Fuller, C.W. and E.W. Hanly. University of Utah, Salt Lake City, Utah. Glutamine synthetase activity in D. melanogaster.

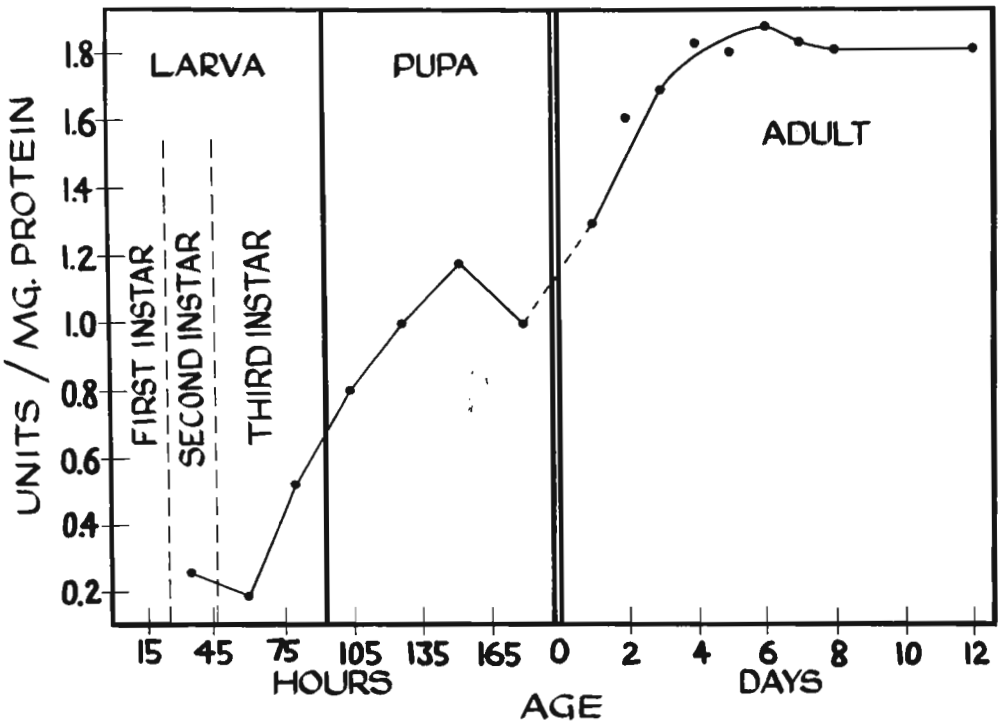
A colorimetric procedure for determining glutamine synthetase activity in various developmental stages of D. melanogaster was established. This involved the modification of a procedure originated by Lipmann and Tuttle (1945) which used a $FeCl_3$-hydroxamate color complex resulting from the following reaction:

$$\text{L-glutamate} + NH_2OH + ATP \xrightarrow{Mg^{++}} \text{L-GHA} + ADP + Pi$$

where GHA is γ-glutamyl hydroxamate which is then complexed with $FeCl_3$.

The assays were done on whole-fly extracts at various developmental stages where the enzyme source was a crude extract prepared in the following manner: 1) One gm frozen flies of various developmental ages was ground in a glass tissue grinder with 10 ml imidazole buffer, pH 7.3 or 10 ml distilled water. 2) Homogenate was centrifuged at 43,600 x g for 20 min. 3) Supernatant stirred with 0.5 gm Norite A for 10 min. 4) Mixture centrifuged at 43,600 x g for 20 min. 5) Supernatant used as enzyme source. All procedures were carried out at 4°C. Pellets of centrifugation steps had no activity.

The routine assay was done at 37°C for 20 min. in an incubation mixture of 2.25 ml containing 25 μmoles sodium ATP (freshly prepared daily), 250 μmoles sodium L-glutamate, 100 μmoles NH_2OH, 100 μmoles $MgSO_4$, 100 μmoles 2-mercaptoethanol, 375 μmoles imidazole buffer and 0.2 ml enzyme extract. These concentrations were determined to be optimum under the conditions used. All components were neutralized to pH 7.3. After the final addition of ATP, the reaction mixture was equilibrated to 37°C for 3 min. before the addition of enzyme extract. The reaction, after 20 min., was terminated by the addition of the $FeCl_3$ reagent (containing HCl and trichloroacetic acid). The tubes were thoroughly shaken and then spun for 15-20 min. in a clinical centrifuge to remove the precipitated protein. The supernatant was removed and absorbance measured at 500 mμ which was determined to be λ_{max}. Protein concentration was determined by the method of Lowry et al. (1951).



Results are reported as GSA (glutamine synthetase) specific activity (units/mg. protein, where one unit is equal to one μmole GHA formed per hour).

The temperature optimum for this reaction was found to be 41°C. The amount of GHA formed was linear with the amount of enzyme and the amount of each substrate at low concentrations of each. The K_m for L-glutamate was found to be about 1×10^{-2} M; for hydroxamate about 6×10^{-4} M.

Whole wild-type (Oregon-R) were used as source of enzyme extracts for larvae, pupae and adults of various ages. The figure shows that there is a slight dip in enzyme activity or concentration (measured on specific activity basis) at approximately 60 hours after hatching and again just prior to emergence from the puparium at 180 hours. These dips are reproducible. There is a slight break in continuity of the curve at emergence but only minor. The activity of the adult increases to a maximum at approximately 4-5 days of age. The slight drop in activity at about day 7 of the adult may not be real, although it occurs in every assay.

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Mutation rates at the loci controling esterase activity of D. virilis.

Recent studies on electrophoresis of single flies have made it possible to know the genetic variabilities of enzymes and their selection mechanisms in populations. Such studies were performed with some Drosophila species and indicated that there were considerable amounts of enzyme variations in most natural populations of Drosophila. No one knows, however, at the present time the exact nature of the mechanisms through which the genetic variations have been maintained in the populations. One of the possible mechanisms, suggested by Lewontin and Hubby (1966), is that selection tends to eliminate alternative alleles but mutation restores them. In order to accept this hypothesis it is necessary to assume the extraordinary high mutation rates or very, very weak selection on the average. The purpose of the present study is to estimate the X-ray induced mutation rates for esterase alleles of D. virilis and to see whether or not the mutation rates are much higher for the esterase alleles than for visible or dysgenic alleles, such as recessive lethals.

The present study consists of two experiments; in Experiment-I the mutation rate from "inactive" to "active" is estimated, while in Experiment-II the reverse is done.

In Experiment-I, male flies of D. virilis taken from "null" strain, which was homozygous for the silent allele at all loci concerned, and therefore had no esterase band, were irradiated with 2,000r of X-rays and thereafter mated with the homozygous females from the same "null" strain. The progenies emerging in the next generation were examined by thin layer agar electrophoresis.

In this experiment the total number of flies examined was 9,372; no mutation was observed at all esterase loci except for Est-2 locus. At this locus we detected 2 mutations from Est-2^O to Est-2^B, where Est-2^O was a silent gene producing no esterase band. This mutation rate was estimated to be 1.05×10^{-7}/r.

In Experiment-II, males homozygous for both the Est-2^B and Est-9 were exposed to 2,000r of X-rays. Immediately after irradiation they were crossed to the females taken from the "null" strain used in Experiment-I. In the next generation F_1 flies heterozygous for "null" and Est-2^B.9 were examined by the thin layer agar electrophoresis.

A total of 14,020 flies were examined in this experiment. At the Est-2 locus 2 mutations from Est-2^B to Est-2^O, and one from Est-2^B to Est-2^D were detected. At Est-9 locus, 7 mutations to "null" were found. The mutation rates were 0.72×10^{-7}/r, 0.36×10^{-7}/r and 2.50×10^{-7}/r, respectively, for Est-$2^B \rightarrow$Est-2^O, Est-$2^B \rightarrow$Est-2^D and Est-9 $\rightarrow$"null". No mutation from the Est-9 band to another esterase band was found. Furthermore, we found 27 cases showing that both of Est-2^B and Est-9 genes mutated together to "null" genes. In this case, it is not obvious that this event is responsible for either point mutation or chromosomal aberration.

Demerec (1934) has reported the mutation rate to be 5.2×10^{-8}/r on the average at 9 loci on the autosome of D. melanogaster. The same order of the mutation rate has been presented by Alexander (1954), i.e., 1.5×10^{-8}/r. At white locus which is located on the X-chromosome of D. melanogaster, Bonnier and Luning (1949) has estimated the mutation rate to be $0.8\sim1.2 \times 10^{-7}$/r. Girvin (1949) has estimated it to be 7.6×10^{-8}/r on the average at 7 visible loci on the sex-chromosome of D. virilis.

Comparing the results obtained in this study with those mentioned above, it seems very unlikely that the X-ray induced mutation rate at isozyme loci is considerably higher than those at visible loci. However, it cannot be determined from the present results that the genes controlling isozyme activity have either an extremely high mutation rate or a very low selective value because of the small number of chromosomes examined. Further studies should be done to accumulate data on this problem.

(The nomenclature of the esterase loci used in this study has been made by Ohba (1968), see Proc. XII Inter. Congr. Genet., Vol. II: 156.)