Baumann, J.L. and W.L. Bischoff. University of Toledo, Toledo, Ohio. A rapid reliable assay for glucose and fructose specific hexokinases in crude extracts of D. melanogaster.

The initiation of a series of experiments designed to cytologically localize the hexokinase loci in D. melanogaster necessitated the development of a satisfactory spectrophotometric assay for this system of enzymes. Published methods designed for use with various mammalian systems proved to be unreliable due to a lack of linearity attributable primarily to inappropriate substrate concentrations and pH optima. These problems have been overcome in our laboratory through the use of the assay procedure described below.

A. Preparation of extracts: Three to ten adult males or females aged 5±1 day post-emergence are ground in 2 ml glass microhomogenizers containing 0.05 ml of 0.03 M tris-HCl buffer at pH 8.5/fly. Homogenates thus prepared are centrifuged at 12,000 xg in the cold for 20 minutes, the supernatant fraction serving as a source of enzyme. Assays were performed within three hours of homogenization. Storage of extracts even at -70°C results in a total loss of activity after 48 hours.

B. Assay: 0.56 ml of a reaction mixture composed of 0.015 M glucose or fructose, 0.02 M $MgCl_2 \cdot 6H_2O$, 0.00013 M NADP, 0.00001 M EDTA (disodium salt) and 0.12 units of glucose-6-phosphate dehydrogenase in 0.02 M tris-HCl at pH 8.5 is mixed with 0.02 ml of the above enzyme extract in a 0.75 ml Helma quartz cuvette and gently agitated. After 1 minute 0.02 ml of 0.03 M ATP is added to initiate the reaction. To assay fructose phosphorylating activity 0.12 units of phosphoglucose isomerase are added to an otherwise identical reaction mixture. Reference cuvettes contain reaction mixture, enzyme, and 0.02 ml of tris-HCl buffer in place of the ATP. The reduction of NADP is monitored at 340 NM, and under the above condition is proportional to enzyme concentration. This assay is linear for at least 30 minutes and has been successfully utilized for the cytological localization of two hexokinase loci. The results of these studies will be reported elsewhere.

Boulétreau, M. and P. Fouillet. University of Lyon, Villeurbanne, France. An accurate and reliable olfactometer.

A new olfactometer was developed in order to measure the behavioral response of flies to various odoriferous substances. The greatest concern was to allow flies to move and fly freely in a sufficient space during the experiment, to prevent the olfactory cue from interfering with other directional signals such as light, drafts or gravity, and to provide an accurate control of the composition and the concentration of the odor to be tested. The device has two main originalities: the use of a large, well aerated cage in which adults can fly and exhibit normal behavior towards an odoriferous source; the use of a gas mixing pump (Wösthoff) which mixes gases in definite ratios and delivers mixtures of reliable composition.

The cages (Fig. 1, 6) are made up of clear plastic boxes (23 x 17 x 10 cm) with large gauze panels arranged on the top and the walls. The traps (Fig. 1) are fitted on the sides of the cages and can be renewed at given intervals without disturbing the flies. Traps are fed with gas mixtures to be tested. The whole arrangement is drawn in Fig. 1: atmospheric air is compressed with a diaphragm pump (1) and dried on silica gel columns (2). It is divided into two flows respectively saturated with vapors of substances A and B in convenient saturators (3) before reaching the pump (4). The pump mixes flows (a) and (b) in the required ratios and delivers two mixing ratios M1 and M2 each of which feeds two traps. The flow in each line is regulated using a flowmeter (5) and a needle valve so as to provide 25 ml/min to each trap.

The reliability of the device was tested by measuring the response of a wild strain of D. melanogaster to various concentrations of ethanol. Flow (a) is first saturated with ethanol vapor by bubbling twice in 100% ethanol and then conveniently diluted with flow (b), which is kept pure. The pump delivers two concentrations of M1 and M2 of ethanol vapor which are expressed as a percentage of concentration in flow (a), which is considered as saturated. Standardized flies, 6 days old and starved for 24 hours on water + agar are put in lots of 500 (250 of each sex) in each cage. Each cage is fitted with only one trap, so that four tests are run simultaneously. The cages are 1.5 m below two 40 W fluorescent lamps, and are kept at 25° and 30% R.H.