When the reversal potential of the IPSP had shifted, its "strength," indicated by the slope of the curve expressing the dependence of inhibitory potential on membrane potential, was not diminished, and convergence of the curves was always present at depolarizing membrane potentials (Fig. 2). A shunt of the inhibitory potential by an increased membrane conductivity was only found when the cells were depolarized during the initial period after injection. After the membrane potentials had recovered, determinations of neuronal input resistance and time constant in these cells revealed no significant deviations from the previous values. The maximum shift in membrane potential seen in the motoneurons studied was from 82 to 63 mv. Repeated injections (up to 10 times the effective dosage) did not surpass this limit. We found exceptions in cells which deleteriously depolarized after injection and in which the internal ionic milieu must have been massively altered. These cells were thus excluded.

The postulated limited pore sizes of the postsynaptic inhibitory membrane, as derived from extensive studies (*3*, *4*), should prevent the penetration of most of the ions studied, except for ammonium and hydrogen ions, since their dimensions in the hydrated form are too large. Ammonium ions show such similarity to potassium ions (*5*) that they could hardly be distinguished by the postsynaptic inhibitory membrane pores. The effects of injections of potassium ions or salt are essentially attributed to intracellular accumulation or diminution of chloride (*3*, *4*). Thus, as with potassium ions, one should expect a small and short-term depolarizing E_{IPSP} shift after injection of ammonium in cationic form or a hyperpolarizing shift if ammonium together with the impermeable acetate is applied by cross-barrel injection. Since both forms of application result in similar effects, it seems unlikely that the effect described here can, in general, be derived from intracellular changes of the concentration of chloride ions. There are direct findings which strengthen this supposition. In contrast to the effect of those ions, such as chloride, that participate in the IPSP ionic current flow, the ions studied here caused IPSP equilibrium shifts that were restricted to a rather fixed upper limit of about 60 mv. This shift was observed without a concomitant membrane potential depolarization, which does not favor the assumption that intracellular chloride ion is retained (*3*). Compared to the values reported for other ions (*3*, *4*), the observed times of decay of the shift are of extreme duration. Whether these different times of decay can occur simultaneously was studied in the separate experiments in which chloride ions were also injected. Even if a maximum shift of the equilibrium potential was obtained, the chloride injection that followed resulted in an additional shift. The recovery time from chloride injection was as fast as in normal cells (1 to 2 minutes).

Since chloride ions still pass and the observed limit of the E_{IPSP} shift is comparable to the proposed electrochemical chloride potential (*3*, *4*), the IPSP potassium channel of the treated cells seems to be inhibited and the chloride channel maintained or favored. Both actions would account for the maintained strength of the inhibitory potential as defined above. An action on the postsynaptic inhibitory membrane by saturation of negative charges at a cationic exchange site perhaps offers an explanation of such an alteration of ionic flow. Although the effects are similar for the organic and inorganic ions studied, it remains in question whether the processes underlying their action are identical.

A nerve cell is less likely to undergo inhibition if it shows a reduction of the postsynaptic inhibitory potential at the normal resting membrane potential. It should be noted that such a diminution of inhibition is produced by those substances (ammonium, hydrogen ions) which are increased in epileptogenic states (*6*).

H. D. Lux
P. Schubert
Max-Planck-Institut für Psychiatrie, Kraepelinstrasse 2, 8 Munich 23, Germany

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A Molecular Basis for Heterosis

Abstract. *Alcohol dehydrogenase allodimers composed of an unstable active subunit and a stable but inactive subunit are both active and stable. The implication of this finding for the problem of heterosis is discussed.*

Heterosis, or hybrid vigor, remains an intriguing problem despite the fact that its genetic basis has been under investigation for the greater part of this century. It is very likely a complex phenomenon which can result from many types of gene and allele interactions. Below we present evidence for a form of allele interaction which results in increased enzyme activity in certain heterozygotes and may thus serve as a model for single gene heterosis.

Maize is polymorphic for the alcohol dehydrogenase (E.C.1.1.1.1.) gene, Adh_1. Four alleles have been described (*1*). The Adh_1^S, Adh_1^F, and $Adh_1^{C(t)}$ alleles specify enzymes which differ in charge. The enzyme is a dimer, and three isozymes are formed in heterozygotes. In zymograms of extracts from mature kernels the two autodimer isozyme bands are of equal intensity and the allodimer (hybrid enzyme) is approximately twice as intense. The fourth allele, $Adh_1^{C(m)}$, produces an enzyme which has the same electrophoretic mobility as that specified by $Adh_1^{C(t)}$ but is much less active. The C^mC^m dimer shows only about 1/30 of the activity of the other three autodimer isozymes. An allodimer enzyme composed of the weakly active C^m protomer and an active protomer is about one-half as active as a dimer composed of two active protomers; and the FC^m and FF isozyme bands in $Adh_1^F/Adh_1^{C(m)}$ heterozygotes are of approximately equal intensity. The same condition is observed in the $Adh_1^S/Adh_1^{C(m)}$ heterozygotes.

Studies on the physical-chemical properties of the isozymes specified by the various alleles have revealed that the C^mC^m isozyme differs strikingly from the isozymes specified by the other three alleles in its stability at high *p*H (*2*). The relatively inactive C^mC^m enzyme is very stable at *p*H 10.0, whereas

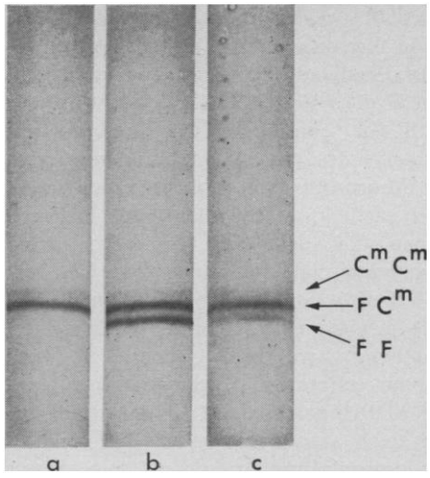

Fig. 1. Alcohol dehydrogenase zymograms of $Adh_1^F/Adh_1^{C(m)}$ kernel extracts. (a) Extract dialyzed against 0.01*M* carbonate-bicarbonate buffer, *p*H 10.0; (b) control, untreated extract; (c) extract incubated with root factor (see text). Origin is at the bottom and the anodal pole at the top.

the active SS, FF, and C^tC^t enzyme forms are irreversibly inactivated at the high *p*H (buffered with carbonate-bicarbonate or glycine-NaOH).

Our recent studies have established that a hybrid enzyme dimer which contains one C^m subunit is both active and stable at *p*H 10.0, as if the stable C^m subunit confers stability to the active subunit in the dimer. This is clearly shown by the following experiment. $Adh_1^F/Adh_1^{C(m)}$ heterozygous kernels were ground in a Wiley mill and the enzyme was extracted by steeping the meal in 0.005*M* sodium phosphate buffer, *p*H 8.0, for 15 minutes at room temperature, with occasional stirring (approximately 1 volume of meal to 2 volumes of buffer). The slurry was centrifuged at 40,000*g* for 10 minutes, and the supernatant was dialyzed overnight at 4°C against 500 volumes of 0.01*M* carbonate-bicarbonate buffer, *p*H 10.0. The dialyzed extract was subjected to starch gel electrophoresis at *p*H 8.3 and the gels were stained for alcohol dehydrogenase at *p*H 8.0 (Fig. 1a) as described in a previous publication (*1*). A control zymogram of an aliquot of the extract which had not been dialyzed at *p*H 10.0 is shown in Fig. 1b. After exposure to high *p*H the FC^m isozyme retains its activity, whereas the FF isozyme is almost completely inactivated. Both the FF and FC^m isozymes remain active when the dialysis is at *p*H 8.0. Similar results are obtained with the $Adh_1^S/Adh_1^{C(m)}$ heterozygotes. The FS, FC^t, and SC^t allodimers do not show this increased stability at high *p*H.

The FC^m dimer is not as stable to high *p*H as the C^mC^m isozyme. After overnight dialysis against the *p*H 10.0 buffer at a higher molarity (0.02*M*) both the FF and FC^m isozymes are completely inactivated while the C^mC^m isozyme retains its low level of activity.

This type of polypeptide interaction whereby an unstable subunit is stabilized by association in a dimer with a stable subunit can be shown to occur in vivo as well. Alcohol dehydrogenase activity in the seedling declines quite rapidly during germination (*3*). Zymograms of extracts from $Adh_1^F/Adh_1^{C(m)}$ seedlings show that the decay of the FF isozyme is considerably more pronounced than the FC^m isozyme as judged from the relative intensities of the two isozyme bands.

This difference in relative intensities of the FF and FC^m bands could also result if the FF and FC^m isozymes decay in vivo at the same rate, but in the seedling the $Adh_1^{C(m)}$ allele is more active in enzyme synthesis than Adh_1^F. If more C^m than F protomers are produced the ratio of FC^m to FF dimers formed would increase. To test this alternative explanation, an enzyme extract from $Adh_1^F/Adh_1^{C(m)}$ mature kernels, in which the FF and FC^m isozyme bands are of equal intensity, was incubated for ½ hour at room temperature with an equal volume of root extract which contains the factor which is responsible for the in vivo inactivation (*3*). Comparison of zymograms of control and treated extracts clearly shows the striking difference in retention of activity between the FF and FC^m isozymes (Fig. 1, b and c).

The implication of these findings to the problem of heterosis is obvious. In general, in order to function effectively in the growth and development of an organism an enzyme must be both active and stable. Through selection, either natural or artificial, alleles which specify such enzyme forms are fixed in the population. However, in the case of some enzymes, and perhaps even many, high stability and activity may be mutually exclusive (that is, no single primary structure can confer both high activity and high stability to the polypeptide). We propose that hybrid vigor may in part result from combining in heterozygotes alleles for active but relatively unstable enzyme forms with alleles which specify stable but inactive enzymes, and that the gene products interact to confer both stability and activity to the hybrid enzyme. It has recently been reported for nitrate reductase in maize that the F_1 hybrid (B 14 × Oh 43) resembles one parent in rate of enzyme synthesis and the other parent in the rate of in vivo decay (*4*).

This advantage of heterozygosity would of course be insured by gene duplication where both alleles are inherited as a unit and transmitted in single gametes. It is of interest in this regard that the only Adh_1 duplication which has been found combines the $Adh_1^{C(m)}$ allele which specifies the stable protomer, and an Adh_1^F allele which specifies a relatively unstable but active protomer. The two genes are very closely linked (*1*).

DREW SCHWARTZ
WILLIAM J. LAUGHNER
Department of Botany,
Indiana University,
Bloomington 47401

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Alcohol Metabolism: Role of Microsomal Oxidation in vivo

Abstract. *A role for microsomal alcohol oxidation cannot be demonstrated in vivo. Factors affecting microsomal drug hydroxylations had no effect on alcohol metabolism in the rat in vivo. The compound SKF 525-A which inhibits, and chronic phenobarbital treatment which enhances microsomal drug hydroxylations did not alter alcohol oxidation.*

Chronic ethanol administration produces a proliferation of the smooth endoplasmic reticulum (*1*) and an induction of microsomal mixed-function oxidase reactions (*1, 2*). Also, microsomal oxidations of alcohols such as ethanol and methanol have been described (*3, 4*). Ethanol oxidation in microsomes depends upon the presence of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) and oxygen, is inhibited by carbon monoxide, and is enhanced by certain inducing agents (*3, 5*). No reversal of carbon monoxide inhibition by light has yet been observed.