conversion factors may be readily calculated: acid-PO = 1.37×10^4; neutral-PO = 1.20×10^4; alkaline-PO = 1.23×10^4. Multiplying raw data of the assays (ΔA_{485} min^{-1}) by the above conversion factors gives the V_{max} PO activity of the 1 ml extract, in picokatals (pkat).

CONCLUSIONS: This assay procedure utilizes total soluble extracts of Drosophila tissue, without prior concentration or isolation of the PO isozymes. Thus, the measurements of enzyme activity cannot be assumed to reflect enzyme concentration alone, but may also be influenced by natural inhibitors and activators of peroxidases, present within the tissue homogenates. The method has the advantage of speed and yields reasonably reproducible results. We are currently analyzing the PO isozymes of Drosophila for developmental changes in activity, tissue distribution and functional significance.

References: Angermueller,S. & H.D.Fahimi 1981, Histochemistry 71:33-44; Frieden,C. 1979, Ann.Rev.Biochem. 48:471-489; Hochachka,P.W. & G.N.Somero 1973, Strategies of Biochemical Adaption, W.B.Saunders Co., Philadelphia; IUPAC-IUB Commission on Biochemical Nomenclature 1976, Handbook of Biochemistry and Molecular Biology, 3rd ed, V2, G.D.Fosman ed., CRC Press, Cleveland, Ohio 1976, pp.84-87.

Poole, J.H. and L.K.Dixon. University of Colorado at Denver, Colorado USNA. Drosophila peroxidases: III. Developmental profile of isozymes.

In this report, we present our findings on the expression of peroxidase (PO) isozyme activity over the lifespan of Drosophila melanogaster.

METHODS: Wildtype stocks were maintained on cornmeal-molasses-agar-yeast medium in half-pint bottles, with a diurnal cycle of 12 hr in the light at 30°C and 12 hr in the dark at 20°C. Imagoes were collected at eclosure and transferred weekly to fresh medium, thereby maintaining distinct age-cohorts. For assay, third instar larvae were collected while wandering outside the medium prior to pre-pupal immobilization. Pupae were collected after completion of body segmentation and wing-bud/leg eversion but prior to initiation of eye pigmentation. Imagoes were collected for assay at eclosure ("week 0") and at weekly intervals thereafter. For each assay 6-10 organisms of a given age and sex were frozen, weighed, homogenized in buffer, centrifuged and photomerically assayed with PDA/H_2O_2 as previously described (see Report II). In addition, protein content of the supernatant was assayed by UV absorbance at 260 and 280 nm (Layne 1957). Three independent extractions and assays were performed for each PO isozyme, at each age and sex (except for the last two weeks of life, when sufficient numbers of organisms survived to perform only 2 independent determinations). Larvae and pupae were not sexed for this series of assays. PO activity is reported in picokatals (picomoles H_2O_2 reduced per second) at V_{max}, per mg body mass (see previous note for conversion factors).

PO ISOZYME ACTIVITIES DURING DEVELOPMENT: The activity of each PO isozyme across the lifespan of wildtype D. melanogaster is shown in Figure 1. Each data point represents the mean and standard error of the mean for PO activity at a given age.

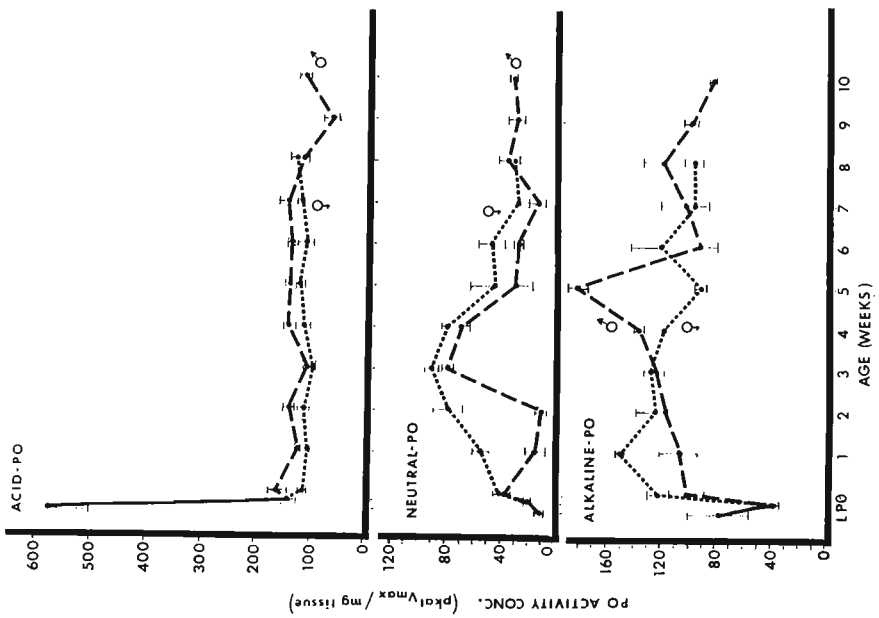

Fig. 1. Activity concentration of peroxidase isozymes vs age (wildtype females and males).

The three isozymes were quite distinct from one another in their developmental expression. Acid-PO showed maximum activity in the pre-pupating larvae. The activity of this isozyme dropped about 80% in pupae and remained low in both sexes for the remaining lifespan. In contrast, neutral-PO activity was quite low in the pre-adult stages, peaked at age 3 weeks in both sexes and then declined by about 60% with age. Alkaline-PO activity was lowest in the pupae. This isozyme appeared to peak briefly (age 1 week in females, age 5 weeks in males), but otherwise remained at relatively constant levels during adult life.

Specific activity (PO activity per mg protein) was also determined for each isozyme. This index of enzyme activity places two of the peaks at slightly later ages than were indicated by activity per mg body mass (neutral-PO in males at age 4 weeks, alkaline-PO in females at age 3 weeks). Otherwise the indices are in general agreement and portray similar developmental trends in the isozyme activity levels.

Armstrong et al. (1978) also measured the PO activity at pH 7.4 (corresponding to neutral-PO) across the lifespan of D.melanogaster. The developmental profile in that study was quite similar to that of neutral-PO in the present study. Armstrong et al. found peak neutral-PO activity at two weeks of age in female imagoes and at three weeks of age in male imagoes. The isozymes activity also declined precipitously with age, as in the present study. It appears that the developmental profile of at least this isozyme is quite reproducible.

We are currently analyzing the functional properties of each PO isozyme and the possible significance of these patterns of isozyme expression during Drosophila development and aging.

References: Armstrong,D., R.Rinehart, L.Dixon, D.Reigh 1978, Age 1:8-12; Layne,E. 1957, Methods in Enzymology, V3, S.P.Colowick & N.O.Kaplan (eds), Academic Press, New York, pp. 451-454.

Preston, C.R. and W.R.Engels. University of Wisconsin, Madison USNA. Movement of P elements within a P strain.

Wild strains of Drosophila melanogaster carry a family of movable genetic elements known as P elements. These elements are known to transpose at a high rate when crossed into laboratory, (M), strains having the condition known as M cytotype (Engels 1983). Though P element activity within a P strain (flies with P cytotype and P elements) is much reduced, the observations reported here demonstrate that transposition and excision still occur at significant and measurable frequencies.

By in situ hybridization of polytene chromosomes, we have seen changes in P element locations in branches of a P strain after the lines had been maintained separately for many generations. These changes presumably represent transpositional activity of P elements within P strains.

Π_2, a wild caught P strain from a Madison population was maintained as described previously (Engels & Preston 1979) through twelve generations of full-sib matings and five subsequent bottle stock generations. At that time, a single pair mating produced the adults that were branched into ten single female lines (lines a-j, see Figure). These branches were kept at room temperature (21°C) by single female matings for three generations and small mass (vial) generations thereafter. At approximately the same time, two more pairs were branched and subsequently maintained at a higher (28°C) and a lower (18°C) temperature in quarter-pint bottle populations. Π_2 20c is yet another line that was branched from the $\Pi_2 c_1$ line at generation 52 and maintained independently for twenty generations as a bottle stock.

After 77-80 generations for the room temperature and 28° lines and 47 generations for the 18° line, larvae were selected for in situ hybridization to P element sequences. The results (see Figure) show the variation of P element positions on the X chromosome within these lines. The number of larvae analyzed per branch line is shown to the right of each chromosome. In cases where more than one larva was examined, there was a possibility of detecting polymorphism (circled points) within the line. We looked at more slides of the "hot" and "cold" lines expecting more variability because of the larger population size. This proved to be especially true in the 28° line where each of the twelve P element sites was missing in at least one case.

Because P element DNA probes were unavailable when the Π_2 strain was isolated in 1977, P element sites of the original line at that time are unknown. However, because these branches all originated from a highly inbred stock, the observed differences must represent