Wicker, C. and J.R. David. National Institute of Applied Sciences (INSA) and University Claude Bernard, Villeurbanne, France. Preliminary characterization of a β-N-acetyl-glucosaminidase from D. melanogaster adults.

Numerous enzymatic activities have been studied in Drosophila (Dickinson and Sullivan, 1975) but, up to now, a single work has been devoted to the analysis of an enzyme capable of using the artificial substrate paranitrophenyl-N-β-D-glucosaminide (PNAG). This enzyme was extracted from D. hydei larvae and considered as a chitobiase (E.C. 3.2.1.29) which, associated with a chitinase, seems to be involved in apolysis during the moulting cycle (Spindler, 1976). We report here a similar activity found in D. melanogaster adults but the responsible enzyme seems better described as a β-N-acetyl-glucosaminidase (E.C. 3.2.1.30).

Drosophila adults from a French population (Villeurbanne) aged 4-6 days and fed with a killed yeast medium were homogenized in 0.1 M citrate buffer (pH 5.0) and then centrifuged. The supernatant crude extract was used in this work. 0.4 ml of the enzymatic extract was added to 0.2 ml of 1.5mM PNAG. After incubating 30 minutes at 38°C, the reaction was stopped and the color developed from the released nitrophenol by adding 0.12 ml of a 1 mM solution of NaOH. The optical density was measured at 410 nm. Results are given in Figs. 1, 2 and 3.

Effects of pH were studied in the range 3.5-6.5. The response curve (Fig. 1) was very symmetric with a single optimum at 5.0 and a slow decrease toward higher values. For temperature (Fig. 2) the optimum was found at about 38°C. Inhibition of the enzymatic activity by an excess of substrate was observed (Fig. 3), the effect being more pronounced when a more concentrated enzymatic preparation was used. With low concentrations of the substrate (5 repetitions for each measure) a linear regression between 1/V and 1/S (Lineweaver and Burk plot) was observed and the Michaelis constant (Km) estimated at 0.73 mM.

Finally, it seemed interesting to characterize the enzymatic activity per fly or per mg of fresh weight. Averages of 30 measures gave the following values, expressed in nM of released p.nitrophenol per minute with a 0.5 mM substrate concentration. Two flies were homogenized in 1 ml of buffer, pH 5.0 and incubated 15 minutes at 38°.

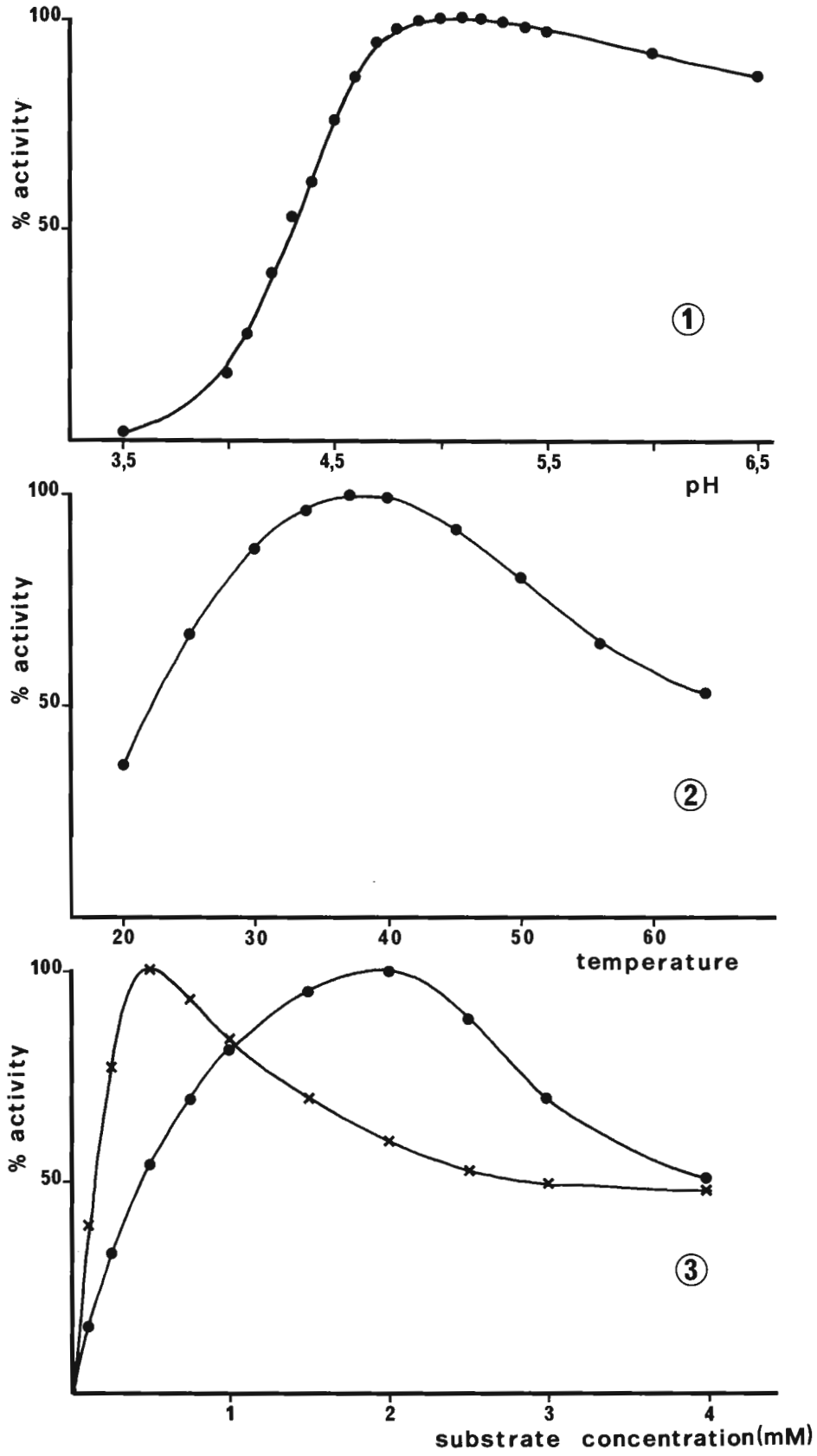
mg of fresh weight: 2.00 ± 0.13 units
individual fly: 2.28 ± 0.18 units

Acrylamide disc electrophoresis using a tris-glycine buffer pH 8.2 and naphtol-ASB-N-acetyl-glucosaminide for staining the gel showed a single enzymatic band, thus suggesting that one enzyme only was involved in this study.

The main problem arising from the above data is to decide if the enzyme of D. melanogaster adults could be homologous to the chitobiase described by Spindler from larvae of D. hydei. Obviously this question deserves further investigation. Some indications, however, exist that the two enzymes are different. First, the pH optima are not the same: 5.0 versus 5.5-6.2 in D. hydei. The temperature optimum found in D. melanogaster was 38°C against 50° in D. hydei. Finally, the Km values for PNAG are very different: 0.73 versus 5.7 mM.

The existence of two different enzymes having an N-acetyl-glucosaminidase activity has been demonstrated in silkworm larvae by Kimura (1977): one is found in the moulting fluid and seems to be involved in apolysis; the second is most abundant in the hemolymph and its function is unknown. In the case of Drosophila, the enzyme here described in adults cannot be involved in apolysis. One possibility is that the larval and imaginal enzymes are not the same, being produced by different genes and having different functions. In that case we have to explain why the D. hydei larval enzyme is so much different from the adult D. melanogaster enzyme. A study of the larval enzyme of D. melanogaster is now in progress to try to answer these questions.

References: Dickinson, W.J. and D.T. Sullivan 1975, Gene enzyme systems in Drosophila, Springer, 163 p.; Spindler, K.D. 1976, Insect Biochem. 6:663-667; Kimura, S. 1977, Insect Biochem. 7:237-245.



Figures 1, 2 and 3: Variation of enzymatic activity in relation to pH (1), temperature (2) and substrate concentration (3). In this last case, two enzymatic preparations were used: x: low concentration, 1 fly per ml; o: high concentration, 4 flies per ml.