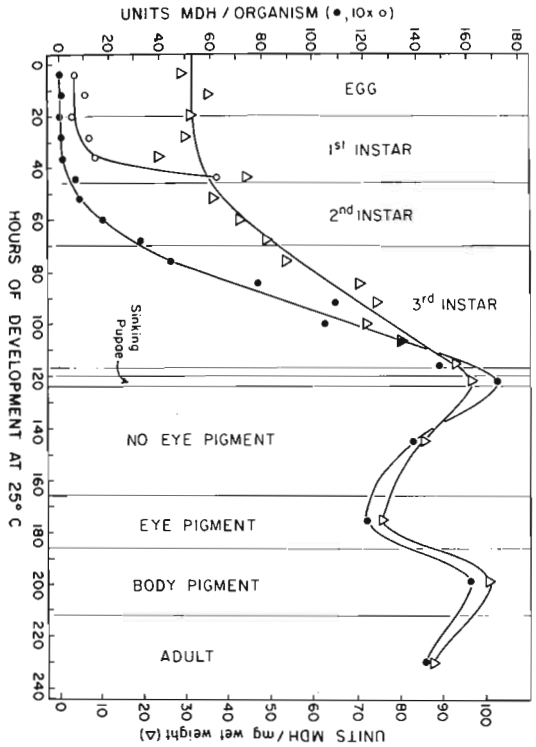
Anderson, M.* The Johns Hopkins University, Baltimore, Maryland. Variations in the level of malate dehydrogenase during development.

The variation in the level of malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37) during development has been studied in Oregon RCH flies. The techniques used to obtain eggs of known age, to raise the eggs and larvae, and to stage pupae, have been described (Rechsteiner, 1970). At various times, eggs and/or larvae were collected by washing them into fine mesh nylon net with distilled water. An aliquot was counted and weighed; the remainder of the organisms was weighed, and the number estimated from the weight of the counted sample. They were homogenized at 100 mg/ml in 0.2 M sodium phosphate, pH 6.4, 0.1% in phenylthiourea. The homogenates were centrifuged for 20 minutes at 17,000 rpm, and the MDH activity of the supernatant solution was immediately determined. For pupal points, pupae were washed from the walls of an established bottle, staged by morphological characteristics, and counted, weighed, homogenized, and assayed. The pupae were found to be significantly lighter than the oldest larvae, presumably because they were raised under more crowded conditions on the standard food. To correct for this in determining enzyme units/organism, the experimentally determined enzymatic activity for the pupal points was multiplied by the ratio of the weight/organism of the oldest larvae to that of the youngest pupae. That the difference in body weight is not merely a reflection of differences in body water, and that some sort of correction factor need be applied, is shown by the fact that enzyme activity/mg. wet weight and enzyme activity/mg. extracted protein were similar for the oldest larvae and youngest pupae, while enzyme activity/organism and wet weight/organism in the pupae were about half of the larval figure.

MDH was assayed at 30°C on a Zeiss monochromator equipped with a Gilford Absorbance Recorder. Two to 25 µl. of homogenate were added to 3 ml. of an assay mixture containing 16.7 mM malate, 2 mM NAD^+, 50 mM glycine, pH 10.0, and the initial rate of change in O.D. at 340 nm. was determined.



It is apparent that the level of MDH in Drosophila undergoes stage-specific variations during development which are regulated independently of total soluble protein. Other Drosophila dehydrogenases show variations which are similar, but not identical. The levels of MDH are approximately constant up to hatching. This has been observed in alcohol dehydrogenase (unpublished observations), isocitrate dehydrogenase (Fox, 1971), α-glycerolphosphate dehydrogenase, (Rechsteiner, 1970), and glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Wright and Shaw, 1970). However, β-hydroxybutyrate dehydrogenase (unpublished observations) and lactate dehydrogenase (Rechsteiner, 1970) show seven- and twenty-fold increases, respectively, in enzyme units per organism during embryogenesis. The drop in MDH activity per organism during metamorphosis (30%) is much less than that observed for alcohol dehydrogenase (500%), β-hydroxybutyrate dehydrogenase (900%) (unpublished observations), and α-glycerolphosphate dehydrogenase (300%, Rechsteiner, 1970), but similar to that seen in $NADP^+$-dependent isocitrate dehydrogenase (40%, Fox, 1971). The measured activity of MDH represents the sum of s-MDH and m-MDH. Separate measurement of the two during development would provide interesting data on the control of the levels of the forms of MDH. If the two are not regulated coordinately, this could bear on the significance of the existence of two forms of the enzyme. E.g., different cell types, or a single cell type at different times

in the life cycle of the cell or organism, could require a different balance between the mitochondrial and supernatant forms of the enzyme. Experiments in other systems (Kitto, 1967; Kitto and Lewis, 1967), including D. virilis (McReynolds and Kitto, 1970), indicate that antibodies to the two forms of MDH do not crossreact; hence immunologic techniques should allow one to measure the activities of the two forms separately during development.

References: Fox, D.J., 1971, Biochem. Genet. 5:69-80; Kitto, G.B., 1967, Biochem. Biophys. Acta 139:16-23. Kitto, G.B., and R.G. Lewis, 1967, Biochem. Biophys. Acta 139: 1-15; McReynolds, M.S., and G.B. Kitto, 1970, Biochem. Biophys. Acta 198:165-175; Rechsteiner, M.C., 1970, J. Ins. Physiol. 16:1179-1197; Wright, D.A., and C.R. Shaw, 1970, Biochem. Genet. 4:385-394.

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A method for the preparation on high molecular weight DNA from adult D. melanogaster.

Twenty-five grams of flies (wet weight) are ground with 120 ml of cold absolute methanol in an all-glass homogenizer in an ice bucket. The homogenate is centrifuged at 12,000 Xg for 10 minutes at 4° C, the supernatant discarded, and the pellet reground in 120 ml of a solution containing 0.15 M NaCl, 0.015 M sodium citrate, and 0.05 M EDTA at pH 7.0 (Solution No. 1). This is centrifuged as before and the pellet is ground again in 120 ml of Solution No. 1, followed by another centrifugation. After this centrfugation the pellet is suspended in 40 ml of 0.1 M NaCl, and 40 ml of 5% Aerosol O.T. in 0.1 M NaCl is added slowly with gentle stirring. The suspension is placed in a water bath at 50° C for one hour. It is then allowed to cool to room temperature and sufficient solid NaCl is added to raise the salt molarity to 1.0 M. After 10 minutes the preparation is centrifuged at 12,000 Xg for 10 minutes at 4° C. The precipitate is now discarded and one volume of cold 2-ethoxyethanol is added to the supernatant. This is placed in a freezer for 15 minutes, and is then centrifuged for 10 minutes at 10,000 Xg.

The precipitate is dissolved in 20 ml of 0.15 M NaCl, and is deproteinized by shaking vigorously for 10 minutes with an equal volume of a solution containing 24 parts chloroform to 1 of isoamyl alcohol. It is then briefly centrifuged to separate the phases, the aqueous phase is removed, and to the aqueous phase is added two volumes of cold ethanol. The DNA is now spooled out on a glass rod and dissolved in 9 ml of 0.015 M NaCl. When it is completely dissolved, 1 ml of 1.5 M NaCl is added.

Removal of contaminating RNA is now carried out by the addition of 1.0 ml of a 0.2% RNase solution (prepared in 0.1 M TRIS, pH 7.6, and heated for 10 minutes at 80° C to inactivate contaminating DNase). This is allowed to incubate in a 37° C water bath for one to three hours. After this, the solution is deproteinized as before. The DNA is then precipitated with either ethanol or 2-ethoxyethanol, deproteinized again, and precipitated once more with either ethanol or 2-ethoxyethanol. Following the last precipitation it is dissolved in 9 ml of 0.015 M NaCl + 1.0 ml 3.0 M NaAcetate containing 0.001 M EDTA, pH 7.0. It is then precipitated by the dropwise addition of 0.60 volumes of cold isopropanol. The DNA is spooled out and dissolved in 9 ml of 0.015 M NaCl + 1.0 ml 3.0 M NaAcetate containing 0.001 M EDTA, pH 7.0, and precipitated by the addition of 0.60 volumes of cold isopropanol. This final precipitate is dissolved in 10 ml of 0.15 M NaCl and is then typically chromatographed on a Sepharose-4B column, eluting with 0.15 M NaCl, collecting and pooling those fractions coming off the column immediately after the void volume.

Orcinol tests for RNA are negative. Melting point determinations show a Tm = 83.6° C with a hyperchromic effect of 40-45% in 0.15 M NaCl. $A_{260\ m\mu}/A_{280\ m\mu}$ = 1.8 - 2.0. Sedimentation equilibrium studies in CsCl show a single narrow main band at ρ = 1.702 with a small shoulder at ρ = 1.687. Prior to Sepharose-4B chromatography an additional smaller but wider band is present at ρ = 1.675. Yield has been as great as 2.5 mg DNA from twenty-five g of flies.

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