Thus, we have added some information to that available about the phylogenic dependent relationship between the thyroid gland pharyngeal IV regions (parathyroid IV, thymus IV) and the neural crest.

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Selective staining of β-esterase of *Drosophila*

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Summary. It is shown that the potent protease inhibitor phenylmethylsulphonylfluoride (PMSF) strongly inhibits the activity of all *Drosophila* esterases but β-esterase. We suggest the application of PMSF for the selective staining of β-esterase in *Drosophila* tissues.

Understanding of the molecular mechanisms of cellular differentiation needs the analysis of cell population heterogeneity in the process of individual development[1]. Determination of the ratio of different forms of the same enzyme at the level of individual cells is one of the most promising methods in this field. A large variety of histochemical methods, as well as microelectrophoresis, have been successfully used for this purpose.

As reported in a number of papers[2-4], the investigation of esterases in *Drosophila* permits us to obtain valuable information about differentiation processes and regulation of genetical expression during the development of this classical object of genetics. However, the investigation of esterases was limited by the ability of many of these enzymes to interact with the same substrates. This allows the determination of esterases only as a group, and important information about the distribution of a particular esterase isozyme in different tissues or in a single cell cannot be obtained. The same is true for the determination of qualitative changes of particular esterases during differentiation and development.

In the present paper we describe a novel approach which permits selective elucidation of *Drosophila* β-esterase. The procedure is based on the inhibitory action of PMSF on all of *Drosophila* esterases except β-esterase.

Materials and methods. Experiments were made with organs of *Drosophila virilis* of the strain $Ce^{-}S^{+}$ which is characterized by high activity of the organ-specific S-esterase in the tissues of the bulbus ejaculatorius. Microelectrophoresis was done as described earlier[5]. Single organs were used to prepare the samples under investigation. Tissues were homogenized in a 0.5% aqueous solution of Triton × 100 (Merck) and the extract obtained was loaded on to a polyacrylamide gel prepared in a glass capillary (0.5 mm ID). The electrophoresis system was Tris-EDTA-borate (Serva) at 220 V, 2 mA. To stain the esterase isozymes after electrophoresis we employed the enzymatic reaction with α-naphthylpropionate and β-naphthylacetate[3].

Histochemical staining of various *Drosophila* tissues was done as described earlier[5]. Three types of incubation mixtures were used: a) containing α-naphthylpropinate; b) containing β-naphthylacetate and c) the mixture of both substrates.

The solution of 0.1 M PMSF (Calbiochem) in dimethylsulfoxide (Serva) was added to the incubation mixtures during histochemical procedures to a final concentration of 1 mM. The same concentration of PMSF was used to extract tissues with 0.5% Triton × 100. During the staining of isozymes after microelectrophoresis the inhibitor was not added to the reaction mixture.

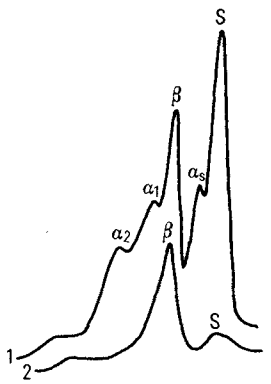

Figure 1. Scanogram of the stained gel after microelectrophoresis of: 1, 0.5% Triton × 100 extract of a single male fly; 2, the same but with the addition of PMSF to the extraction solution.

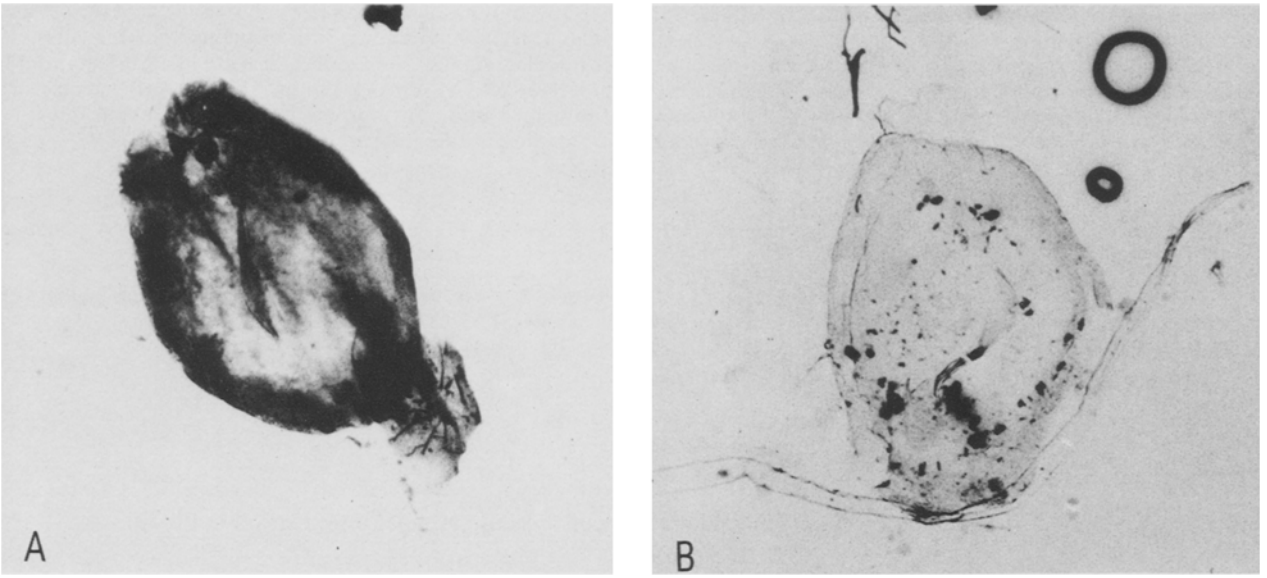

Figure 2. Ejaculatory bulb of *Drosophila virilis* male fly stained in the abscence (A) and in the presence (B) of 1 mM PMSF.

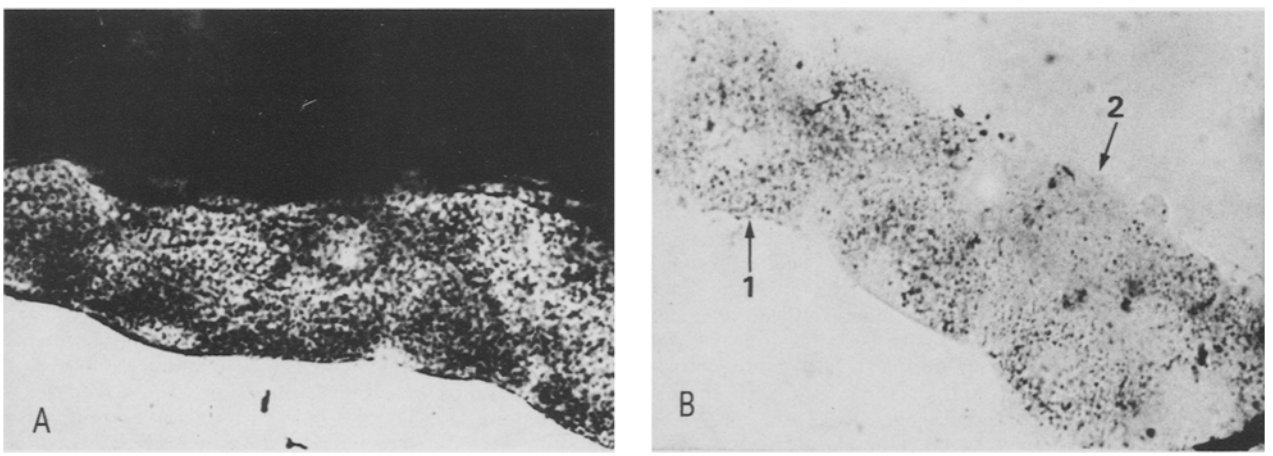

Figure 3. *Drosophila* Malpighian tubule tissue preparation stained without (A) and with(B) the addition of PMSF.

Results and discussion. The electrophoretic pattern of *Drosophila virilis* esterases is presented in figure 1: esterases α_1, α_2 and α_S (reacting preferentially with α-naphthylpropionate) as well as S-esterase and β-esterase (reacting preferentially with β-naphthylacetate) could clearly be shown as distinct peaks on the scanogram. The addition of PMSF during the extraction of tissues with 0.5% Triton $\times$ 100 solution inhibited the activity of all esterases but β-esterase (fig. 1). One can see, though, that a slight activity of S-esterase was preserved in this case as well.

The electrophoretic data presented here show the inhibitory action of PMSF on most of *Drosophila's* numerous esterases and the resistance of β-esterase to its action. These observations led us to try the application of PMSF in histochemical staining of esterases, for studying β-esterase distribution in different cells and tissues.

Figure 2 shows the results of such a staining procedure in a male organ of *Drosophila virilis* - the ejaculatory bulb. One can see that treatment with PMSF greatly decreased the staining of this organ (fig. 2, A and B). These data are in a good agreement with the results obtained earlier[3] where it was shown that this organ contains very low amounts of β-esterase and that the organ specific S-esterase prevails in its tissue. As is shown in figure 1, S-esterase preserved some of its activity following PMSF treatment.

The results of histochemical staining of Malpighian tubule preparations of both male and female flies (fig. 3) demonstrated that in controls the staining was strong, and about the same in all cells of this organ (fig. 3, A). However, in preparations treated with PMSF the staining was less, and its intensity varied between different cells (fig. 3, B, compare cells 1 with cells 2). Uneven distribution of β-esterase among different cells of this organ has previously been suggested by one of us[1,3] on the basis of microelectrophoretic analyses.

The data presented in this paper allow us to propose the treatment of tissues with PMSF for histochemical studies on β-esterase distribution in tissues and cells of *Drosophila.* This method is more simple and direct than microelectrophoresis in polyacrylamide gels and permits us to obtain more detailed information.

Furthermore, the results obtained in this work with PMSF suggest, that β-esterase differs from other *Drosophila* esterases, not only in its substrate specificity.

Another important implication of this work is the necessity of very cautious application of PMSF in biochemical studies of *Drosophila* esterases. This agent is most widely used as an inhibitor of proteases in various preparative and analytical manipulations with proteins and enzymes, in pariticular. As was shown here, the use of PMSF to inhibit protease activity during the purification of esterases of *Drosophila* and most probably of other insects may be misleading for the interpretation of the results.

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Time course study of the changes in blood glutathione induced by acute ethanol intoxication in the rat

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Summary. Acute ethanol treatment of rats (5 g/kg) has a biphasic effect on the glutathione content of the erythrocyte. After 3 h of intoxication there is a diminution in total GSH equivalents, followed by a recovery to basal values 6 h after treatment. The decrease of total GSH equivalents is mainly due to a diminution of the oxidized form of the tripeptide. Concomitantly a marked increase in the plasma level of glutathione was found at 3 h, followed by a diminution to values obtained at time zero.

Previous studies by our group[2,3] and others[4] have shown that both acute and chronic ethanol administration to rats were able to induce a marked diminution of the levels of reduced glutathione (GSH) in the liver, and a moderate decrease in kidney. Liver GSH depletion induced by acute ethanol ingestion is partially due to a conversion of GSH into glutathione disulfide (GSSG) in the tissue, which accounts for 20% of the total decrease in GSH[5]. In this condition, the biliary excretion of GSH and GSSG is decreased, while the levels of both metabolites were found to be enhanced in blood plasma[5]. This increase in the plasma content of glutathione induced by ethanol was suggested not to be a consequence of a release from the erythrocyte, since total GSH equivalents ($GSH+2\,GSSG=GSH_T$) in red blood cells were found to be comparable at zero and 6 h of intoxication[5]. Despite this observation, the glutathione status of the erythrocyte did seem to be altered by acute ethanol ingestion, as its GSH content was elevated and that of GSSG was reduced after 6 h of treatment[5]. This indicates a change in the redox state of the glutathione couple, the GSH/GSSG ratio being markedly enhanced[5]. The studies presented here were undertaken in order to clarify the changes induced by acute ethanol ingestion in the glutathione status of the erythrocyte during the period of intoxication of 6 h, for comparison with liver and kidney[2,3]. The time courses of changes in GSH and GSSG levels in plasma and whole blood were also determined under the same conditions.

Materials and methods. Male Wistar rats (División de Ciencias Médicas Occidente, Facultad de Medicina, Universidad de Chile) weighing 150-200 g were fasted overnight (16 h) prior to the treatment. Animals receiving ethanol were intubated with 5 g of ethanol/kg b.wt as a 40% w/v solution in saline. Control animals received isocaloric amounts of glucose 8.75 g/kg b.wt p.o. as a 50% w/v solution. All rats were kept in a warm environment (25-28 °C) during the period of intoxication. The time course study of changes in erythrocyte, plasma and whole blood GSH and GSSG was carried out 1, 2, 3, 4, 5, and 6 h after treatment, on blood samples obtained by cardiac puncture with heparinized syringes, from animals under light ether anesthesia.

For determination of glutathione in plasma and erythrocytes, the blood samples were centrifuged at 2500×g for 10 min at 4 °C. Erythrocytes were washed once with cold 154 mM NaCl and then resuspended in a 1:1 ratio with 154 mM NaCl. Plasma, erythrocyte and whole blood samples were deproteinized with 1 N $HClO_4$ and neutralized with 1.75 M K_3PO_4 prior to the determination of GSH and GSSG as described by Bernt and Bergmeyer[6]. Proteins were determined according to Lowry et al.[7], and hemoglobin was measured as described by Drabkin and Austin[8]. All

Changes in the content of total GSH equivalents (GSH_T) in red blood cells and whole blood from rats given a single dose of ethanol

Period of treatment	0 h GSH_T (nmoles/mg hemoglobin)[a]	3 h	Effect (%)	p
Whole blood	8.11±0.63 (11)[b]	10.09±0.37 (4)	+24.4	<0.02
Red blood cells	7.51±0.30 (12)	5.56±0.41 (10)	−25.8	<0.002

[a] GSH and GSSG were determined enzymatically in erythrocytes and whole blood samples from rats given 5 g of ethanol/kg, and were expressed as total GSH equivalents ($GSH_T=GSH+2\,GSSG$). [b] Number of animals used.