# GENETIC CONTROL OF ALCOHOL DEHYDROGENASE— A COMPETITION MODEL FOR REGULATION OF GENE ACTION*

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Received July 16, 1970

THE past two decades have witnessed tremendous advances in the science of genetics; determination of the structure of the genetic material, deciphering the genetic code, etc. An important problem still remaining concerns the mechanism(s) by which gene action is regulated in the development and differentiation of higher organisms. The elegant induction/repression scheme of JACOB and MONOD (1961) is an all-or-none process, a gene is either repressed or derepressed. This scheme does not account for differential gene activity in diverse cell types of an organism. While qualitative differences in protein profiles are observed in some tissues with specific proteins either present or absent, striking quantitative differences are also seen, i.e., differences in relative concentration of the same protein. For example, the $Ldh^A$ and $Ldh^B$ genes show approximately equal activity in some tissues, while in others, their relative activities are very dissimilar. In the kidney, the relative activities of these two genes change strikingly during development (MARKERT 1961). The same condition can be shown to exist between two alleles of the same gene, as in the case of the $E_1$ esterase in maize. In plants heterozygous for prime and standard alleles which produce electrophoretically distinct isozymes, as in $E_1^{N'}/E_1^{S}$, one observes that $E_1^{S}$ is more active than $E_1^{N'}$ in the late stages of endosperm development, $E_1^{S}$ is less active than $E_1^{N'}$ in the aerial root, and both alleles show approximately equal activity in the root and shoot of the seedling (SCHWARTZ 1962, 1964). As is the case with the kidney LDH, a change in the relative activity occurs during development. In the young endosperm, $E_1^{N'}$ and $E_1^{S}$ are equally active, but as the endosperm matures, the activity of $E_1^{N'}$ is drastically reduced relative to the activity of $E_1^{S}$. Thus while genes may be turned off and on by repression and derepression, we feel that some additional mechanism is required to regulate the level of activity of the derepressed genes.

In this paper we propose a model to account for the regulation of the level of gene activity. The model is an outgrowth of our studies on the genetic control of alcohol dehydrogenase (ADH) in maize. It is a competition model of gene regulation whereby the level of activity of a gene is dependent upon interaction and competition with other genes. We will first present the scheme and then discuss in detail the experimental evidence for each point in the regulation model. This is

* This work was supported by National Science Foundation grants GB 6945 and GB 8107.

not proposed as a universal model for the control of the activity of *all* genes, and there is no reason to expect that all genes should be regulated in the same manner. The proposed regulatory mechanism was developed to explain the control of alcohol dehydrogenase but it could be more general and hold for other genes as well.

## THE MODEL

We propose the following model: (1) The amount of enzyme synthesized in a particular cell is limited by the concentration of some specific factor. (2) The limited factor acts at the gene level to control activity; the more factor, the higher the gene activity. Although we can only speculate at the moment as to the manner in which this is brought about, a number of possibilities come to mind. The limited factor may displace histones on the gene allowing the locus to uncoil and become active; the more histones displaced, the greater the gene activity. Alternatively, the limited factor may be an initiator of messenger RNA transcription, either the polymerase or a cofactor which confers specificity to the polymerase (BURGESS *et al.* 1969). (3) A group of genes can be "activated" by the same limited factor, but the cell contains a number of different factors and the specificity is very high such that any one gene can be activated only by a particular limited factor. (4) The unrepressed genes within each group compete with each other for the limited factor. The sum total of the activity of a set of genes is determined by the concentration of the group-specific limited factor, but the activity of each gene within the group is determined by its capacity to compete (competitive ability) with other genes in the same group for the limited factor. (5) The relative competitive abilities of the genes in a group are not constant and fixed, but can vary greatly depending on the internal cellular environment. If *A* and *B* represent two genes comprising a group, *A* may be able to compete for the limiting factor much better than *B* in one tissue, but in another tissue where the cellular conditions are different, the situation may be reversed and *B* can compete better than *A*, or both may compete equally well and show the same level of activity. (6) Repressed genes do not compete for the limited factor.

## MATERIALS AND METHODS

The evidence to be presented in support of the model is based for the most part on studies with two alcohol dehydrogenase alleles, $Adh_1^F$ and $Adh_1^S$, but some experiments will be reported on other alleles (SCHWARTZ and ENDO 1966).

Immature kernels were harvested at varying numbers of days after pollination, and stored in plastic bags at −20°C until use. The kernels may be stored in this manner for a few months without detectable change in ADH activity in the endosperm or embryo. Pollen enzyme assays and electrophoretic analyses were conducted within a few hours after the pollen was shed. Kernels were germinated on moistened filter paper in Petri dishes in a 30°C incubator.

Total enzyme activity was measured by following the rate of reduction of nicotinamide-adenine dinucleotide (NAD) in the presence of ethanol as described in an earlier publication (EFRON and SCHWARTZ 1968). Proteins were estimated by the method of LOWRY *et al.* (1951). Relative activity of the two alleles in heterozygotes was determined both by visual comparison of isozyme band intensities in the zymogram and by densitometry. The techniques for electrophoresis and development of zymograms are described elsewhere (SCHWARTZ and ENDO 1966).

## RESULTS

*Evidence for competition for a limited factor:* Maize ADH behaves as a dimer (SCHWARTZ and ENDO 1966). The $Adh_1^F$ and $Adh_1^S$ alleles produce subunits that differ in charge. In each homozygote, only the single ADH isozyme SS or FF is produced. In heterozygotes, the three dimer combinations, FF, FS, and SS are formed. Using a particular $Adh_1^F$ allele in a heterozygote with $Adh_1^S$, one observes that in some but not all tissues the relative concentrations of the three isozymes are strikingly different from those expected if the two alleles and their products are equally active. With equal activity, the three isozymes should occur in a 1:2:1 ratio in diploid material, with the allodimer FS twice as active as the FF and SS autodimers. However in the seedling zymograms, a strikingly different ratio of band intensities is obtained, with the FF band being much more intense than SS (Figure 1). A similar situation is observed in the endosperm. Endosperm tissue is triploid receiving two sets of genes from the female parent and one set from the male. Endosperm of the genotype $Adh_1^F/Adh_1^F/Adh_1^S$ should form the three isozymes in a ratio of 4FF:4FS:1SS, however, the FF band is seen to be

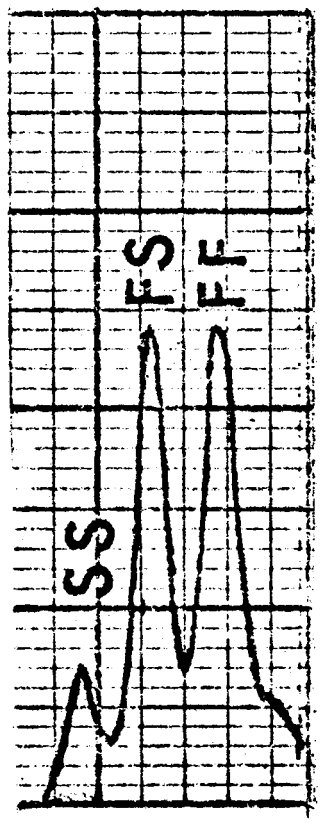


FIGURE 1.—Densitometer scan of ADH isozyme bands in zymograms of $Adh_1^F/Adh_1^S$ plumules from two day old seedlings.

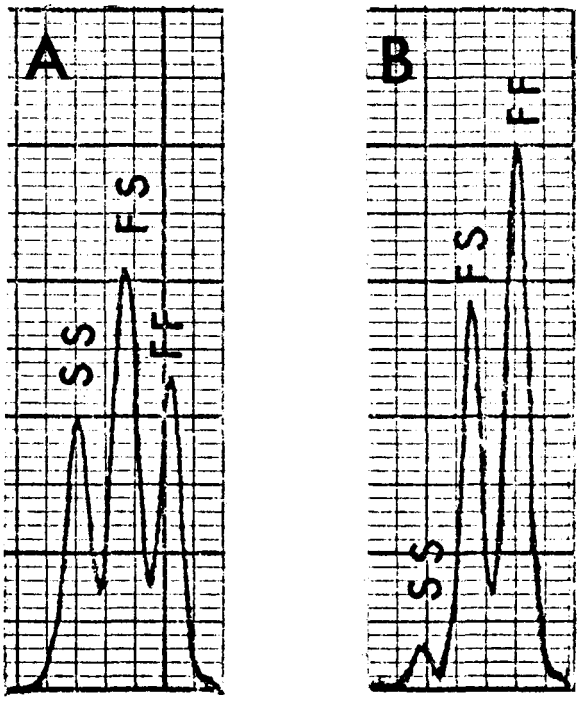


FIGURE 2.—Densitometer scan of ADH isozyme bands in zymograms of 16 day immature endosperm. A = $Adh_1^F/Adh_1^S/Adh_1^S$; B = $Adh_1^F/Adh_1^F/Adh_1^S$.

considerably more intense than FS, and the SS band is very faint. In the reciprocal heterozygotes $Adh_1^F/Adh_1^S/Adh_1^S$, the ratios should be 1FF:4FS:4SS if $Adh_1^F$ and $Adh_1^S$ are equally active, but the observed isozyme ratio approximates 1FF:2FS:1SS (Figure 2). Since there are twice as many $Adh_1^S$ as $Adh_1^F$ genes in this triploid material, this ratio is consistent with the $Adh_1^S$ allele being only half as active as $Adh_1^F$ in producing ADH protomers.

From these results one cannot determine whether it is the $Adh_1^F$ allele or its product which is more active. The differential activity is found only in heterozygotes where both alleles are present in the same cell. In homozygotes, both genotypes show very similar activities. This was first determined by comparing the staining intensities of the ADH bands in zymograms of sibling $Adh_1^F/Adh_1^F$ and $Adh_1^S/Adh_1^S$ segregants in an $F_2$ population; however, quantitation of enzyme level was required to definitely establish this point. Comparison of enzyme levels was determined in the following way: $Adh_1^F/Adh_1^S$ heterozygotes were self-pollinated producing $F_2$ progeny of the following three constitutions: $Adh_1^F/Adh_1^F$, $Adh_1^F/Adh_1^S$, and $Adh_1^S/Adh_1^S$. The genotypes show no detectable phenotypic differences so that classification of the seedlings into the three genotypes requires electrophoretic analysis. In each experiment, approximately 120 $F_2$ kernels were soaked for 48 hr then germinated another 48 hr on moistened filter paper in a 30°C incubator. The shoot and root of each seedling were excised and stored individually on moistened filter paper at 4°C. The scutellum was separated

TABLE 1

*Comparison of ADH activity in $F_2$ seedlings*

| Genotype | Activity units/seeding* | | | | |
|---|---|---|---|---|---|
| *F/F* | 172 | 134 | 192 | 101 | 113 |
| plumule *F/S* | 178 | 121 | 184 | 92 | 101 |
| *S/S* | 176 | 117 | 182 | 97 | 105 |
| *F/F* | 76 | 40 | 76 | 38 | 58 |
| root *F/S* | 80 | 44 | 70 | 42 | 43 |
| *S/S* | 68 | 38 | 67 | 36 | 44 |

* Each column represents a separate $F_2$ population.

from the endosperm and analyzed electrophoretically for classification of the seedling genotype. Shoots and roots of identical genotypes were pooled (to minimize variability due to modifying genes which might also be segregating), homogenized in 0.005 M sodium phosphate buffer at pH 7.0 (0.2 ml per root or shoot), centrifuged, and the extracts were analyzed for total ADH activity. Comparisons are presented in units of activity per seedling, although identical results were obtained when comparisons were made on the basis of activity per mg protein. For the immature endosperm analyses, 16 day old $F_2$ kernels were used. A small portion of each endosperm was removed and tested electrophoretically to determine its genotype. All four genotypes, $Adh_1^F/Adh_1^F/Adh_1^F$, $Adh_1^F/Adh_1^F/Adh_1^S$, $Adh_1^F/Adh_1^S/Adh_1^S$, and $Adh_1^S/Adh_1^S/Adh_1^S$, can be scored in this manner since, as described above, the two heterozygotes are distinguishable on the basis of isozyme intensities. The endosperms were then separated from the pericarp and embryo, and identical genotypes pooled and homogenized in phosphate buffer. Since no precautions were taken to remove equal portions of endosperm for genotype classification, the comparison is made on the basis of activity per mg endosperm protein. Results of these analyses are presented in Tables 1 and 2.

The results clearly show that enzyme levels are almost identical in all of the genotypes. However, electrophoretic analysis of the extract from the pooled heterozygous material reveals considerably more activity associated with the $Adh_1^F$ specified subunit than $Adh_1^S$. The zymograms are similar to those shown in Figures 1 and 2. The possibility of an electrophoretic artifact such that the band intensities in electrophoresis do not give a true picture of the intensities of the FF,

TABLE 2

*Comparison of ADH activity in endosperm of $F_2$ kernels*

| Genotype | Activity units/mg protein |
|---|---|
| *F/F/F* | 420 |
| *F/F/S* | 374 |
| *F/S/S* | 366 |
| *S/S/S* | 350 |

FS, and SS isozymes can be ruled out. When FF and SS extracts ($F_2$ seedlings) of equal ADH activity, as determined by rate of NAD reduction, are mixed in a 1:1 ratio and subjected to electrophoresis, the two bands are of equal intensity. Also, as will be discussed later, extracts from other tissues of the same heterozygotes show the expected 1:2:1 ratio.

In seedlings and endosperm, the $Adh_1^F$ allele or its product is more active than $Adh_1^S$ only in heterozygotes where the two alleles are present in the same cells. In homozygotes, the two alleles show equal activity. One possible explanation for these results is that the $Adh_1^F$ allele or its product in some manner suppresses the activity associated with the $Adh_1^S$ allele so that it is the dominant form in heterozygotes; or in reverse, that the $Adh_1^S$ allele or its product causes an activation of the $Adh_1^F$ allele. This scheme is ruled out by the observation that total alcohol dehydrogenase activity in the heterozygote is equal to that in each homozygote. If $Adh_1^F$ suppresses $Adh_1^S$, one would expect the heterozygote to be lower in activity. If $Adh_1^S$ causes an enhancement of $Adh_1^F$ activity, the heterozygote should be higher. Equal activity in $Adh_1^F/Adh_1^F$, $Adh_1^F/Adh_1^S$, and $Adh_1^S/Adh_1^S$ genotypes with $Adh_1^F$ more active than $Adh_1^S$ in the heterozygote can best and perhaps only be explained by competition between $Adh_1^F$ and $Adh_1^S$ or their products for some factor essential for activity which is present in limited quantity in the tissue, and $Adh_1^F$ would compete for this factor better than $Adh_1^S$.

The difference between $Adh_1^F$ and $Adh_1^S$ or their products, which is responsible for the differential competitive abilities, can be shown to be separable and distinct from that which leads to alteration in the net charge of the isozyme subunit. Some alleles which produce the F type subunit show the same competitive ability as $Adh_1^S$. The band intensities for the three isozymes are 1FF:2FS:1SS in the diploid seedling material and 4FF:4FS:1SS or 1FF:4FS:4SS for reciprocal triploid endosperm tissue.

*Evidence that competition occurs at the gene level:* Having established that competition for some factor limits the amount of active enzyme synthesized, the question arises as to the step in enzyme synthesis at which this competition occurs. The results presented above can be accounted for (1) if there is competition between F and S polypeptides for some limited cofactor essential for activity, or (2) if there is competition between F and S messenger RNA for a limiting transfer RNA species, or (3) if competition exists between genes for some sort of activator. It is ordinarily difficult to make a distinction experimentally between these alternative hypotheses. However in this case, it can be proven unequivocally that competition exists at the transcription level, in that we can show that the competition occurs prior to enzyme synthesis.

ADH is synthesized during maturation of the pollen and can be assayed in mature pollen grains. $Adh_1^F/Adh_1^F$, $Adh_1^F/Adh_1^S$, and $Adh_1^S/Adh_1^S$ plants produce the same amount of enzyme in the pollen, that is, ADH activity per gram pollen grains is the same for all three genotypes. This was established in tests with the $F_2$ progeny of a selfed $Adh_1^F/Adh_1^S$ plant. The genotype of each plant was determined electrophoretically by analyzing pollen extracts. Shedding pollen was collected and pooled from plants belonging to each of the three genotypes. Equal

TABLE 3

*ADH activity in pollen from $F_2$ plants*

| Genotype | Activity units/g protein |
|---|---|
| *F/F* | 1780 |
| *F/S* | 1820 |
| *S/S* | 1740 |

weights of pollen were homogenized in phosphate buffer and ADH activity was determined per gram pollen. As shown in Table 3, the specific activities are identical in all three genotypes.

Pollen extracts from $Adh_1^F/Adh_1^F$ and $Adh_1^S/Adh_1^S$ plants show the single ADH bands as expected. Pollen extracts from $Adh_1^F/Adh_1^S$ heterozygotes show only two bands, the FF and SS isozymes; no hybrid enzyme is formed. The FF band is considerably more intense than SS (Figure 3). This observation, considered in conjunction with the finding that the three genotypes produce the same total amount of enzyme, shows that the competition occurs in the synthesis of ADH in the pollen as well as in the endosperm and seedling. However, the absence of the hybrid FS band in pollen ADH establishes that the enzyme was synthesized after haploidization and segregation of the $Adh_1^F$ and $Adh_1^S$ alleles at meiosis. If the enzyme in the pollen was formed prior to haploidization, all three enzymes, FF, FS, and SS, would be present in each haploid pollen grain. Thus we can clearly show that the competition occurs in the diploid stage prior to enzyme synthesis, and therefore the competition must be at the transcription level. The

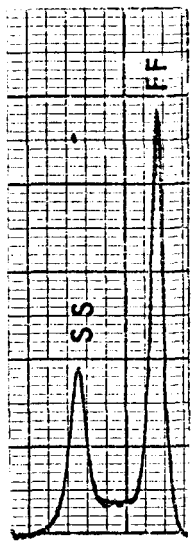


FIGURE 3.—Densitometer scan of ADH isozyme bands in zymograms of mature pollen from $Adh_1^F/Adh_1^S$ plants.

experiments performed do not shed any light on the nature or mechanism of the genic competition and speculation is fruitless at this stage.

The absence of the FS hybrid band in haploid pollen from $Adh_1^F/Adh_1^S$ heterozygotes is the sole basis for the conclusion that the enzyme is made after haploidization and segregation at meiosis. One could argue that this is an erroneous conclusion; that the enzyme is in fact synthesized prior to haploidization but for some reason in this tissue dimerization is limited to identical subunits, a situation similar to the restriction in LDH tetramer formation observed in some organisms where no heterotetramers are produced even though both LDH genes are active (SALTHE, CHILSON and KAPLAN 1965). Fortunately a compound $Adh_1$ locus (duplicate genes) containing two alleles which specify different ADH isozymes is available to allow a critical test of this alternative. In the case of the compound locus, two $Adh_1$ alleles are present in individual haploid pollen grains. According to the alternative hypothesis that the hybrid dimers cannot be formed in the pollen, one would not expect to see the hybrid band in zymograms of pollen carrying the duplication. If, however, hybrid dimers can be formed, and the absence of the hybrid band in pollen from $Adh_1^F/Adh_1^S$ plants results from the fact that the enzyme is not synthesized until after haploidization, then one should observe the hybrid band in pollen containing the duplication. This is precisely the condition which was found. The duplication includes an $Adh_1^F$ and an $Adh_1^{C(m)}$ allele. Pollen extracts from $Adh_1^{\overline{FC(m)}}/Adh_1^{\overline{FC(m)}}$ duplication homozygotes clearly show the presence of the $FC^m$ hybrid band. Pollen from plants of the genotype $Adh_1^S/Adh_1^{\overline{FC(m)}}$ are of two types, half of the pollen grains contain only the $Adh_1^S$ allele and the other half receive the $Adh_1^F$ and the $Adh_1^{C(m)}$ alleles in the duplication. Pollen from such heterozygous plants show the $FC^m$ hybrid enzyme but no heterodimer enzyme hybrids involving the S subunit.

The inequality in amounts of FF and SS isozymes found in pollen from heterozygous plants is not due to unequal proportions of the two types of pollen grains. When heterozygous $Adh_1^F/Adh_1^S$ plants are self-pollinated and the genotypes of the $F_2$ progeny kernels scored, a close fit to the expected 1:2:1 ratio is observed (440 $Adh_1^F/Adh_1^F$ : 975 $Adh_1^F/Adh_1^S$ : 456 $Adh_1^S/Adh_1^S$). These data indicate that equal numbers of $Adh_1^F$ and $Adh_1^S$ pollen grains are produced.

These results suggest that the limited factor is not synthesized after meiosis. It stands to reason that if this were not so, $Adh_1^F$ and $Adh_1^S$ pollen grains would synthesize equal amounts of enzyme since each microspore contains only one $Adh_1$ allele and there is no competition. The effect of interallelic competition for the limited factor in the diploid must persist into the haploid microspore such that even in the absence of further competition, the two alleles maintain their differential gene activity in enzyme synthesis. Either the level of gene activity is preset in the diploid stage, or the limited factor is unequally distributed into the $Adh_1^F$ and $Adh_1^S$ microspores at meiosis. Presetting could come about if transcription is initiated but not completed during meiosis so that the nascent mRNA remains associated with the $Adh_1$ genes until after gene segregation and haploidization. If synthesis of a number of mRNA chains can be initiated on a single gene and more nascent mRNA molecules are associated with $Adh_1^F$ than $Adh_1^S$, then the

$Adh_1^F$ microspores would contain more ADH mRNA than the $Adh_1^S$ microspores after chain completion. This would result in a difference in enzyme level in the classes of microspores if no new ADH mRNA synthesis is initiated after meiosis. The association of many RNA molecules with single DNA chains in meiosis has been demonstrated for the lampbrush chromosomes in amphibian oocytes (GALL 1956). Unequal distribution of the limited factor into the microspores could result if the factor complexes with the $Adh_1$ gene and segregates with it at meiosis.

*Evidence that competition can also occur between nonallelic genes:* The data presented above show differential competition between different alleles of the same gene for a limited activator factor. The model presented for regulation of the level of gene activity proposes competition between nonallelic genes. Such competition can be shown to occur between the $Adh_1$ and $Adh_2$ genes. The $Adh_2$ gene forms a polypeptide which associates in a dimer with $Adh_1$ subunits (SCHWARTZ 1966, 1969). The product of the $Adh_2$ gene shows only very slight ADH activity but heterodimers composed of $Adh_1$ and $Adh_2$ subunits are active. The $Adh_2$ gene is normally repressed showing only little activity, but under certain conditions it is derepressed. Since its product is similar enough to the $Adh_1$ product to allow for cross-dimerization, it is a good candidate as a competitor with $Adh_1$ for the same limited factor.

The evidence for this nonallelic gene competition is as follows: In the seedling, $Adh_1^F$ is a better competitor than $Adh_1^S$ for the limited factor. If $Adh_1$ is the only locus competing for this factor, then the level of enzyme in $Adh_1^F/Adh_1^F$ and $Adh_1^S/Adh_1^S$ homozygotes should be identical and limited by the concentration of the factor. If, however, another gene is competing with $Adh_1$ for the limited factor, then the $Adh_1^F/Adh_1^F$ and $Adh_1^S/Adh_1^S$ seedlings should not show equal ADH activity. Assume that $X$ competes with $Adh_1$ and has the same competitive ability as the $Adh_1^F$ allele, whereas the $Adh_1^S$ allele has only half the competitive ability of $Adh_1^F$ or $X$. In $Adh_1^F/Adh_1^F$ homozygotes, 50% of the limited factor would be available for $Adh_1$ activation, the rest being utilized by the $X$ gene. However, in $Adh_1^S/Adh_1^S$ homozygotes only 33% of the limited factor would be available for $Adh_1$ since $Adh_1^S$ is a poorer competitor than $X$. Thus in the presence of other nonallelic genes which compete for the same factor, alleles with different competitive abilities should show dissimilarity in the total amount of enzyme produced in homozygotes.

In four day old seedlings, the $Adh_2$ gene is derepressed in the mesocotyl (the region of the shoot between the scutellum and the base of the coleoptile), but is still repressed in the epicotyl (the region of the shoot above the point of attachment of the coleoptile). Competition between the nonallelic $Adh_1$ and $Adh_2$ genes was tested experimentally in the following way: An $F_2$, four day old, seedling population segregating for $Adh_1^F/Adh_1^F$, $Adh_1^F/Adh_1^S$ and $Adh_1^S/Adh_1^S$ was grown, each shoot excised and cut in two at the node. After determining the seedling genotypes by testing the scutellum, the mesocotyls and epicotyls of each genotype were pooled, homogenized, and assayed for total ADH activity. The results are shown in Table 4. In the epicotyls, where $Adh_2$ is repressed, there is no competition with $Adh_1$; and $Adh_1^F/Adh_1^F$, $Adh_1^F/Adh_1^S$, and $Adh_1^S/Adh_1^S$

TABLE 4

*Comparisons of ADH activity in 4 day $F_2$ seedlings*

| Genotype | Activity (units/seedling) Epicotyl | Mesocotyl |
|---|---|---|
| *F/F* | 94 | 88 |
| *F/S* | 102 | 67 |
| *S/S* | 100 | 53 |

plants show equal ADH activity. In the mesocotyls where $Adh_2$ is active, the ADH level in the $Adh_1^S/Adh_1^S$ plants is considerably lower than in the $Adh_1^F/Adh_1^F$ sibs.

*Evidence that the relative competitive ability of the gene is not constant and can vary strikingly in different cells:* In contrast to the situation encountered in the seedling, endosperm, and pollen, the $Adh_1^F$ and $Adh_1^S$ alleles show equal activities in heterozygous embryos from mature kernels. The FF, FS, and SS bands occur in the 1:2:1 ratio expected for equal activity of both alleles (Figure 4). Thus, the same two alleles show different relative activities in different plant parts. This could be accounted for either by a change in competitive abilities or by postulating that in the mature embryo the factor is present in excess so that there is no competition, and the limitation in ADH synthesis depends upon other

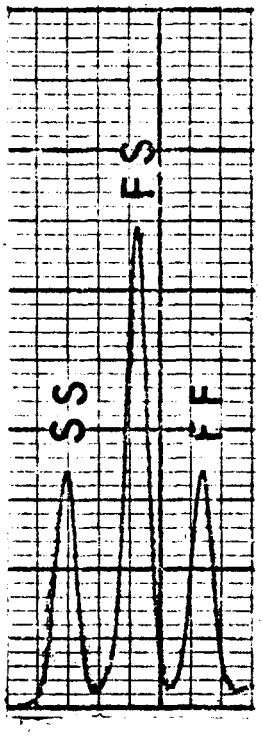


FIGURE 4.—Densitometer scan of ADH isozyme bands in zymograms of $Adh_1^F/Adh_1^S$ embryos from mature kernels.

factors which restrict protein synthesis in general. These alternatives can be distinguished by comparing the amount of ADH synthesized in homozygotes and in hemizygotes. If the factor is not limited, the hemizygotes, which contain only a single *Adh* gene, should show only half as much ADH as the homozygotes where the gene is present twice. However, if the factor is still limited in the mature embryo and the 1:2:1 band intensity ratio results from equal competition between $Adh_1^F$ and $Adh_1^S$ alleles, the hemizygote should have as much enzyme as the homozygotes since the genes do not function to their full capacities and enzyme level is set by the concentration of the limited factor.

The $Adh_1$ gene has been localized on the long arm of chromosome *1* approximately 1.6 map units from $lw_1$ (SCHWARTZ, unpublished). Since small deletions of the region of the chromosome which include the $Adh_1$ locus are not available for the two-gene *vs.* one-gene comparison, the gene dosage test was made with the use of the *TB-1a* translocation involving the long arm of chromosome *1* and a *B* chromosome. The $B^1$ chromosome carries the $Adh_1$ locus and regularly undergoes nondisjunction in the second microspore division so that one sperm in the pollen grain contains zero and the other contains two $B^1$ chromosomes (ROMAN 1947). The test involves crossing an $Adh_1^S/Adh_1^S$ plant as the female parent by a plant heterozygous for the translocation but homozygous for the $Adh_1^F$ allele. Almost all of the pollen which functions in fertilization is of two types: (a) nontranslocated with a normal chromosome *1* in both sperm; and (b) translocated, with two $B^1$'s in one sperm and none in the other. One sperm fertilizes the egg giving rise to the diploid embryo, while the second sperm fertilizes the polar nuclei giving rise to the endosperm. The egg may be fertilized by either the sperm with or without the $B^1$ chromosomes. Thus three classes of embryos are formed on the ears from the above cross: (a) normal diploids with an $Adh_1^S$ and an $Adh_1^F$ allele arising from fertilization with normal pollen; (b) hypoploid with only the $Adh_1^S$ allele from the egg; (c) hyperploid with the $Adh_1^S$ allele from the egg and two $Adh_1^F$ alleles on the translocated $B^1$ chromosomes. The hyperploid embryos can be recognized easily due to the fact that the associated hypoploid endosperm is defective, and this class is discarded. Kernels with normal or hypoploid embryo genotypes are identical in appearance and can be distinguished only by electrophoretic sampling of their ADH constitution. Normal embryos contain both $Adh_1^S$ and $Adh_1^F$ alleles and show three ADH bands, while the hypoploid embryos contain only a single $ADH^S$ band. The kernels were classified by excising a small part of the scutellum from each dry kernel, soaking these for 24 hr and then testing for the ADH isozyme patterns. Equal numbers of normal and embryo hypoploid kernels were pooled, ground to a meal in a Wiley Mill, extracted with equal volumes of phosphate buffer for 15 min, centrifuged, and ADH activity of the extracts were compared. The enzyme levels in kernels with embryos that contain either one or two doses of the $Adh_1$ gene are approximately equal (Table 5). Specific activity comparisons of the two extracts show a 24% difference, but this is not unexpected since the two samples are genotypically dissimilar. The kernels with hypoploid embryos have hyperploid endosperms which probably contain more protein than the normal triploid endosperm.

This experiment shows that the factor is still limiting in the embryo of the

TABLE 5

*Comparison of ADH activity in mature kernels carrying one or two* $Adh_1$ *genes*

| | Activity (units/ml) | Protein (mg/ml) | Specific activity |
|---|---|---|---|
| hypoploid (1 $Adh_1$ gene) | 2500 | 2.96 | 845 |
| diploid (2 $Adh_1$ genes) | 2680 | 2.41 | 1116 |

mature kernel; however, the $Adh_1^F$ and $Adh_1^S$ alleles compete equally well at this stage of development in contrast to the endosperm, seedling, and pollen.

The relative competitive ability of the gene is not tissue specific but depends upon the stage of development. In the scutellum from the mature kernel, $Adh_1^F$ equals $Adh_1^S$ in competitive ability; whereas the scutellar tissue in the immature developing kernel (about 20–25 days after fertilization) shows $Adh_1^F$ to be a better competitor than $Adh_1^S$, similar to the condition in the seedling. Also, in the plumule of the germinated seedlings, $Adh_1^F$ is a better competitor than $Adh_1^S$; however, $Adh_1^F$ is equal to $Adh_1^S$ in competitive ability in the embryo plumule prior to germination. A reasonable explanation for these results is that the relative competitive abilities of genes depend upon the environmental conditions in the cell. In the late stages of maturation of the kernel, either some substance is produced which alters the limited factor such that it can now be competed for equally well by the two alleles, or cellular conditions are established in which $Adh_1^F$ and $Adh_1^S$ can both compete equally well for the limited factor.

A combination of spectrophotometric and electrophoretic analyses was used to establish that the $Adh_1^F$ and $Adh_1^S$ alleles compete unequally in the seedling and endosperm but equally in the mature embryo for the limited factor. Quantitation of enzyme level in isozyme bands is not precise. To eliminate the possibility of electrophoretic artifact, a different type of analysis was performed which does not require comparison of intensities of isozyme bands in the zymograms. This study used a $CRM^+$ mutant $Adh_1$ allele which produces a catalytically inactive enzyme. This mutant, designated $Adh_1^o$, was derived from $Adh_1^S$ by treatment with the mutagen ethyl methanesulfonate. If there is no competition between $Adh_1^F$ and $Adh_1^o$ for the limited factor, or if the two alleles compete equally well, the enzyme level in the $Adh_1^F/Adh_1^o$ heterozygotes should be one-half of that in sibs which contain two alleles which specify active enzyme. However, if there is competition, and if the $Adh_1^F$ allele competes for the limited factor better than $Adh_1^o$, the relative enzyme level in the $Adh_1^F/Adh_1^o$ heterozygote should be higher, since more than half of the limited factor would be used in the synthesis of the active F subunit.

Plants heterozygous for $Adh_1^S$ and $Adh_1^o$ were crossed to $Adh_1^F/Adh_1^F$ homozygotes to obtain sibling material in which $Adh_1^F$ is heterozygous with alleles that produce active and inactive enzyme subunits. Enzyme and protein determinations were made on mature kernels and on seedling roots of pooled $Adh_1^F/Adh_1^S$ and $Adh_1^F/Adh_1^o$ genotypes as described previously. The results are given in Table 6.

TABLE 6

*Comparison of ADH activity in* $Adh_1^F/Adh_1^S$ *and* $Adh_1^F/Adh_1^0$ *genotypes*

| Plant part | Genotype | Activity units/ml | Protein mg/ml | Specific activity |
|---|---|---|---|---|
| Mature kernel | $Adh_1^F/Adh_1^S$ | 2280 | .65 | 3507 |
| Mature kernel | $Adh_1^F/Adh_1^0$ | 1100 | .67 | 1642 (47%) |
| Root | $Adh_1^F/Adh_1^S$ | 490 | 2.03 | 241 |
| Root | $Adh_1^F/Adh_1^0$ | 350 | 2.1 | 167 (69%) |

In the experiment with mature kernels it was observed that the $Adh_1^F/Adh_1^0$ heterozygotes had approximately half the ADH activity of the $Adh_1^F/Adh_1^S$ heterozygotes, indicating no difference in competitive abilities of the $Adh_1^F$ and $Adh_1^0$ alleles in this stage of development. On the other hand, the $Adh_1^F/Adh_1^0$ roots had 69% of the ADH activity of the $Adh_1^F/Adh_1^S$ roots, a result expected only if the $Adh_1^F$ allele competes for the limited factor about twice as well as $Adh_1^0$. Since $Adh_1^0$ was derived from $Adh_1^S$ and is expected to have the same competitive ability, these results are in close agreement with those obtained in the electrophoretic studies.

## DISCUSSION

The results of the studies on alcohol dehydrogenase in maize cannot be considered as evidence in favor of the proposed model for the regulation of gene activity since the model was formulated on the basis of these results. The evidence presented, however, shows that the model is feasible and the various postulates put forth have factual rather than purely hypothetical bases.

It is fortunate that only the alleles of the $Adh_1$ locus (except for the cases where $Adh_2$ is derepressed) compete for the limited factor. If this were otherwise, and if other genes competed for the same factor; it would have been almost impossible to prove allelic competition, since ADH levels in the $Adh_1^F/Adh_1^F$ and $Adh_1^S/Adh_1^S$ homozygotes would not have been equal.

The competition model readily accounts for the regulation of activity of the $Adh_1$ gene. This scheme may not be limited to *Adh* and could represent the mechanism whereby the activities of a number of different genes are controlled. The striking difference in behavior of the prime and standard $E_1$ alleles (SCHWARTZ 1964) could be explained by this model if we assume that other nonallelic genes compete with $E_1$ for its limited "activating" factor.

The $E_1$ prime alleles differ from the standard alleles in relative activity in various tissues of the plant. The prime and standard alleles show approximately equal activity in the seedling and young endosperm. At about 14 days after fertilization, the prime alleles become either completely or partially (depending on the allele) inactive in the endosperm, whereas the standard alleles retain high activity throughout maturation of the endosperm. This difference holds even in heterozygotes where both standard and prime alleles are present in the same nuclei. It is especially striking in the triploid endosperm which contains two doses

of the prime allele and one dose of the standard allele. In young endosperm, one finds a preponderance of the esterase isozymes specified by the prime allele whereas in older endosperm only the isozyme specified by the standard allele is formed. Some prime alleles show higher activity than the standard alleles in the aerial roots. The $E_1$ esterase behavior differs from alcohol dehydrogenase in that the prime alleles show the drastic reduction in activity in the older endosperm even in homozygotes. However, this is precisely the condition which would be expected to exist if the $E_1$ gene were competing with other nonallelic genes for the same limited factor. We propose that the prime alleles have poor competitive ability in older endosperm, but can compete well in young endosperm, seedling, etc. In homozygous condition, the prime alleles are inactive, since although there are no competing standard $E_1$ alleles present, they still have to compete with the other nonallelic genes in the group for the limited factor. This competition hypothesis is at present not testable for the $E_1$ alleles since we have no idea which nonallelic genes compete with $E_1$ for the same limited factor.

Preliminary results indicate that the competitive abilities of the $Adh_1$ alleles can be altered by treatment with specific plant growth regulators. When seedling root sections are cultured on nutrient agar (MILLER 1965) with 10 ppm of 2,4-dichlorophenoxy acetic acid (2,4-D), ADH is synthesized in these cultures. Cultures of $Adh_1^F/Adh_1^S$ roots show the typical skewed ratio of FF, FS, and SS isozymes observed in roots from intact seedlings, i.e., high FF and low SS. If, however, as low as 1 ppm abscissic acid (kindly supplied by Dr. BLONDEAU of Shell Development Co.) is added to the culture medium, the relative proportions of the isozymes is altered and the ratio approximates the 1FF:2FS:1SS ratio found in mature embryos (Figure 5). Abscissic acid is a "dormancy" hormone found in fruit and other organs during late stages of development, and probably occurs in mature maize kernels. The change in relative competitive abilities of the $Adh_1^F$

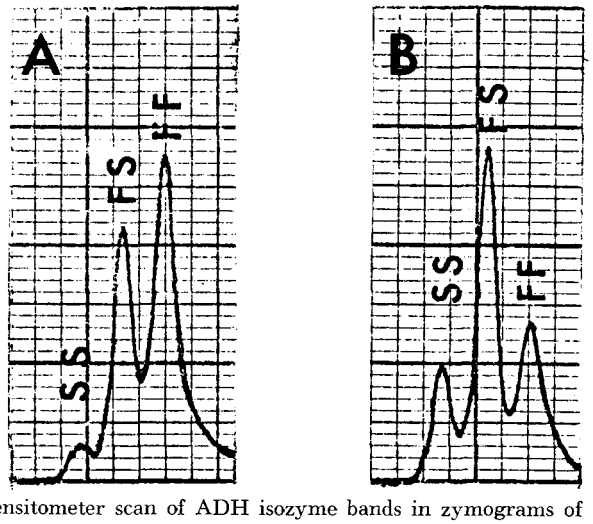


FIGURE 5.—Densitometer scan of ADH isozyme bands in zymograms of $Adh_1^F/Adh_1^S$ root cultures grown in (A) absence of and (B) presence of 1 ppm abscissic acid.

and $Adh_1^S$ alleles by the abscissic acid probably results from an alteration of the physiological condition of the cells.

The invaluable assistance of EILEEN O. SMITH and MARILYN CAMPBELL is gratefully acknowledged.

## SUMMARY

Evidence is presented which established that two alleles of the alcohol dehydrogenase gene in maize compete differentially for a limited factor which is essential for activity. Competition is at the gene level. The competitive abilities of the alleles vary with stage of development. A competition model for the regulation of gene action in differentation is presented and discussed.

## LITERATURE CITED

BURGESS, R. R., A. A. TRAVERS, J. J. DUNN and E. K. F. BAUTZ, 1969 Factor stimulating transcription by RNA polymerase. Nature **221**: 43–46.

EFRON, Y. and D. SCHWARTZ, 1968 *In vivo* inactivation of maize alcohol dehydrogenase by a two-factor system. Proc. Natl. Acad. Sci. U.S. **61**: 586–591.

GALL, J. G., 1956 On the submicroscopic structure of chromosomes. Brookhaven Symp. Biol. **8**: 17–32.

JACOB, F. and J. MONOD, 1961 Genetic regulatory mechanisms in the synthesis of protein. J. Mol. Biol. **3**: 318–356.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, 1951 Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265–275.

MARKERT, C. L., 1961 Isozymes in kidney development. pp. 54–64. In: Proc. 13th Ann. Conf. Kidney. Edited by J. METCOFF. Northwestern Univ. Press, Evanston, Illinois.

MILLER, C. O., 1965 Evidence for the natural occurrence of zeatin and derivatives: Compounds from maize which promote cell division. Proc. Natl. Acad. Sci. U.S. **54**: 1052–1058.

ROMAN, H., 1947 Mitotic nondisjunction in the case of interchanges involving the *B*-type chromosome in maize. Genetics **32**: 391–409.

SALTHE, S. N., O. P. CHILSON and N. O. KAPLAN, 1965 Hybridization of lactic dehydrogenase *in vivo* and *in vitro*. Nature **207**: 723–726.

SCHWARTZ, D., 1962 Genetic studies on mutant enzymes in maize III: Control of gene action in the synthesis of pH 7.5 esterase. Genetics **47**: 1609–1615. ——, 1964 Tissue specificity and the control of gene action. (ERWIN BAUER Gedächtnisvorlesungen III, 1963) pp. 201–203. In: *Structur und Funktion des genetischen Materials*. Akademie Verlag, Berlin. ——, 1966 The genetic control of alcohol dehydrogenase in maize: gene duplication and repression. Proc. Natl. Acad. Sci. U.S. **56**: 1431–1436. ——, 1969 Alcohol dehydrogenase in maize: Genetic basis for multiple isozymes. Science **164**: 585–586.

SCHWARTZ, D. and T. ENDO, 1966 Alcohol dehydrogenase polymorphism in maize—simple and compound loci. Genetics **53**: 709–715.