dosage effect of C, it is difficult to detect regularly.

Experiments are currently underway in which c Bz ear parents will be crossed with C bz-x3m individuals to produce c Bz/c Bz/C bz-x3m offspring. The frequency of C-c variegation in these progeny will represent the frequency of breakage in chromosome 9. Studies are also in progress to detect chromosome breakage cytologically.

John P. Mottinger

UNIVERSITY OF SOUTH CAROLINA
Genetics Laboratory, Columbia, South Carolina
and
MSU/AEC PLANT RESEARCH LABORATORY
East Lansing, Michigan

Purification and properties of genetically determined malate dehydrogenase isozymes — A large number of inbred lines have been examined, and eight strain-specific malate dehydrogenase (MDH) zymogram patterns have been found. The general isozyme pattern does not vary spatially or temporally within any given inbred strain, though quantitative differences are found. However, of the total number of isozymes present in any inbred strain, some are associated with the soluble cytoplasm (cytosol), some with mitochondria and some with glyoxysomes. The latter were found to be present only in scutella, while NADP-dependent MDH was found in leaf chloroplasts.

In strain W64A two soluble MDH isozymes (s-MDH), five mitochondrial MDH isozymes (m-MDH) and one glyoxysomal MDH (g-MDH) were found in scutella of etiolated seedlings. Both s-MDHs and m-MDHs were precipitated in 50-65% saturated ammonium sulfate. Fractionation on Sephadex G-150 columns shows that, by test tube assay, s-MDHs and m-MDHs come off as a single peak and therefore should have similar molecular weights. Detailed analyses of the MDH zymograms of the "peak fractions" indicate that m-MDHs come off a bit earlier than do the s-MDHs. The MDH isozymes thus partially purified were applied to DEAE cellulose columns. The seven MDH isozymes were separated into three major peaks by a linear salt gradient into two s-MDHs (s-MDH-1 and s-MDH-2), the three most anodal m-MDHs and the two most cathodal m-MDHs. The three peaks were then pooled separately and concentrated. Through the above mentioned purification steps the MDH isozymes in each peak were purified 200-300 fold. Using these three groups of partially purified MDH isozymes, experiments with reducing agents (100 mM mercaptoethanol), low pH treatments (pH 2.0) and high salt concentration treatments (7.5 M guanidine-HCl), along with

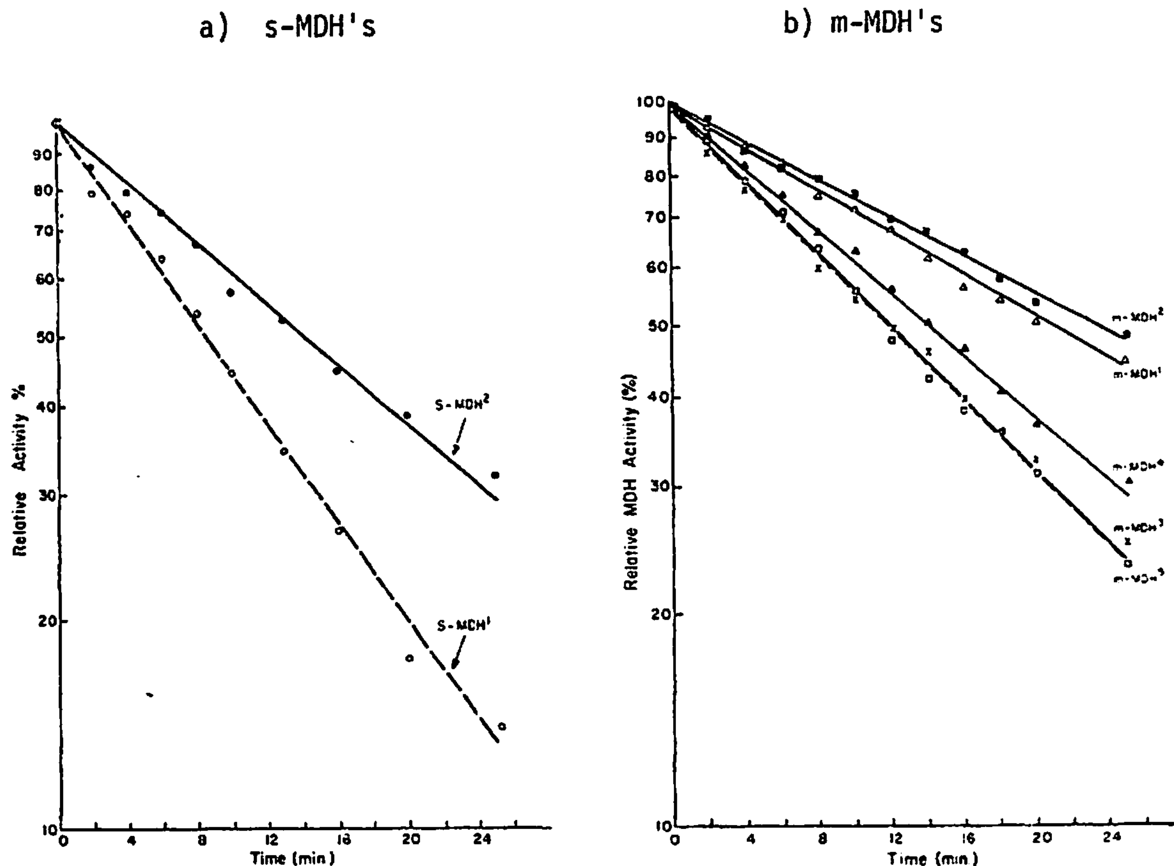
the genetic evidence so far obtained in our laboratory, have eliminated the possibility of conformational alterations to account for MDH multiplicity in maize. The isozymes of maize MDH are genetically determined. Molecular weight and isoelectric point determinations of the MDH isozymes were made on three highly concentrated MDH isozyme fractions. Using eight non-enzymatic protein molecular weight markers, gel filtration on Sephadex G-150 shows that s-MDHs and m-MDHs have molecular weights of 70,800 ± 1650 and 79,500 ± 1860, respectively. The pI for the different MDH isozymes ranges from pH 4.92 to pH 5.17.

The three MDH isozyme fractions were then subjected to starch gel electrophoresis; the MDH isozymes at the same peak on DEAE cellulose were thus separated clearly from one another. Each isozyme was then eluted from the gel by high-speed centrifugation. The seven isozymes were separated and highly purified in this manner; the biochemical properties of the seven forms were then examined.

It was found that both s-MDHs and the five m-MDHs have a pH optimum of about 8.5. Thermal inactivation curves indicate that the MDH isozymes vary in their thermolability (Figure 1). Michaelis constants (K_m) for OAA, malate, NAD^+ and

Fig. 1. Thermal inactivation of maize malate dehydrogenase isozymes at 53°C.

a) s-MDH's b) m-MDH's



and NADH of each isozyme were determined at pH 7.5, pH 8.5 and pH 9.5. In general the K_ms for OAA increase when pH increases, but the K_ms for OAA of the s-MDHs increase to a much greater extent than those of the m-MDHs. The K_ms for NADH are less dependent on pH. The K_ms for malate decrease when pH increases; however, the K_ms of m-MDHs are more affected by higher pH. For s-MDHs the K_ms for NAD^+ are almost independent of pH, but those of m-MDHs increase when pH increases. High concentrations of OAA (250 um) preferentially inhibit s-MDHs, but NAD^+ at high concentrations (500 um) seems to inhibit both s-MDHs and m-MDHs similarly. Both inhibitions are pH-dependent.

s-MDHs and m-MDHs are quite different in their capacity to use three NAD analogs as coenzymes (Table 1). Citric acid and isocitric acid inhibit only

Table 1. Catalytic activity of maize malate dehydrogenase isozymes in the presence of NAD or NAD analogs.

Coenzyme	Isozymes (Relative activity)*						
	s-MDH^1	s-MDH^2	m-MDH^1	m-MDH^2	m-MDH^3	m-MDH^4	m-MDH^5
NAD^+	100	100	100	100	100	100	100
Deam-NAD^+	54.6	44.8	20.7	28.5	24.4	26.5	21.1
3-Ap-NAD^+	96.9	100.6	188	200	216.8	192.3	182.8
TN-NAD^+	241	253	18.3	21.4	21.8	21.1	21.5

*The data are the percentage of the reaction rates relative to NAD^+. Spectrophotometric assays were conducted with 0.75 mM NAD^+ or analog at 5 mM malate, 0.025 M Glycylglycine buffer pH 8.5.

m-MDHs; however, cis-aconitic acid and alpha-ketoglutaric acid inhibit both s-MDHs and m-MDHs. Reducing agents, chelating agents and monovalent metal ions are not required for the enzymatic activities of either s-MDHs or m-MDHs, but divalent metal ions may enhance MDH activities, especially for m-MDHs.

N.S. Yang* and J.G. Scandalios

*Roche Institute of Molecular Biology
Nutley, N.J.

<u>On the regulation of alcohol dehydrogenase</u> — The activity of many enzymes increases sharply after germination of maize seeds. However, alcohol dehydrogenase (ADH; EC 1.1.1.1) activity in the scutellum declines rapidly during this developmental period, indicating that there might be a unique mechanism for the control of ADH activity. Two possible ways for this to occur are (1) faster degradation (or inactivation) than formation of active enzyme molecules or