the middle of the smear often show a more rapid and intense reaction than those at the end and sides. We believe that the speed and intensity of reaction depend upon physical phenomena related to the proximity of the sperm to the substrate.

In addition to rabbit spermatozoa, experiments have been carried out on ejaculated spermatozoa from humans and epididymal sperm from rats, mice, guinea pigs, and hamsters. There are considerable differences between species in the capacity of sperm acrosomes to react on the gelatin membranes. So far it appears that human sperm are the only ones comparable to rabbit sperm in the rate of reaction (Fig. 6). The human spermatozoon is much smaller than that of the rabbit [4.6 μm as opposed to 8.4 μm in head length (*6*)], but its "reaction circle" is proportional in size. The reaction is somewhat more rapid and intense at the higher *p*H's (7.5 to 9.5) than at lower ones, but this difference is not so clear as in the case of rabbit sperm. In their favorable *p*H range, human spermatozoa show a maximum reaction in less than 1 hour. Washing them twice in calcium-free Ringer solution and resuspending in Hanks solution did not significantly alter their capacity to depolymerize the membranes. Human seminal plasma from which the sperm had been removed showed a definite capacity to digest the gelatin.

When rat spermatozoa react on the membranes, the zone of activity is invariably confined to the convex surface of the head (Fig. 7). The reaction rarely occurs except at *p*H 8.55 to 9.5. It does not begin until at least 1 hour after the preparation is made and seldom achieves maximum intensity until about 24 hours. Mouse spermatozoa show a similar reaction (beginning on the convex aspect of the head), but the reaction is more rapid and occurs over a wider *p*H range compared to the reaction of rat sperm. In the hamster and the guinea pig strong reactions may occur also in 4 to 6 hours from *p*H 7.5 to 9.5 and only slight or moderate reactions below *p*H 7.5. The guinea pig spermatozoon displays a large reaction circle similar to that of the rabbit.

To our knowledge this is the first demonstration of a proteolytic enzyme in the individual mammalian spermatozoon. Reaction between the spermatozoon and gelatin membrane begins in the region of the acrosome in a manner which is species-specific.

The features of the enzyme noted so far (its proteolytic activity, high *p*H optimum, inhibition by a trypsin inhibitor, and localization in the acrosome) suggest that it may be the "trypsin-like" enzyme identified by others as the primary agent effecting dissolution of the zona pellucida (*2, 7*). If this is true, we have a useful method for studying the potential of different populations of spermatozoa to effect penetration of the zona.

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Alcohol Dehydrogenase in Maize: Genetic Control of Enzyme Activity

Abstract. *A gene* (Adh_r) *which controls the activity of alcohol dehydrogenase in the scutellum of maize has been found. This gene is not allelic to the* Adh_1 *locus, which specifies the charge of the enzyme molecule and hence its migration rate. The two genes are linked and located about 17 crossover units apart. The* Adh_r^N *allele specifies equal activities of both the* Adh_1^S *and* Adh_1^F *products. The* Adh_r^L *allele gives lower activities of the* Adh_1^S *products only and operates in both the* cis *and the* trans *configurations.*

Variation in enzyme activities between different inbred lines of maize has been demonstrated recently for a number of enzymes (*1–3*). Hybrids between lines with different activities usually showed intermediate activities (*1*). Unfortunately, these studies showed only an average activity of the products of the two alleles contributed by the two parental lines. This is probably the reason for the absence of information concerning the mode of inheritance of differences in enzyme activities. Allelic isozymes having different electrophoretic mobilities were used in the present study to investigate this problem.

Maize is polymorphic for the alcohol dehydrogenase (E.C.1.1.1.1) gene Adh_1. Four alleles have been described thus far (*4*): Adh_1^S, Adh_1^F, and $Adh_1^{C(t)}$, which specify enzymes that differ in charge; and $Adh_1^{C(m)}$, which produces an enzyme having the same electrophoretic mobility specified by $Adh_1^{C(t)}$ but is much less active. The enzyme is a dimer and three isozymes are formed in heterozygotes (*4*).

Two homozygous lines of maize were used: $N,Adh_1^F/N,Adh_1^F$, a line with normal activity of alcohol dehydrogenase (ADH) in the scutellum of the mature kernel (N) and with a fast migrating isozyme form (Adh_1^F); and $L,Adh_1^S/L,Adh_1^S$, a rare line with lower ADH activity (L) and slow migrating isozyme form (Adh_1^S). The difference in activity between the two lines is easily detected visually (Fig. 1A) after starch-gel electrophoresis and specific staining for ADH (*4*). In quantitative tests (*5*) the intensities of the ADH bands that were developed after 1 hour of staining were measured in 18 different gels from each line by a densitometer (*6*) fitted with a filter of 545 nm (Fig. 2A). Mean curve areas of 98.8 ± 13.6 mm^2 (100 percent) and 62.2 ± 14.7 mm^2 (63 percent) were found for the $N,Adh_1^F/N,Adh_1^F$ and the $L,Adh_1^S/L,Adh_1^S$ lines, respectively. The difference in activity as measured by the curve area between the two lines is significant ($\alpha = .001$). When the enzymes from the two lines were extracted from mature dry kernels and assayed spectrophotometrically as described (*7*), similar results were found. The $L,Adh_1^S/L,Adh_1^S$ line had 60.7 percent of the activity shown by the $N,Adh_1^F/N,Adh_1^F$ line.

As judged by electrophoretic mobility, three isozyme bands were found in Adh_1^F/Adh_1^S heterozygotes since the

Table 1. Segregation for the activities of the FF, FS, and SS isozyme bands in scutellum of Adh_1^F/Adh_1^S heterozygotes in F_1 and F_2 generations and in test cross families in crosses involving three lines of maize with different ADH activities. Activity distribution type I—expected activity distribution of 25 : 50 : 25 percent for the FF, FS, and SS isozyme bands, respectively; activity distribution type II—expected activity distribution of 37.7 : 47.4 : 14.9 percent for the FF, FS, and SS isozyme bands, respectively. Abbreviations are: F, Adh_1^F; S, Adh_1^S; L, low ADH activity; and N, normal ADH activity.

Cross	Seeds with activity distribution type I			Seeds with activity distribution type II		
	Number	Percent	FF : FS : SS mean ratio (%)	Number	Percent	FF : FS : SS mean ratio (%)
NF/NF × LS/LS	0	0.0		18	100	35.6 : 52.2 : 12.2
NF/NF × NS/NS	20	100.0	27.7 : 45.8 : 26.5	0	0.0	
NF/LS × NF/NF	13	17.1	25.2 : 50.4 : 24.8	63	82.9	35.4 : 49.6 : 15.0
NF/LS × LS/LS	0	0.0		65	100.0	34.1 : 50.0 : 15.9
NF/LS × NF/LS	16	14.0	25.7 : 50.3 : 24.0	98	86.0	36.8 : 48.4 : 14.8

enzyme is a dimer: the two autodimers FF and SS and the hybrid FS allodimer, which showed intermediate electrophoretic mobility. If we assume random dimerization of the F and S monomers into dimers and if equal numbers of F and S monomers are synthesized and both have equal potential activity, a ratio of 25:50:25 percent activity is expected for the FF, FS, and SS isozyme bands, respectively. However, if unequal numbers of F and S monomers are formed or if the F and S monomers have different potential activity, the ratio of the relative activity of the FF, FS, and SS isozyme bands will be changed accordingly. Thus, according to the activities found for the $N,Adh_1^F/N,Adh_1^F$ and $L,Adh_1^S/L,Adh_1^S$ parental lines, a ratio of 37.7:47.4:14.9 percent activity is expected for the FF, FS, and SS isozymes, respectively, in hybrids between the two lines.

In $N,Adh_1^F/L,Adh_1^S$ heterozygotes, the FF autodimer band stained more intensely than the SS autodimer (Fig. 1B). Quantitative densitometer measurements of zymograms of 18 such heterozygotes (Table 1) showed mean relative intensities of 35.6:52.2:12.2 percent for the FF, FS, and SS isozymes, respectively. The similarity between the observed and the expected ratios indicated that, in the hybrid, each of the parental factors retained its own activity. This was also indicated by the fact that in the hybrid the total activity was intermediate between that of the two parents (Table 2). When the $N,Adh_1^F/N,Adh_1^F$ parent was crossed with an $N,Adh_1^S/N,Adh_1^S$ line, both with the same activities, the resulting $N,Adh_1^F/N,Adh_1^S$ heterozygotes (Fig. 1C) had activities similar (Table 1) to the expected 25:50:25 percent activity for the FF, FS, and SS isozymes, respectively. Thus, both the assumption of random dimerization and the validity of the testing technique were confirmed.

To study the mode of inheritance of the differences in activities of the ADH enzyme, segregation tests in the F_2 and back-cross generations were performed on Adh_1^F/Adh_1^S heterozygotes only where the FF, FS, and SS isozymes were comparable in the same extract. In this way possible technical errors, leading to mistakes in classification, were minimized.

In the test cross $(N,Adh_1^F/L,Adh_1^S) \times (N,Adh_1^F/N,Adh_1^F)$ one-half of the progeny were expected to be Adh_1^F/Adh_1^S. If the charge of the enzyme molecules and the activities are specified by two different genes, two heterozygous Adh_1^F/Adh_1^S types are expected: $N,Adh_1^F/N,Adh_1^S$ and $N,Adh_1^F/L,Adh_1^S$. The first one is expected to show a 25:50:25 percent activity for the FF, FS, and SS isozyme bands (activity distribution type I) and the second a ratio of 37.7:47.4:14.9 percent for the same three isozyme bands, respectively (activity distribution type II). However, if the same gene controls both the charge and the activity of the enzyme, all the Adh_1^F/Adh_1^S heterozygotes should be $N,Adh_1^F/L,Adh_1^S$, with activity distribution type II. Both type I and type II activity distributions were found among the progeny of the above cross (Fig. 2B), an indication that two different genes are involved. However, the two types were not found in the expected 1:1 ratio for two independent genes (Table 1). Only 17.1 percent type I segregants were found, an indication that the two genes are linked and located about 17 crossover units apart.

Activity distribution type II can be produced in two ways. First, the allele which reduces enzyme activity (L) affects both the S and F polypeptides but in the *cis* configuration only. Alternatively, only the S polypeptide is influenced by the L allele. In the $(N,Adh_1^F/L,Adh_1^S) \times (L,Adh_1^S/L,Adh_1^S)$ test cross, two Adh_1^F/Adh_1^S heterozygous types are expected: the parental type $N,Adh_1^F/L,Adh_1^S$ (83 percent) and a recombinant type $L,Adh_1^F/L,Adh_1^S$ (17 percent). If the L allele does affect both the S and F polypeptides, the recombinant $L,Adh_1^F/L,Adh_1^S$ should have activity distribution type I. However, if only the S polypeptides are affected, the recombinant should have activity distribution type II. All the progeny of

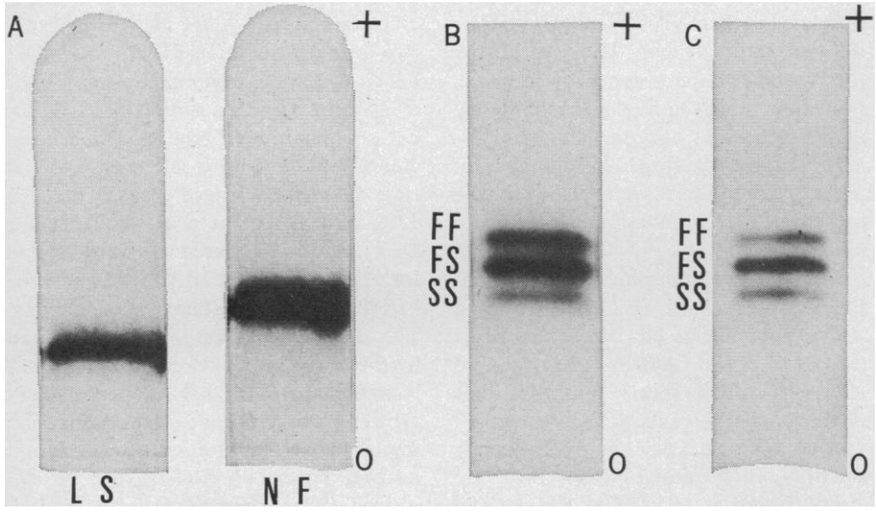

Fig. 1. Starch-gel electrophoresis of alcohol dehydrogenase extracted from scutellum of maize. (A) Comparison of activities in two different lines of maize: NF, $N,Adh_1^F/N,Adh_1^F$—homozygous Adh_1^F/Adh_1^F with normal ADH activity; LS, $L,Adh_1^S/L,Adh_1^S$—homozygous Adh_1^S, with low ADH activity. (B) Zymogram of heterozygous $L,Adh_1^S/N,Adh_1^F$ scutellum having activity distribution type II. (C) Zymogram of heterozygous $N,Adh_1^S/N,Adh_1^F$ scutellum having activity distribution type I.

this test cross showed activity distribution type II (Table 1), an indication that only the S polypeptides are affected by the L allele.

Four different heterozygous Adh_1^F/Adh_1^S types are expected in the F_2 generation—$N,Adh_1^F/L,Adh_1^S$, $L,Adh_1^F/L,Adh_1^S$, $N,Adh_1^F/N,Adh_1^S$, and $L,Adh_1^F/N,Adh_1^S$. With a distance of 17.1 crossover units between the two genes, the expected frequencies of the four genotypes are 68.9, 14.1, 14.1, and 2.9 percent, respectively. It has earlier been concluded that the $N,Adh_1^F/L,Adh_1^S$ and the $L,Adh_1^F/L,Adh_1^S$ genotypes are expected to have activity distribution type II, while the $N,Adh_1^F/N,Adh_1^S$ shows type I. In the fourth genotype ($L,Adh_1^F/N,Adh_1^S$), activity distribution type I would be obtained if the L allele operates only in the *cis* configuration, and type II would be obtained if it operates in both the *cis* and the *trans* configurations. Thus, 17.0 and 14.1 percent type I segregants are expected for the two alternatives in the F_2 generation. The result of 14.0 percent type I segregants (Table 1) which were found among the F_2 segregating progeny strongly suggests that the L allele operates both in the *cis* and the *trans* configuration, even though the difference of 2.9 percent expected in order to distinguish between the two alternatives is too small.

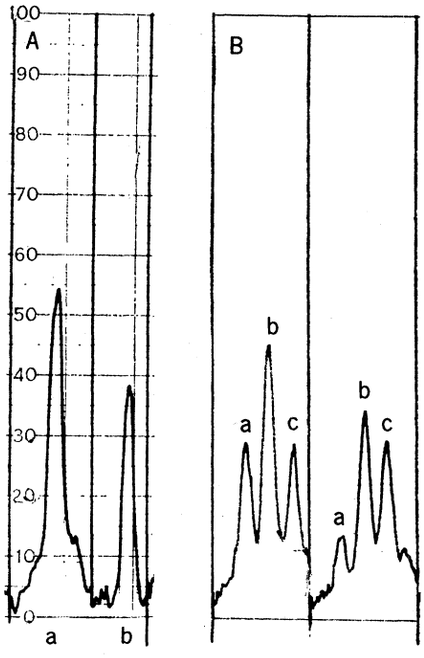

Fig. 2. Densitometer scan of maize scutellar alcohol dehydrogenase zymograms. (A) Comparison of bands' intensities from (*a*) $N,Adh_1^F/N,Adh_1^F$ and (*b*) $L,Adh_1^S/L,Adh_1^S$ homozygous scutella. (B) Two segregants from the $L,Adh_1^S/N,Adh_1^F \times N,Adh_1^F/N,Adh_1^F$ test cross with activity distribution type I (left) and activity distribution type II (right). (*a*) SS autodimer; (*b*) FS allodimer; and (*c*) FF autodimer.

Table 2. Phenotypic expression of alcohol dehydrogenase in various genotypes of maize. (a to c) Homozygous parental lines; (d to g) heterozygous Adh_1^S/Adh_1^F with different combinations of the N and L alleles of the Adh_r gene. Activity in heterozygous Adh_1^S/Adh_1^F was determined spectrophotometrically as described before (*7*) in the F_1 generation only. Not tested, N.T.

Genotype	Activity	Isozyme bands		Activity distribution
		Number	Dimers	
a) $N,Adh_1^F/N,Adh_1^F$	Normal	1	FF	
b) $N,Adh_1^S/N,Adh_1^S$	Normal	1	SS	
c) $L,Adh_1^S/L,Adh_1^S$	Low	1	SS	
d) $N,Adh_1^S/N,Adh_1^F$	Normal	3	FF;FS;SS	Type I
e) $L,Adh_1^S/N,Adh_1^F$	Intermediate	3	FF;FS;SS	Type II
f) $L,Adh_1^S/L,Adh_1^F$	N.T.	3	FF;FS;SS	Type II
g) $N,Adh_1^S/L,Adh_1^F$	N.T.	3	FF;FS;SS	Type II

The results obtained in the F_2 generation (Table 1) are in good agreement with those obtained in the two test crosses. Also, the mean activity distributions (Table 1) in the above crosses are similar to the activity distribution obtained in the F_1 generation and to that expected from the relative activity of the two parental lines. Therefore, it is concluded that the gene which specifies the activity of the ADH enzyme of the scutellum of maize is not allelic to the Adh_1 structural gene which specifies the charge of the enzyme molecule. The symbol Adh_r is proposed for this gene. The Adh_r^L allele is dominant, but it reduces activities of the S polypeptides only. The Adh_r^N allele specifies higher activities for both the S and the F polypeptides. It is not known yet whether the Adh_r gene operates directly on the structural Adh_1 gene or on its products.

Schwartz and Endo (*4*) reported on reduced activity of the Adh_1^S allele in heterozygous Adh_1^F/Adh_1^S endosperm but not in the scutellum of the same genotype. There are no reports on the ADH activities in endosperms and scutella of Adh_1^F/Adh_1^F and Adh_1^S/Adh_1^S homozygous plants or on genetic segregation tests. The Adh_r gene described here is active in the scutellum as well as in the very young roots and plumules (*8*) as long as detectable amounts of ADH activity are present (*7*). Furthermore, the reduced activities of ADH in the scutellum occur only when both the Adh_1^S and the Adh_r^L alleles are present, while in the endosperm (*4*) the products of the Adh_1^S allele are always less active. However, it is of interest that in both cases the activities of the Adh_1^S products are reduced.

The Adh_r gene also differs in its nature from the E_1 prime regulatory mutant alleles described for the *p*H 7.5 esterase in maize (*9*). These mutants operate only in the *cis* configuration and are found at or very close to the site of the structural gene. They were considered (*9*) to be similar to the operator in the β-galactosidase system in bacteria (*10*). By analogy, though not proven, the Adh_r gene is comparable with the β-galactosidase *i* regulatory gene.

The effects of variation in the activities of an enzyme are probably more significant for the organism than variation in the charge of the enzyme molecule. Hence, variation in genes like the Adh_r gene could have significant evolutionary consequences. The presence of genes of this nature should be taken into consideration in future studies such as studies dealing with quantitative genetic variation on the molecular level or studies on heterosis.

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