

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 centrifugation 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 $T_m = 83.6^\circ \text{C}$ with a hyperchromic effect of 40-45% in 0.15 M NaCl. $A_{260 \text{ m}\mu} / A_{280 \text{ m}\mu} = 1.8 - 2.0$. Sedimentation equilibrium studies in CsCl show a single narrow main band at $\rho = 1.702$ with a small shoulder at $\rho = 1.687$. Prior to Sepharose-4B chromatography an additional smaller but wider band is present at $\rho = 1.675$. Yield has been as great as 2.5 mg DNA from twenty-five g of flies.

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