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A rapid spectrophotometric assay of triglycerides in Drosophila.

For the purposes of a study of genetic variation in triglyceride storage, we needed a rapid assay sensitive enough to quantitate the triglycerides in a single fly. Most commonly the analysis of lipids begins with an extraction in organic solvents such as methanol-chloroform (Christie 1982). Solvent extraction is not practical for single fly measures, but the following protocol yields consistent and accurate determinations of triglycerides.

The procedure follows Bucolo & David (1973), and uses the Sigma Chemical Co. serum triglyceride kit 335. The reagent containts lipase to cleave the fatty acids from the triglycerides, glycerokinase and ATP to phosphorylate the resultant glycerol to glycerol-l-phosphate, pyruvate kinase and phosphoenol pyruvate to generate pyruvate, and finally it contains LDH and NADH to reduce the pyruvate to lactate. The end reaction is followed spectrophotometrically by measuring the change in absorbance at 340nm due to the oxidation of NADH to NAD+. Live flies are homogenized in 72-well tissue plates, with 200 ul distilled water per fly. The enzyme reagent is dissolved in 30 ml of distilled deionized water and 1.5ml is dispensed into a series of 10x75mm test tubes. To each tube is added 10ul of the homogenate, and the solution is briefly vortexed. The tubes are allowed to incubate for 1 hr at 25° and then the absorbance at 340nm is measured. Triglyceride standards containing 10, 25 and 50 ul of 1mg/ml solution are run in parallel. The effect of turbidity is negligible in such small samples, so clearing is not necessary.

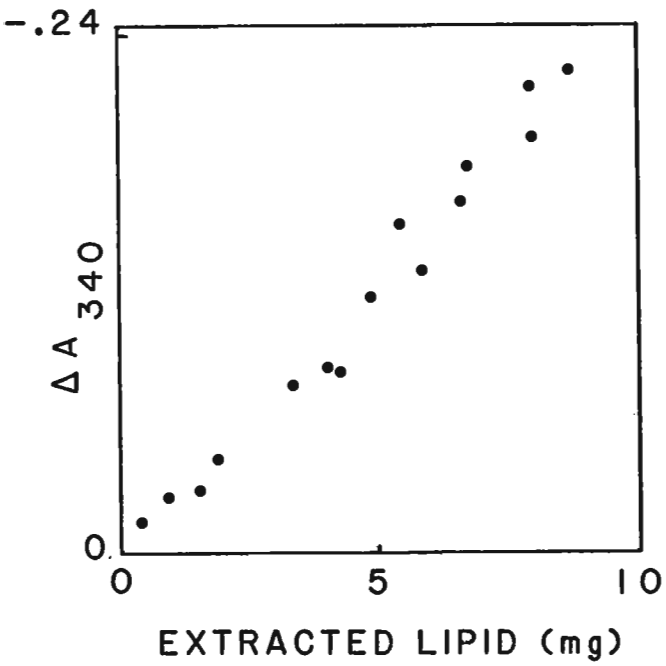

Figure 1. An analysis of lipid contents in 15 split samples of Drosophila by methanol-chloroform extraction and by an enzymatic procedure.

The triglyceride assay procedure was developed for serum rather than whole-organisms, and its accuracy must be tested in this context. Variation in quantitites of free glycerol, PEP, pyruvate or NADH would affect the apparent measure of triglycerides, so the accuracy of the method depends on the relative quantities of these residual substrates and triglycerides. A test of this was done by splitting a series of Drosophila samples and analyzing them both for triglycerides (using the above procedure) and for total lipids (using a methanol-chloroform extraction). Samples of 10 to 150 flies were used for the extractions, and lipids were quantified by the difference in dry weights before and after extraction. The extraction measures total lipids rather than just triglycerides, but in this homogeneous sample of flies, triglycerides represent a constant proportion of total lipids. Figure 1 clearly indicates that the two procedures give comparable results, and the correlation coefficient among these data is 0.97. The enzymatic test is very linear over the range from 0.5 to 40ug of triglyceride, with a coefficient of variation of 3.5%. An excellent correspondence is also seen between the enzymatic procedure and quantitation of triglycerides by TLC followed by sulfuric acid charring (Marsh & Weinstein 1966). The influence of endogenous NADH and substrates was tested by adding 10ul of fly homogenate to a series of buffers with OmM, 0.5mM, .25mM and .5mM NADH and following the change in absorbance. In no case was the change greater than 0.003, suggesting that the endogenous products do not grossly distort results. In conclusion, the enzymatic procedure appears to be a fast and accurate means of quantitating triglycerides in individual Drosophila.

References: Bucolo, G. & H. David 1973, Clin. Chem. 19:476; Christie, W.W. 1982, in: Lipid Analysis: Isolation, Separation Identification and Structural Analysis of Lipids, 2nd ed., Pergamon Press, Oxford; Marsh, J.B. & D.B. Weinstein 1966, J. Lipid Res. 7:574-576.