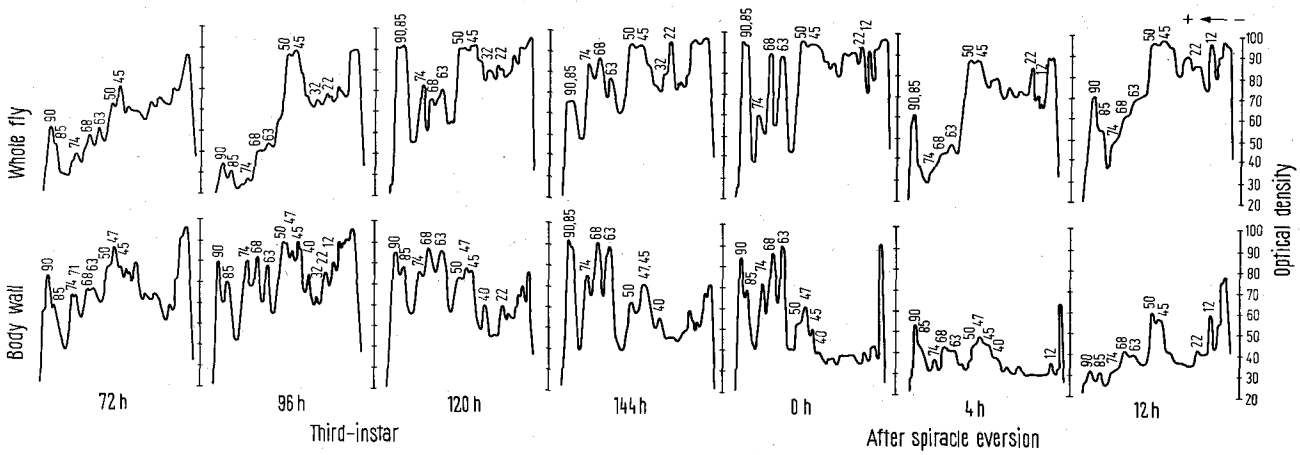

Fig. 2. As for Figure 1 but for whole-fly and body-wall proteins.

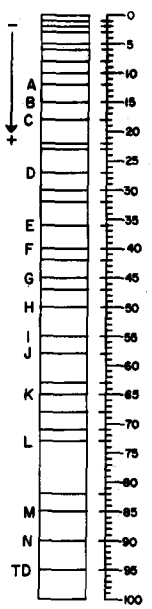

Fig. 3. Diagramatic representation of hypothetical microgel containing all protein bands regardless of developmental stage or tissue or complex of tissues. Numbers represent the position of each protein band in the gel. Letters correspond to designations used in previous reports.

This increase coincides with the period of active protein synthesis which has been observed in other Diptera[5,7]. However, with the exception of band 65, these protein bands are all present in the hemolymph, and part of the increase in optical density could be due to an uptake of hemolymph proteins[7,10,11]. In the salivary gland bands 82 and 85 which appear only at the 16 h after spiracle eversion stage (just a few hours before the complete histolysis of the glands) are of particular interest. It is not known if these bands correspond to newly synthesized proteins or to proteins of high molecular weight which are being broken down. However, *D. pseudoobscura* salivary glands show synthesis and secretion of material in the lumen from 8 h after spiracle eversion on[12]. Additionally, the formation of some new puffs has been described to occur very late in development on the polytene chromosomes of other species of *Drosophila*[13]. In *D. pseudoobscura* the activity of two puffs increases dramatically at 14 h after spiracle eversion[14]. Whether these puffs are associated with the production of protein bands 82 and 85 remains unknown.

Whole-fly and body-wall homogenates present distinct similarities: Figure 2 shows that protein bands 63, 68, 74, 85 and 90 of whole flies originate mainly from the integument since they are approximately of the same optical density in the homogenates of both. The slower moving proteins (12, 22, 30, 45, 50, etc.) come partly from the body-wall, but probably mostly from hemolymph and fat body. The body-wall proteins of the 12 h after spiracle eversion stage are in fact the pupal body-wall proteins while those of the preceding stages are those of the larval body-wall. Pupal and larval body-wall proteins do not differ much in quality but they do so in quantity.

From our study of *D. pseudoobscura* proteins, it becomes clear that all studied tissues have a characteristic electrophoretic protein pattern. These patterns disclose both qualitative and quantitative variations from stage to stage. In some cases these developmental variations can be correlated with known biological events.

Résumé. Les protéines présentes à différents moments de l'ontogenèse dans les glandes salivaires, l'hémolymphe, le corps adipeux, la paroi du corps et les individus entiers de *Drosophila pseudoobscura* ont été analysées grâce à une technique nouvelle de micro-électrophorèse discale.

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