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.

↓

2. Homogenate centrifuged 45 minutes (27,000 x g).

↓

3. Supernatant dialyzed 16 hours (4°C) against 0.025 M Citrate-phosphate buffer, pH 5.2, containing 4×10^{-4} M EDTA.

↓

4. Dialysate centrifuged 30 minutes (27,000 x g).

↓

5. Dry $(NH_4)_2\ SO_4$ 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 $(NH_4)_2\ SO_4$. 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-