9 February 1973, Volume 179, Number 4073 **SCIENCE**

The Barrier to Internal Rotation in Ethane

A qualitative, intuitively useful explanation emerges from a comparison of different theoretical approaches.

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Scientists have long sought to understand the sources of barriers to internal rotation about single bonds. Recognition of the importance of molecular conformation to biochemical activity has increased interest in the problem in recent years. Despite the many theoretical papers published, no general agreement has yet been reached on an explanation for such barriers. In this article, I compare some of the theoretical approaches that have been used and show that a simple and useful explanation for the barrier in ethane appears to be possible.

Classification of Theories

Recent theoretical analyses of the barrier mostly fall into one of the following three categories (see Fig. 1).

1) Decomposition of the total energy change with rotation into various groupings of the change in internuclear repulsion (ΔV_{nn}), the change in nuclear-electronic attraction (ΔV_{ne}), the change in interelectronic repulsion (ΔV_{ee}), and the change in kinetic energy (ΔT). Barriers are categorized depending on how these groups compare in magnitude.

2) Explanations based on delocalized, or canonical molecular orbitals (MO's), frequently with a heavy emphasis on symmetry.

3) Explanations based on localized MO's, produced by intermixing occupied canonical MO's to form new orbitals which can be labeled "lone pair," "inner shell," "C-H bond," and so forth.

It is useful to distinguish two classes of approximation common to all three categories. Rigid-rotor (RR) calculations are those in which all bond distances and angles (except the torsional angle) are identical for all conformations. In geometry-optimized (GO) calculations the energy is minimized for all bond distances and angles in each conformation.

Groupings used in the category 1 approaches have varied. Fink and Allen (*1*) originally proposed combining ΔV_{nn}, ΔV_{ne}, and ΔT in one group, ΔV_{ee} being the other. More recently, Allen and his collaborators have come to prefer $\Delta V_{\mathrm{nn}} + \Delta V_{\mathrm{ee}} + \Delta T$ as one group (called "repulsive") with ΔV_{ne} as the other ("attractive") (*2*). When the change with internal rotation is greater for the repulsive group than for the nuclear-electronic attraction (V_{ne}), the barrier is said to be "repulsive dominant." In the opposite situation, it is "attractive dominant." Very recently, an alternative grouping ($\Delta V_{\mathrm{nn}} + \Delta V_{\mathrm{ee}}$ as repulsive, $\Delta V_{\mathrm{ne}} + \Delta T$ as attractive) has been proposed (*3*). This grouping has the advantage of being fairly insensitive to scaling of the wave function.

A number of barrier calculations have been analyzed in terms of such groupings, and serious difficulties have emerged. It has been found that the qualitative description of a barrier (as repulsive or attractive dominant) can change depending on whether the calculation is carried out in the rigid-rotor or the geometry-optimized approximation. Thus, the ethane barrier is repulsive dominant in the RR case and attractive dominant in the GO case (*4*). Hydrogen peroxide has been shown to possess similar instability (*5*). Even when two calculations are based on identical geometries, it appears that the use of different basis sets can change a calculated barrier from attractive to repulsive dominant (*5*). The sensitivity of components of the total energy to small geometry changes was pointed out in 1963 by Pitzer and Lipscomb (*6*) and has been reemphasized several times since then (*4, 7*). It arises from the fact that the variation method leads to a minimum on the total energy curve, but not to minima for the components. Hence, if we are at or near the equilibrium geometry, the total energy will be much less sensitive to small geometry changes than the components will be. A similar argument applies for scaling (*8*) or basis set change.

Another difficulty with the category 1 approach is its failure, to date, to lead to a model that enables us to guess a barrier in advance of experiment or calculation. The terms ΔT, ΔV_{ee}, and ΔV_{ne} involve integrations over the entire molecule. Unless these are broken down, region by region, we do not know why a barrier is attractive or repulsive dominant in terms of specific intramolecular interactions. It does appear, from examining analyses published so far, that a barrier is usually repulsive dominant when it is in phase with V_{nn}, and attractive dominant when

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it is out of phase. However, the sensitivity to the basis set mentioned above indicates that this rule cannot always be relied on.

The instability of group 1 categorizations with geometry and basis set variations, together with the lack of insight provided by the labels attractive dominant and repulsive dominant, leaves the usefulness of this approach open to serious question. For the remainder of this article, I will be concerned with relations between categories 2 and 3.

At the outset, it is useful to subdivide category 2 (explanations based on delocalized MO's) into two classes. One class involves MO's resulting from Hückel-type methods, wherein the total energy is simply the sum of one-electron energies, and the wave function is a simple product of MO's. The other class involves ab initio self-consistent field (SCF) MO calculations, wherein the total energy is not the sum of one-electron energies, and the wave function is an antisymmetrized product of MO's.

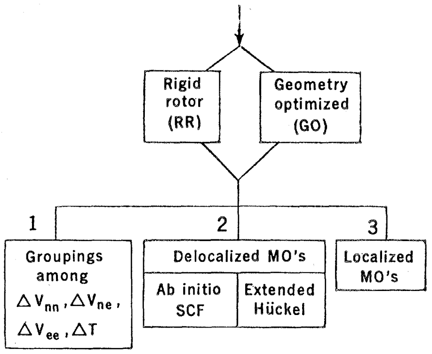


Fig. 1. Theoretical approaches to barriers to internal rotation about single bonds.

Repulsion between Helium Atoms

Before treating ethane itself, it is instructive to consider the interaction between two helium atoms from the viewpoints of localized and delocalized orbitals. This system serves as a model for treating repulsions with MO theory.

In approaches involving delocalized orbitals (extended Hückel and ab initio) the lowest-energy MO is He-He bonding ($1\sigma_g$) and the MO with the next lowest energy is antibonding ($1\sigma_u$). Each of these MO's is occupied by two electrons. The antibonding character of $1\sigma_u$ dominates the bonding character of $1\sigma_g$ because the presence of a nodal plane necessitates larger coefficients for the atomic orbitals (AO's) in $1\sigma_u$ (see Fig. 2A). This has long been recognized and has been reemphasized recently (*9, 10*). Our first point, then, is that (I) the repulsion between two helium atoms is seen, from the point of view of delocalized orbitals, as being due to the dominance of the antibonding member in a bonding-antibonding pair of MO's (*11*). This dominance arises from the requirement that the orbitals be simultaneously orthogonal to each other and normalized. Coulson (*9*) has shown that, "provided the overlap integrals are small and positive, we expect all bond orders in the case of a completely filled MO shell to be negative."

The description in terms of equivalent localized orbitals has a different appearance. Localized orbitals are produced by mixing together the $1\sigma_g$ and $1\sigma_u$ MO's of Fig. 2A to form new functions, which are localized mostly on one helium or the other (*12*). It can be seen that combining the delocalized MO's of Fig. 2A will produce MO's of the general appearance shown in Fig. 2B. The difference in coefficient sizes in $1\sigma_u$ and $1\sigma_g$ MO's leads to the existence of "tails" in the localized orbitals. As the helium atoms come together, the tail of a particular localized orbital grows larger and interacts with the rest of the localized orbital in an antibonding way. The net interaction between helium atoms is still repulsive because localized orbitals are completely equivalent to delocalized orbitals in terms of total energy and total electron density distribution. Thus, we have that (II) the net antibonding between two helium atoms is manifested in the localized orbitals as an antibonding, or destructive, interference between the main part of a localized orbital and its tail.

The repulsion between two helium atoms is one example of what chemists refer to as "steric repulsion." What I have discussed, then, are the descriptions in terms of equivalent localized and delocalized orbitals of steric repulsion between two closed-shell atoms.

Ethane in the Rigid-Rotor Approximation

When we consider the barrier to internal rotation in ethane, we are no longer concerned with completely filled MO shells. Nevertheless, there is much that is similar to the He-He situation.

Consider first the occupied delocalized MO's of ethane shown in Fig. 3. These occupied MO's are all C-H bonding. The valence atomic orbitals also produce an equal number of empty C-H antibonding MO's (not shown). Thus, compared to He_2, ethane repre-

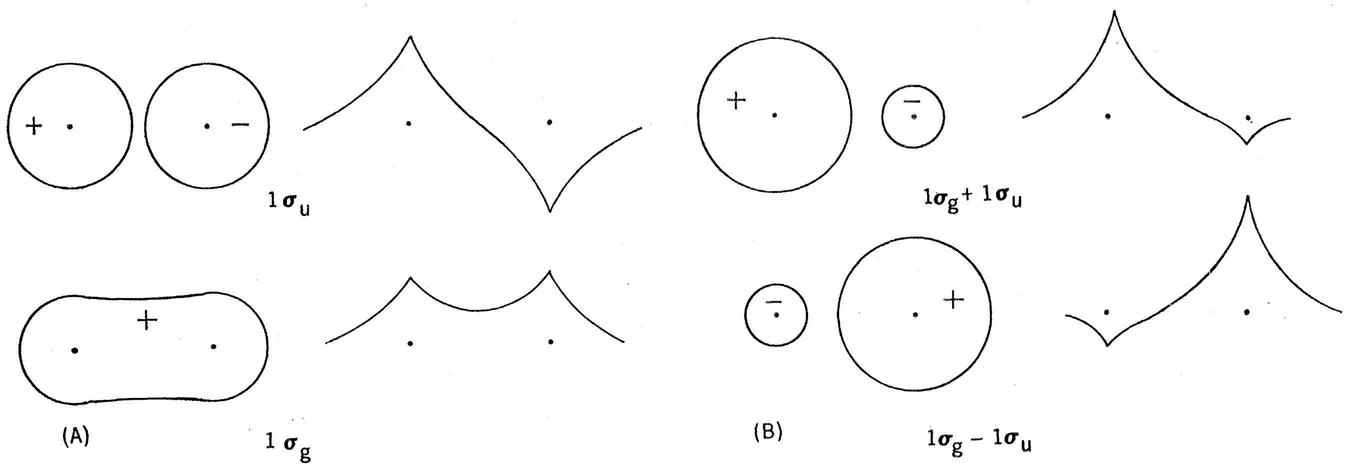


Fig. 2. (A) The $1\sigma_g$ and $1\sigma_u$ MO's of He_2. The $1\sigma_u$ MO has a larger absolute value near the He nuclei because of the presence of the node. (B) Localized orbitals formed by taking linear combinations of $1\sigma_g$ and $1\sigma_u$ of (A).

sents a system with a half-filled valence MO shell. The general nature and energy order of these valence MO's is quite similar in Hückel-type and ab initio calculations. The orbital energy increases as we go from a particular type of C-C bonding MO to its antibonding mate. Also, MO's involving carbon 2*s* AO's tend to be lower in energy than those involving 2*p* AO's.

If we seek the reason for the relative stability of staggered ethane within the framework of Hückel methods and delocalized orbitals (making the RR approximation) we quickly find that the only significant energy changes in the valence MO's come from $1E_u \rightarrow 1E'$ and $1E_g \rightarrow 1E''$ orbitals (*13*). The lower-energy set stabilizes eclipsed ethane, the higher-energy set stabilizes staggered ethane (see Fig. 3). The higher set dominates because it has an extra node between the carbons, leading to larger coefficients and net antibonding between vicinal eclipsed C-H bonds in eclipsed ethane (*14*). Notice the resemblance between the *E*(b) MO's in Fig. 3 and the familiar pi MO's of butadiene. The argument (*15*) that the greater stability of *trans*- as opposed to *cis*-butadiene is due to net antibonding between terminal carbons is similar to our argument for ethane. We conclude that (III-a) the explanation based on extended Hückel delocalized MO's for the relative instability of eclipsed ethane is that the methyl groups experience a greater net antibonding interaction in the eclipsed conformation.

Note that the interaction between vicinal hydrogens is antibonding when they are *cis* coplanar and that it becomes bonding as they become *trans* coplanar. It is important to bear in mind that the vicinal H-H interactions in ethane as depicted by delocalized MO's are not always antibonding (*16*).

The bonding or antibonding interactions between vicinal hydrogens are brought about by the need for orbital orthogonality and normality, just as was true for the He-He interaction. But here we have a half-filled, rather than a completely filled, MO shell. If we admit this as a kind of steric interaction (and there seems no fundamental reason for not doing so) we can say that (III-b) an explanation based on extended Hückel delocalized MO's for the relative instability of eclipsed ethane is that there is greater steric repulsion between eclipsed methyl groups than staggered methyl groups.

Returning momentarily to the butadiene analog mentioned above, we can make a similar kind of statement: (IV) an explanation based on simple Hückel MO's for the relative instability of *cis*-butadiene is that there is steric repulsion between terminal carbons due to pi-electronic interactions. This example makes it evident that our use of the term "steric" is somewhat broader than is traditional. There is no clear demarcation between what has traditionally been called steric repulsion and what has been called orbital control (*17*).

If we look to ab initio calculations for confirmation of these ideas based on Hückel-type calculations, we face difficulties that have not yet been overcome directly. The basic problem is that the one-electron energies do not have the same meaning in the two methods. Hückel one-electron energies are supposed to sum to the total energy, whereas SCF one-electron energies count V_{ee} twice and do not include V_{nn} at all. Thus, there is no compelling reason to expect orbital energy changes to be comparable in the two methods (*18*). We must seek other ways to see if the more reliable SCF method "agrees" with the explanations given above.

Table 1. Bond orders between hydrogen A and its vicinal relative *j*. For H_A-C-C-H_j dihedral angles 0°, 60°, 120°, and 180°, *j* = 1, 2, 3, and 4, respectively. The ab initio values are from (*7*).

	Bond order	
	EHMO	Ab initio
1	− 0.2267	− 0.3037
2	− 0.1486	− 0.1423
3	+ 0.0337	+ 0.1710
4	+ 0.1416	+ 0.3234

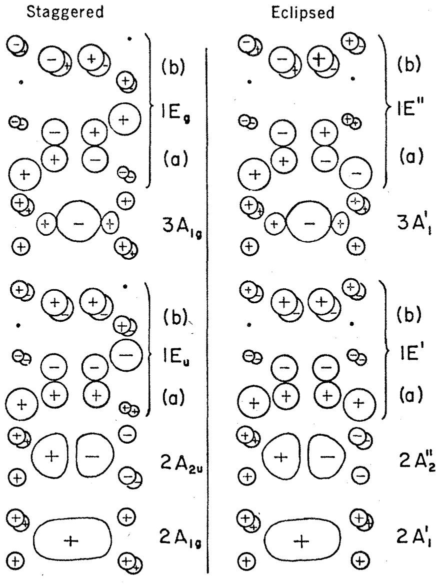


Fig. 3. Occupied MO's of ethane. The energy increases upward.

One way is to compare long-range bond orders, which give an indication of how much bonding there is between atoms. By this measure there is qualitative agreement between ab initio and extended Hückel (EHMO) methods. Table 1 indicates that both methods predict an antibonding interaction between *cis* coplanar vicinal hydrogens and a bonding interaction between *trans* hydrogens.

Another way these methods have been compared is through localized orbitals. It is possible to transform the ab initio delocalized MO's of ethane into localized orbitals which may be identified as C-H bonding, C-C bonding, and inner shell. This has been done by Pitzer (*7*) and England and Gordon (*19*), the latter workers using SCF wave functions obtained in the approximation involving intermediate neglect of differential overlap (INDO) (*20*). In analyzing the contributions of localized orbitals to the barrier, England and Gordon included two positive charges on nuclei with each electron pair in a localized orbital, thereby including V_{nn} in a systematic and reasonable way. Their analysis for a rigid rotation led them to state that the barrier in ethane is almost entirely due to the C-H bond localized orbitals, which exhibit the greatest destructive interference between the bond and its vicinal tails in eclipsed ethane. A C-H bond localized orbital for each conformation of ethane is sketched in Fig. 4. Note how the nature of the tails in these orbitals is strongly reminiscent of the *E*-type delocalized MO's of Fig. 3. England and Gordon have traced their explanation based on localized orbitals back to the starting delocalized MO's and have indeed found that most of the interference between the C-H bond and its tails arises from the *E*-type MO's that are central to the extended Hückel explanation. This supports the contention that the explanation pertaining to extended Hückel calculations of the barrier is also largely applicable to ab initio SCF calculations, where it is disguised by complications mentioned earlier. The residual tails in the localized C-H orbitals result from the fact that the $1E_g$-$1E''$ delocalized MO's have a node that is absent from the $1E_u$-$1E'$ set. The existence of an additional node and its proximity to the carbons (*15*) leads to larger coefficients for the hydrogens in the higher-energy set. Thus, some parallelism with He_2 is maintained.

On the basis of these remarks, we may tentatively (*21*) state that (V) the localized-orbital explanation of the relative instability of eclipsed ethane is that there is repulsion between eclipsed C-H bonds. Moreover, this view is not inconsistent with the SCF delocalized MO's from which the localized orbitals are derived. We emphasize that this C-H bond repulsion is not analogous to electrostatic repulsion, because the C-H bonds have a bonding interaction at certain angles.

A related study in terms of localized orbitals was described by Sovers *et al.* (*10*), who calculated energies for staggered and eclipsed ethane by using completely localized C-H and C-C bond functions as a basis set. They first made their calculations by using as the wave function a Hartree-type product of these nonorthogonal bond functions. This led to a lower calculated total energy for eclipsed than for staggered ethane. Then they recalculated the energies, but required an antisymmetrized wave function and obtained a reasonable barrier. The antisymmetry requirement, in effect, forces the orbitals to become orthogonal to each other, and they accomplish this by becoming a little delocalized, that is, by growing tails. These authors concluded that (*10*) "the overlap (repulsive) interaction between bond orbitals due to the Pauli exclusion principle is the primary factor in the rotational barrier. Thus, the dominant term in the ethane barrier can be considered analogous to the closed-shell repulsion between helium atoms." We have seen that there is indeed a strong analogy with the He_2 interaction, but that there is an important distinction. The He-He interaction is always antibonding in simple MO theory, whereas, in ethane, the analogous C-H bond interaction may be antibonding or bonding depending on the angular relationship. This bonding-antibonding nature of vicinal hydrogen atoms was also found in staggered ethane by Pople and Santry (*22*), who used a perturbation approach.

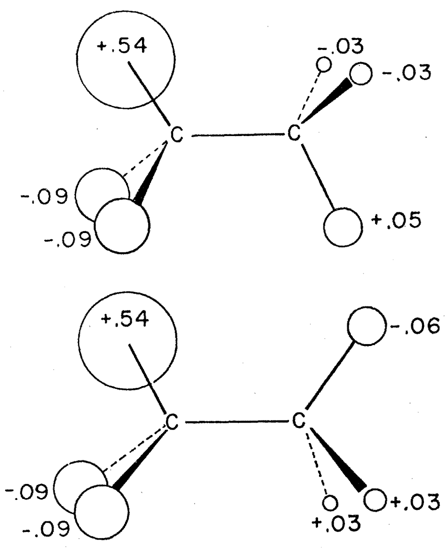
Ethane in the Geometry-Optimized Approximation

The fairly recent capability for minimizing the energy of both eclipsed and staggered conformations with respect to all bond distances and angles has produced new insights and problems. We have already seen that the approaches of category 1 appear particularly sensitive to geometry optimization. We will now examine how the explanations based on delocalized and localized MO's are affected.

It has been found that, starting with geometry-optimized staggered ethane, a rigid rotation to eclipsed ethane (which we will call step 1) gives an ab initio calculated barrier of about 3 kilocalories per mole. Subsequent geometry optimization of eclipsed ethane (step 2) causes the molecule to expand. [The C-C bond lengthens and the C-C-H angles increase (*5*, *23*). Notice that this expansion is consistent with the end-to-end repulsive interaction already described.] The energy lowering associated with step 2 is very small compared to the energy change in step 1 (*23*). It is tempting to hope that this means that inclusion of step 2 will not effect the explanation for the barrier, but this is not necessarily the case. It seems inescapable that some of the increased C-H bond repulsion energy of step 1 will be transformed to energy of stretching of the C-C bond and energy of opening of the C-C-H angles in step 2 (*24*). England and Gordon (*19*) performed a localized orbital analysis for a GO calculation (INDO method). A somewhat opened C-C-H bond angle was observed, and a noticeable change in the C-C bond energy (favoring eclipsed ethane) due to increased 2*s* character was noted. The interaction between C-H bond orbitals and vicinal tails was still found to dominate the energy changes producing the barrier. However, their optimized eclipsed molecule did not exhibit the stretched C-C bond which more accurate calculations show, so this conclusion is not final.

Stevens and Karplus (*25*) have repeated the calculation of Sovers *et al.* (*10*), but have used the optimized geometries of Stevens (*23*). They obtain similar results as in the RR case, and they argue that this means that the barrier is produced by the same factor in each case. However, this doesn't answer the question of the detailed way in which interaction energies differ in distribution among various parts of the molecule for RR and GO calculations.

In part, at least, there is confusion here over what constitutes an adequate explanation of the barrier in ethane. Most chemists will probably be satisfied with an explanation of this sort: If we treat the ethane barrier as a two-step process, the energy of the barrier is almost entirely due to increased C-H bond repulsion in eclipsed ethane reached by a rigid rotation (step 1). Subsequent relaxation (step 2) causes almost no energy change but does redistribute the C-H bond repulsions somewhat into C-C bond stretching, and so forth. The quantitative nature of the redistribution of step 2 is not yet known in detail in localized terms. In short, the barrier is "caused" by eclipsed C-H bond repulsion (or by eclipsed H---H antibonding) in the eclipsed form (*26*).



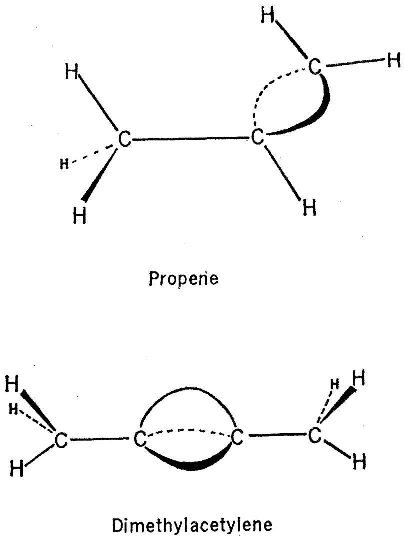


Fig. 4 (left). Coefficients of hydrogen 1*s* AO's in a localized C-H bond for staggered and eclipsed ethane [data from (*7*)]. Fig. 5 (right). Staggered adjacent bonds of Pauling's model for propene and dimethylacetylene.

Comparison with Pauling's Model

Pauling (*27*) has proposed a model for predicting stable conformations and rough barrier values in a large range of molecules. The central feature of this model is the preference for a staggered configuration of bonds emanating from adjacent atoms. For double or triple bonds, the bent-bond description is used (see Fig. 5). Pauling's rationalization of this model in terms of the participation of *d* and *f* orbitals in bonds has not been supported by ab initio calculations (*6*), but this does not necessarily mean the model itself should be discarded. In fact, the model was constructed to fit observed facts about conformations and barriers, and should be judged independently of its rationalization. The use of Pauling's model makes it possible to get around difficulties in extending the localized bond picture to barriers in certain other molecules.

For example, Fig. 6 indicates how Pauling's model predicts that dimethylacetylene should prefer an eclipsed conformation. The same prediction is given by the delocalized MO arguments. The pi-type MO's sketched in Fig. 6 should give a net bonding *cis* H---H interaction (albeit over a very long distance, so the barrier should be very small). Here, then, is a case where the eclipsed C-H bonds exhibit what is analogous to a steric attraction (*28*). This is certainly different from what one might have guessed from the extension of an oversimplified bond-bond repulsion model, and indicates that the extension of models based on localized interactions will require some caution (*29*).

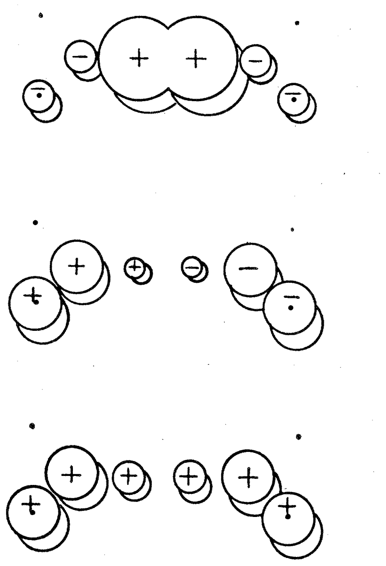


Fig. 6. The three occupied *E*(b)-type MO's in eclipsed dimethylacetylene.

Conclusions

The internal rotation barrier in ethane appears susceptible to "explanation" at a qualitative, intuitively useful, reasonably correct level. At this level, localized and delocalized MO's each produce a description.

For a rigid rotation, the delocalized description is basically the "orbital control" type familiar from Woodward-Hoffmann rules (*30*), Walsh's rules (*31*), and the formation of diatomic molecules (*32*). This description indicates that long-range eclipsed H---H antibonding is responsible for the barrier. The equivalent localized-bond description invokes destructive interference or repulsion between eclipsed C-H bonds. The extension of this kind of description to other molecules requires care.

Subsequent to rigid rotation, the relaxation of ethane into its optimum eclipsed geometry produces almost no energy change but produces an unknown degree of energy "redistribution."

I have emphasized that the theoretical distinction between "orbital control" and "steric interaction" is not precise and that a clarification in terminology may be desirable in this connection.

The discussion of barriers given here applies only to a limited class of molecules, exemplified by ethane. No doubt, additional factors enter into a proper description of barriers in molecules having lower symmetry or more polar bonds, or both (*33*).

Finally, I should point out that approaches other than those in categories 1 to 3 have been made in efforts to rationalize or predict barriers to internal rotation (*34*). The Hellmann-Feynman theorem has been applied to find the torque on ethane at conformations between staggered and eclipsed (*35*). The integral Hellmann-Feynman theorem has served as a basis for discussing barriers in terms of transition densities (*36*) and has led to an electrostatic model for barriers which has given some remarkably successful barrier predictions (*37*). These approaches are mathematically valid, and it is perfectly legitimate to try to extract physical explanations from them also. Thus, several valid explanations for the barrier to internal rotation in ethane are possible. However, I feel that the explanations proposed above in terms of delocalized or localized orbitals are preferable at present inasmuch as they are couched in terms and concepts currently in the mainstream of chemical thinking.

References and Notes

1. W. H. Fink and L. C. Allen, *J. Chem. Phys.* **46**, 2261, 2276 (1967).
2. L. C. Allen, *Chem. Phys. Lett.* **2**, 597 (1968); ——— and H. Basch, *J. Amer. Chem. Soc.* **93**, 6373 (1971).
3. A. Liberles, B. O'Leary, J. E. Eilers, D. R. Whitman, *J. Amer. Chem. Soc.* **94**, 6894 (1972).
4. I. R. Epstein and W. N. Lipscomb, *ibid.* **92**, 6094 (1970).
5. A. Veillard, *Theor. Chim. Acta* **18**, 21 (1970).
6. R. M. Pitzer and W. N. Lipscomb, *J. Chem. Phys.* **39**, 1995 (1963).
7. R. M. Pitzer, *ibid.* **41**, 2216 (1964).
8. L. Pedersen and K. Morokuma, *ibid.* **46**, 3941 (1967).
9. C. A. Coulson, *Mol. Phys.* **15**, 317 (1968).
10. O. J. Sovers, C. W. Kern, R. M. Pitzer, M. Karplus, *J. Chem. Phys.* **49**, 2592 (1968).
11. This does not mean that this is the only interpretation consistent with MO theory. One may also examine energy components such as ΔV_{nn}, for example. But, for a comparison of theories, the statement in the text seems more useful.

→ J. E. Lennard-Jones and J. A. Pople, *Proc. Roy. Soc. London Ser. A* **202**, 166 (1950); C. Edmiston and K. Ruedenberg, *Rev. Mod. Phys.* **35**, 457 (1963).

13. R. Hoffmann, *J. Chem. Phys.* **39**, 1397 (1963).
14. J. P. Lowe, *J. Amer. Chem. Soc.* **92**, 3799 (1970); R. Hoffmann, *Pure Appl. Chem.* **24**, 567 (1970).
15. The dominance of hydrogen coefficients in $1E_g + 1E''$ MO's occurs even in calculations in which overlap is neglected. This comes about because the node exists between the carbon atoms, so the electron density is shifted away from the carbon to the hydrogen atoms. For further discussion of this point, see J. P. Lowe, *J. Amer. Chem. Soc.* **94**, 3718 (1972).
16. We shall sometimes refer to interactions having long-range bonding indexes as "attractive," those with antibonding indexes as "repulsive." However, it is not obvious that an intramolecular bonding index always corresponds to a true attraction. (In fact, a rigorous useful theoretical definition of intramolecular attraction might be difficult to produce.) But it seems safe to say that the interaction between two antibonding parts of a molecule is more repulsive than that between similarly disposed bonding parts.
17. This does not imply that intramolecular steric interactions are describable by the same parameters and radii as intermolecular interactions.
18. The sum of one-electron energies in the SCF calculation of (*8*) changes by roughly five times the barrier value on internal rotation. The total energy change is in good agreement with the barrier.
19. W. England and M. S. Gordon, *J. Amer. Chem. Soc.* **93**, 4649 (1971).
20. J. A. Pople and D. L. Beveridge, *Approximate Molecular Orbital Theory* (McGraw-Hill, New York, 1970).
21. Statement (V) is tentative because the SCF method used by England and Gordon is an approximation to the ab initio method.
22. J. A. Pople and D. P. Santry, *Mol. Phys.* **9**, 301 (1965).
23. R. M. Stevens, *J. Chem. Phys.* **52**, 1397 (1970).
24. Epstein and Lipscomb (*4*) have pointed out that the SCF orbital energy changes in the GO calculation are quite different from those in the RR calculation. These differences are mostly understandable with the observation that, for the stretching of step 2, C-C bonding MO's tend to increase in energy and antibonding MO's tend to decrease. However, as we indicated earlier, SCF orbital energies do not yet provide a satisfactory basis for understanding total energy changes. (For step 2, even though the total energy change is almost zero, the sum of SCF one-electron energies changes by about twice the barrier value.)

25. R. M. Stevens and M. Karplus, *J. Amer. Chem. Soc.* **94**, 5140 (1972).
26. One could just as well focus on effects stabilizing the staggered form, but the viewpoint taken here seems more natural.
27. L. Pauling, *The Nature of the Chemical Bond* (Cornell Univ. Press, Ithaca, N.Y., ed. 3, 1960), pp. 130–142.
28. Note that this attraction is not the van der Waals attraction due to correlated electron motion.
29. The stable form and barrier in dimethylacetylene have not been determined experimentally or by ab initio calculation. Extended Hückel calculations (with hydrogen orbital exponent 1.2) predict that the eclipsed form should be stable by 0.0025 kcal/mole.
30. R. B. Woodward and R. Hoffmann, *The Conservation of Orbital Symmetry* (Academic Press, New York, 1970).
31. A. D. Walsh, *J. Chem. Soc. London* (1953), pp. 2260, 2266, 2288, 2296, 2301, 2306, 2321.
32. R. S. Mulliken, *Rev. Mod. Phys.* **4**, 1 (1932).
33. L. Radom, W. J. Hehre, J. A. Pople, *J. Amer. Chem. Soc.* **94**, 2371 (1972).
34. For reviews, see J. P. Lowe, *Progress in Physical Organic Chemistry*, A. Streitwieser and R. W. Taft, Eds. (Interscience, New York, 1968), vol. 6, p. 1; E. B. Wilson, Jr., *Advances in Chemistry Physics*, I. Prigogine, Ed. (Interscience, New York, 1959), vol. 2, p. 367.
35. J. Goodisman, *J. Chem. Phys.* **44**, 2085 (1966); L. C. Allen and J. Arents, *ibid.* **57**, 1818 (1972).
36. R. E. Wyatt and R. G. Parr, *ibid.* **41**, 3262 (1964); *ibid.* **43**, S217 (1965); *ibid.* **44**, 1529 (1966).
37. J. P. Lowe and R. G. Parr, *ibid.* **43**, 2565 (1965); *ibid.* **44**, 3001 (1966); J. P. Lowe, *ibid.* **45**, 3059 (1966); *ibid.* **51**, 832 (1969); *ibid.* **52**, 3314 (1970).

Hemolytic Anemia and G6PD Deficiency

Physiologic activity, not in vitro activity, of enzymes is related to the severity of genetic diseases.

Akira Yoshida

The discovery that primaquine-induced hemolytic anemia was associated with an inherited deficiency of glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate: NADP oxidoreductase, E.C. 1.1.1.49) (G6PD) in red blood cells (*1*) led to many investigations of the genetic variants of this enzyme in man (*2*). This enzyme, G6PD, catalyzes the initial step in the hexose monophosphate oxidation pathway of carbohydrate metabolism causing reduction of NADP to NADPH. Under normal physiologic conditions glucose is metabolized primarily (more than 90 percent) via the Embden-Meyerhof pathway to produce lactate. During this process no net generation of NADH occurs since 1 mole of NAD is reduced to NADH by glyceraldehyde 3-phosphate dehydrogenase while 1 mole of NADH is oxidized to NAD by lactate dehydrogenase. Lactate can be oxidized by the aerobic oxidation pathway producing NADH and NADPH in the nucleated cells of various tissues. However, since the matured human red cells lack the oxidative enzymes of the Krebs cycle, the hexose monophosphate shunt pathway in red cells has a particular importance in generating NADPH. NADPH is required by the red cell glutathione reductase (E.C. 1.6.4.2) to maintain glutathione in the reduced state. Reduced glutathione appears to be necessary for maintaining sulfhydryl groups within the red cell and perhaps in the red cell membrane. Thus, severe genetic deficiency of G6PD is frequently associated with a low concentration of reduced glutathione and with hemolytic anemia.

More than 80 variants of G6PD, which are distinguishable from one another by their kinetic characteristics, electrophoretic mobilities, and substrate specificities, have been reported (*3*). The amino acid substitution has been elucidated in only two of these variant enzymes, the common Negro variant G6PD A+ (*4*) and G6PD Hektoen (*5*) which is associated with overproduction of the enzyme (*6*). By analogy with the structure of many human hemoglobin variants, most of the G6PD variants are presumed to be caused by single amino acid substitutions.

About 40 variants have normal activity or mild enzyme deficiency in red cells and therefore are not associated with any clinical manifestations. Another group of variants causes severe enzyme deficiency in red cells, but requires exogenous agents such as drugs, infections, or fava beans for hemolysis to occur. Other variants (about 20) are associated with chronic nonspherocytic hemolytic anemia even in the absence of exogenous agents. Deficiency of G6PD is the commonest genetically determined enzymatic abnormality in human beings, probably affecting more than 100 million males. Because the gene determining the structure of the G6PD molecule is located on the X chromosome (*7*), almost twice as many females carry gene coding for variants of G6PD.

In contrast to genetic defects located on the autosomal chromosomes, the location of the gene for G6PD on the X chromosome results in males that carry a single G6PD gene. In the study of the molecular abnormality, enzymatic characteristics, and physiologic disorders caused by genetic mutation of G6PD, one can avoid some of the difficulties that arise when studying a heterogeneous mixture of normal and variant enzymes. Thus, G6PD deficiency has become a model system for understanding the molecular pathology of genetic diseases, and much valuable knowledge has been obtained which advances not only the understanding of G6PD disorders, but also the understanding of other genetic disorders [see, for example, the review by Kirkman (*8*)].

The degree of enzyme deficiency does not correlate well with the clinical severity of the disease, not only in the case of G6PD variants but also in other human enzyme abnormalities. This is one of the problems in the molecular pathology of genetic disorders. Thus, some variant subjects associated with severe G6PD deficiency, such as Gd Markham (*9*) and Gd Union (*10*), cause no hemolytic problem while other variants associated with less severe G6PD deficiency, such as Gd Manchester (*11*), Gd Alhambra (*12*), and Gd Tripler (*13*), cause chronic hemolytic anemia even in the absence of exogenous agents. Kinetic characteristics (affinity

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