Baird, M.B., H.V. Samis, H.R. Massie and R.J. Nicolosi. Masonic Medical Research Laboratory, Utica, New York. A method for the determination of catalase activity in individual Drosophila.

We have recently begun to study the regulation of catalase activity in Drosophila melanogaster. The assay procedure used for these studies[1] was essentially a slight modification of the spectrophotometric assay method which had been developed for homogenates derived from mammalian organs[2]. Because of the relatively low catalase activity in single flies, it was necessary to assay fly homogenates which contained large numbers (1000-150) of individual organisms in order to obtain reproducible data. However, it is advantageous to know enzyme levels of individual flies, especially in aging studies, where one may be interested in enzyme activity in the individual organism rather than mean values for a population. The purpose of this note is to describe a method for the assay of catalase activity in individual Drosophila.

Individual flies are collected on cold plates and placed in glass homogenizers containing 0.5 ml of a solution containing 0.1% Triton-X, which insures solubilization of all catalase activity[3], and 0.1% ethanol, which prevents formation of inactive catalase complex-II[4]. The flies are then homogenized with glass beads and rendered devoid of chitin as described elsewhere[5].

Fly whole homogenates were assayed for catalase activity by a slight modification of the polarographic technique described by Goldstein[6]. In this assay method, catalase activity is measured by determining the rate of oxygen production during the enzymatic decomposition of either sodium perborate or hydrogen peroxide. All assays were performed at 30°C with a YSI Model 53 Biological Oxygen Monitor, equipped with an externally thermostated circulation bath and Bausch and Lomb VOM 8 linear-log recorder. Three (3.0) ml of degassed substrate (0.033M sodium perborate in 0.05M sodium phosphate buffer, pH 7.0) were pipetted into each of the incubation chambers of the oxygen monitor. The chamber contents were allowed to equilibrate for 3 minutes with constant magnetic stirring. The Clark-type oxygen probes were then inserted into the chambers and the reaction was initiated by adding a suitable volume (25-50 µl) of a homogenate into the chambers through PE50 intramedic tubing (Adams) inserted into the overflow groove of the Lucite probe plunger. Oxygen production was recorded for 30 seconds. One unit of catalase activity is defined as that amount of catalase activity which releases 1.0 µM of O_2 per minute under these conditions[6]. Protein was determined on fly homogenates according to the method of Lowry et al.[7].

The results of assays of at least four individual flies on three separate occasions are shown in Table 1. There were no significant differences in mean enzyme activity in single Drosophila when assayed in three separate experiments. The results in Table 1 show that catalase activity per fly is about 13.4 units, and that there are approximately 90 units/mg protein in individual flies.

Table 1. Catalase activity in individual three-week old Drosophila males. One unit of activity is defined as that amount of catalase which releases one µM of O_2 per minute from 0.033M sodium perborate at 30°C.

Experiment #	units/fly $\bar{X}$ ± 1S.E.	units/mg protein $\bar{X}$ ± 1S.E.
1	13.599 ± 0.968	93.5 ± 2.4
2	13.553 ± 0.664	----------
3	13.210 ± 1.131	87.6 ± 3.7

These results indicate that the polarographic determination of catalase activity is applicable to the determination of activity in individual Drosophila. The results obtained indicate high reproducibility of catalase assays both with samples from single flies and between samples of different flies.

References: 1. Samis, H.V., M.B. Baird, and H.R. Massie 1972 J. Insect Physiol. (in press); 2. Price, V.E., W.R. Sterling, V.A. Tarantola, R.W. Hartley, Jr. and M. Rechcigl, Jr. 1962 J. Biol. Chem. 237:3468; 3. Adams, D.H. 1950 Brit. J. Cancer 4:183; 4. Adams, D. H. and E.A. Burgess 1959 Enzymologia 20:341; 5. Samis, H.V. and F.C. Erk 1969 DIS 44:132; 6 Goldstein, D.B. 1968 Anal. Biochem. 24:431; 7. Lowry, O.H., M.J. Rosebrough, A.L. Farr and R.J. Randall 1951 J. Biol. Chem. 193:265.