There is evidence suggesting that Class II seedlings contain a "modifier" which rapidly reacts with catechol oxidase during extraction, so that the enzyme no longer migrates in electrophoresis. The existence of a non-migrating enzyme in extracts of untreated Class II seedlings can be demonstrated by electrophoresis on cellulose acetate strips. Zymograms from these strips show a band of catechol oxidase activity remaining at the origin.

The amount of "modifier" present in untreated Class II seedlings is limited, but sufficient to prevent the appearance of the cathodally migrating band. Thus, when shoots from Class II seedlings carrying the $\underline{Cx}^{F}$ allele are homogenized in the presence of increasing concentrations of S enzyme extract (partially purified on a sephadex column), the F enzyme band is eventually revealed in electrophoresis.

We suggest that the difference between Class I and Class II lines is due to the amount of "modifier" present. The amount of "modifier" is sufficient in Class II lines to inhibit the migration of all of the catechol oxidase, but insufficient in Class I, thus allowing the appearance of an enzyme band in zymograms from untreated seedlings. On this basis, the treatment of seeds with maleic hydrazide in some way affects the amount or availability of "modifier" in shoots, so that the migration of enzyme in Class II seedlings is not affected and extracts give rise to a cathodal band in electrophoresis.

Tony Pryor

2. Substrate inhibition of allelic isozymes of alcohol dehydrogenase in maize.

Differences in the pH optima of the enzyme forms specified by the $\underline{Adh}_1^{C(m)}$ and $\underline{Adh}_1^{S}$ alleles were reported previously (Schwartz & Laughner, Maize News Letter 42:83, 1968). Further studies on the kinetic properties of these alcohol dehydrogenase isozymes have revealed striking differences in their inhibition by specific substrates.

Alcohol dehydrogenase (E.C. 1.1.1.1.) catalyzes the following reaction:

$$\text{Ethanol} + \text{NAD} \underset{\text{foward}}{\overset{\text{backward}}{\rightleftharpoons}} \text{Acetaldehyde} + \text{NADH}$$

Oxidation of ethanol to acetaldehyde is accompanied by reduction of NAD to NADH and the reverse. When the activity of specific isozymes at different substrate concentrations was determined in the back and forward reactions (by following the absorbance of NADH at 340mu), it was found (fig. 1) that the enzyme specified by the $\underline{Adh_1}^{C(m)}$ allele was inhibited by ethanol concentrations which were not inhibitory to the enzymes specified by the $\underline{Adh_1}^{F}$, $\underline{Adh_1}^{S}$, and $\underline{Adh_1}^{C(t)}$ alleles.

Similarly, the enzymes specified by the $\underline{Adh_1}^{F}$, $\underline{Adh_1}^{S}$, and $\underline{Adh_1}^{C(t)}$ alleles were inhibited by concentrations of acetaldehyde which were not inhibitory to the C^m enzyme. Although $ADH^{C(t)}$ and $ADH^{C(m)}$ show the same migration rate in starch gel electrophoresis, the two enzymes differ much more from each other than do the three electrophoretically distinguishable enzymes specified by the $\underline{Adh_1}^{F}$, $\underline{Adh_1}^{S}$, and $\underline{Adh_1}^{C(t)}$ alleles.

William Laughner

Figure 1. Relative activity (calculated by setting the activity at .01M substrate concentration as 1) vs substrate concentration.