

Cole, Thomas A. Wabash College, Crawfordsville, Indiana. Alcohol dehydrogenases in the pupae of *D. melanogaster* (Oregon R-C).

Alcohol dehydrogenases of carefully aged pupae of *Drosophila melanogaster* (Oregon R-C) were studied by polyacrylamide gel electrophoresis in small tubes (disc electrophoresis). This method reveals

that alcohol dehydrogenase is a highly polymorphic enzyme. A total of fourteen different types have been detected. Nine different bands have been identified with eight bands being the largest number in any one type and two being the smallest number. This system in Oregon R-C, when studied with disc electrophoresis, appears to be more complex than the alcohol dehydrogenase system in other strains as reported in the literature. (Supported by Research Grant GM-11860, United States Public Health Service)

MacIntyre, Ross J. and M. R. Dean. Cornell University, Ithaca, New York. In vitro dissociation and reconstitution of acid phosphatase-1 from *D. melanogaster*.

Zymograms of heterozygotes containing alleles specifying acid phosphatase-1 (Acph-1) molecules with different electrophoretic mobilities show three bands or zones of activity (MacIntyre, Genetics 53: 461). This probably means that the enzyme,

in its active form, is at least a dimer. However, in order to verify the multimeric nature of this enzyme and to obtain some information about the bonding between the polypeptide subunits, we attempted to reversibly dissociate the extracted enzyme. These attempts, which utilized a variety of known dissociating agents, were unsuccessful until the enzyme was partially purified by ammonium sulfate fractionation. The procedure is outlined in Figure 1.

Figure 1

1. Ten grams (wet weight) of flies homogenized in 50 ml 0.025 M Citrate-phosphate buffer, pH 7.0, with 0.5 g Norit A.
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2. Homogenate centrifuged 45 minutes (27,000 x g).
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3. Supernatant dialyzed 16 hours (4°C) against 0.025 M Citrate-phosphate buffer, pH 5.2, containing  $4 \times 10^{-4}$  M EDTA.
- ↓
4. Dialysate centrifuged 30 minutes (27,000 x g).
- ↓
5. Dry  $(\text{NH}_4)_2 \text{SO}_4$  added to supernatant to bring to 60% of saturation (calculated for 0°C). Stirred at 4°C for 30 minutes.
- ↓
6. Centrifuged 20 minutes (27,000 x g).
- ↓
7. Precipitate suspended in 20 ml of 0.025 M Citrate-phosphate buffer, 45% saturated with  $(\text{NH}_4)_2 \text{SO}_4$ . Stirred at 4°C for 1 hour.
- ↓
8. Centrifuged 20 minutes (27,000 x g).
- ↓
9. Supernatant dialyzed successively against distilled water for 4 hours and against 0.05 M Na Cl for 10 hours.

This results in about a 40 - 50 fold increase in the specific activity of acid phosphatase. Enzyme activity was measured during purification and in these experiments by the spectro-

photometric determination of *p*-nitrophenol released by the enzymatic hydrolysis of *p*-nitrophenyl phosphate at pH 5.0 (Bergmeyer, *Methods of Enzymatic Analysis*, p. 783). The ability of Acph-1 enzymes to hydrolyze this substrate was demonstrated by developing zymograms in stain solutions containing *p*-nitrophenyl phosphate,  $\text{Mg Cl}_2$ ,  $\text{Pb (NO}_3)_2$  and  $(\text{NH}_4)_2 \text{S}$  (Allen and Hyncick, *J. Histochem. Cytochem.* 11:169).

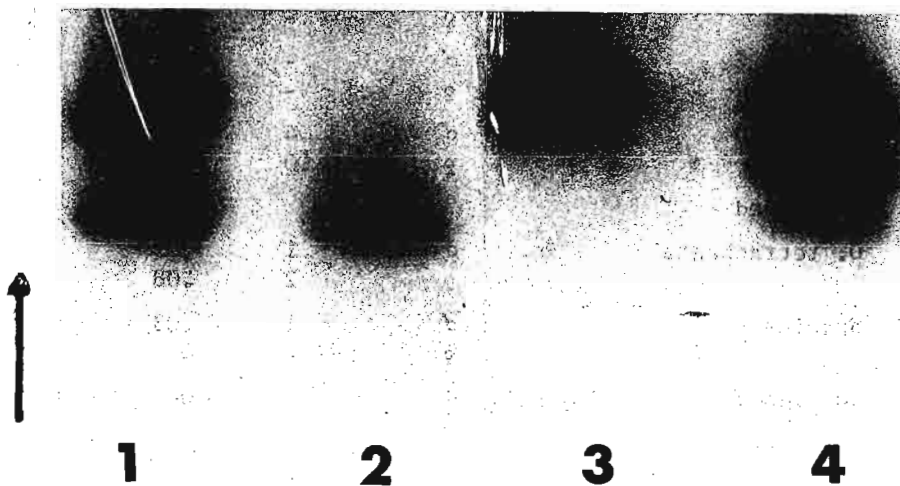
It was found that acid phosphatase activity of partially purified preparations from stocks monomorphic for the "slow" electrophoretic variant (Acph-1<sup>AA</sup>) decreases sharply between pH 2.9 and 2.5 when dilute HCl is added. No detectable activity was found in preparations below pH 2.5. On the other hand, the acid phosphatases in preparations from stocks monomorphic for the "fast" variant (Acph-1<sup>BB</sup>) are more susceptible to hydrogen ion concentration. Activity begins to decrease at pH 3.3 and is gone at pH 2.9.

When partially purified and acid-inactivated extracts are dialyzed against or diluted in various buffers between pH 6.0 and 7.5, some acid phosphatase activity can be regained. Tris-maleate at pH 6.5 seems to be the best buffer for the recovery of activity from acid-inactivated preparations. At best, however, only about 30% of the activity of the untreated control has been obtained.

If solutions of acid-inactivated extracts from stocks monomorphic for Acph-1<sup>AA</sup>, Acph-1<sup>BB</sup> and a mixture of the two are dialyzed (72 hours, 4°C) against Tris-maleate buffer at pH 6.5, concentrated and electrophoresed, the patterns shown in Figure 2 are obtained. This zymogram clearly shows that the electrophoretic mobilities of the reconstituted Acph-1<sup>AA</sup> and Acph-1<sup>BB</sup> enzymes do not differ from those of the untreated molecules. In addition and most significant is the appearance of the "hybrid" enzyme or Acph-1<sup>AB</sup> in the treated extract of a mixture of flies from stocks monomorphic for the two electrophoretic variants, Acph-1<sup>AA</sup> and Acph-1<sup>BB</sup>. It seems certain, then, that acid-inactivation of Acph-1 involves dissociation into inactive but intact polypeptide subunits, and, with the increase in pH, reactivation is brought about by the reaggregation of these subunits into enzymatically active molecules. Furthermore, since high hydrogen ion concentrations alone will dissociate Acph-1, there are probably no disulfide bonds holding the subunits together (Schachman, *Cold Spring Harbor Symp. Quant. Biol.* 28:409).

Figure 2

Zymogram of reconstituted Acph-1 enzymes. Slot 1 is an untreated, partially purified extract of a mixture of flies from stocks monomorphic for Acph-1<sup>AA</sup> and Acph-1<sup>BB</sup>. Slots 2 - 4 are inactivated, reconstituted and concentrated extracts of flies from stocks monomorphic for Acph-1<sup>AA</sup> (slot 2), Acph-1<sup>BB</sup> (slot 3) and Acph-1<sup>AA</sup> and Acph-1<sup>BB</sup> together (slot 4). Treatment consisted of the fol-



lowing steps. Ten ml of the partially purified extracts were taken to pH 2.4 by adding 0.1 N HCl. After 30 minutes at 0°C, the extracts were assayed to confirm the absence of acid phosphatase activity. They were then dialyzed at 4°C first for 72 hours against 0.1 M Tris-maleate buffer at pH 6.5 and then for 4 hours against the same buffer containing 15% (by weight) polyethylene glycol (carbowax 4000). The final volume of each extract was about 2 ml. The arrow indicates the direction of migration.