MOLECULAR PHARMACOLOGY, 7, 328–336

Theoretical Considerations of *Alpha* and *Beta* Adrenergic Activity

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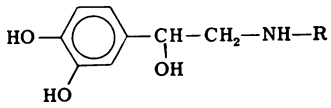
(Received December 2, 1970)

SUMMARY

It appears that *N*-alkyl groups necessary for *beta* adrenergic activity act directly on the receptor rather than by influencing the conformation of the side chain relative to the catechol ring or the charge distribution around the nitrogen. While *alpha* activity probably involves hydrogen bond formation between an onium proton and a negatively charged receptor, *beta* activity is likely to involve dispersion binding between the alkyl group and receptor such that the nitrogen may not be necessary for *beta* activity.

INTRODUCTION

In 1948 Ahlquist (1) proposed that adrenergic agonists could be divided into two general classes, designated *alpha* and *beta*, on the basis of target organ response. Since that time, it has become generally recognized that there are adrenergic receptors responding to different patterns of structure in active molecules. The catecholamine ring and aliphatic hydroxyl and amino groups have all been shown to be important for adrenergic activity and presumably interact with the receptors. A perplexing problem has been the observation that structural changes in the agonist that diminish *alpha* activity may increase *beta* activity, and vice versa. Thus, in the catecholamine series (I), norepinephrine (Ia) is one of the most potent *alpha* adrenergic agonists. Monosubstitution with methyl (epinephrine, Ib), ethyl (Ic), and isopropyl (isoproterenol, Id) leads to decreasing *alpha* agonist potency but to increasing potency of *beta* agonist activity.



I. Adrenergic catecholamine
(a) R = H
(b) R = CH_3
(c) R = CH_3CH_2
(d) R = $(CH_3)_2CH$

An unresolved question has been whether the R group exerts its influence on the relative degrees of *alpha* and *beta* activity by altering the preferred conformation of the side chain relative to the catechol ring, by influencing the reactivity of the amino group, or by direct interaction with the receptor. Here we report the results of a theoretical study of the influence of the R group on the conformation of the side chain and the charge distribution on the onium group.

This study was supported by Grants FR 5409-07 and GM 16312 and by Research Career Development Award AM 1159 to J. M. G. from the National Institutes of Health.

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The extended Hückel theory as proposed by Hoffmann (2) was used to perform the conformational calculations. Briefly, the molecular orbitals ψ_m are written as a linear combination of atomic basis functions, φ:

$$\psi_m = \sum_j C_{mj}\varphi_j$$

Assuming an effective 1-electron Hamilton operator, H, we write $H_{ij} = \int \varphi_i H \varphi_j \, d\tau$. The overlap integrals $S_{ij} = \int \varphi_i \varphi_j \, d\tau$ are also required. Minimization of the molecular energy with respect to the coefficients C_{mj} then leads to the secular equation

$$\det (\mathbf{H} - E\mathbf{S}) = 0$$

and **H** and **S** are the matrices formed from the integrals H_{ij} and S_{ij}, respectively. The calculations consider only the valence electrons, and the diagonal elements of **H** are approximated by valence state ionization potentials. All the overlap integrals, S_{ij}, are computed exactly for a Slater orbital basis set, and the off-diagonal elements of **H** are approximated by the equation

$$H_{ij} = 0.5K(H_{ii} + H_{jj})S_{ij}$$

with K set at 1.75 (Table 1). The Hückel energy is computed as the sum of the occupied orbital energies found from the solution of the secular equation, multiplied by an occupation number. The molecules considered in this study contain an even number ($2k$) of electrons, and these are then placed two at a time in the lowest-lying k molecular orbitals, so that the extended Hückel energy is $E_{EH} = 2\sum_{s=1}^{k} E_s$, where E_s is the energy level corresponding to the molecular orbital ψ_s.

Extended Hückel theory has been rather successful for the prediction of preferred conformations of molecules (2–12), although rotational barriers and energy differences between *trans* and *gauche* conformations tend to be exaggerated. The rationale for the success of the extended Hückel theory for conformational studies is found in the work of Fink and Allen (13), who considered the conditions under which the theory should yield results similar to those of studies *ab initio*. These conditions are not stringent, and apply to the types of internal rotational modes studied in this paper. The work of Fink and Allen also shows that barriers computed by using sums of orbital energies as an approximation to molecular energies will generally yield exaggerated results.

TABLE 1

Extended Hückel theory parameters

Coulomb integrals			Slater exponents	
Electron		Value	Atom	Value
		eV		
N	2*s*	−26.00	H	1.000
N	2*p*	−13.40	C	1.625
O	2*s*	−35.30	N	1.950
O	2*p*	−17.76	O	2.275
C	2*s*	−21.40		
C	2*p*	−11.40		

It is important before presenting our findings to emphasize that the methods used here have limited accuracy, and this fact must always be kept in mind in assessing our conclusions. First, the extended Hückel theory utilized for conformational calculations exaggerates steric effects, so that preferred conformations are generally predicted accurately, but rotational barriers are generally too large, as are energy differences between various conformations of the same molecule. These shortcomings can be minimized by comparing a series of results for similar molecules and also by use of experimental information. Second, the CNDO (Complete Neglect of Differential Overlap) calculation of charge distributions should be much more adequate on an absolute basis than the conformational results. This method gives good results for dipole moments and for trends in dipole moments within a series of related molecules. Therefore, the discussion of trends in charge distributions below is on firm ground. Further justification for this conclusion lies in the parallel results obtained for trends in charge distribution found by calculations *ab initio*.

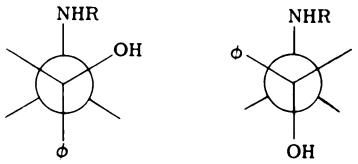
For the calculations of the catecholamines (Ia–d) in this study, it was necessary

to know precise three-dimensional coordinates for each atom. This was accomplished by means of a computer program that was obtained from the Indiana University Quantum Chemistry Program Exchange. The program performs a series of vector summations with previously determined planes of atoms as references. The program can be keyed to output the atom coordinates on punched cards, in the fields required for the extended Hückel program. The bond angles and lengths used in the coordinates program were assumed to be of conventional dimensions. Bond lengths were assumed not to vary in the different conformational models.

CONFORMATION OF SIDE CHAIN

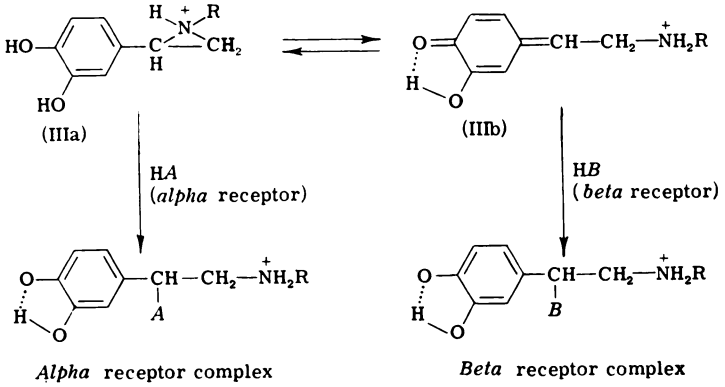
Although the barrier energies are expected to be exaggerated with respect to their absolute values, a comparison of calculated barriers in a closely related series is justifiable and should give an indication of differences in a series of molecules and the ordering of the barrier heights. As the nitrogen of adrenergic agonists is largely protonated at physiological pH values, this was the form considered. The phenolic hydroxyl groups were assumed to be *trans* to each other in relation to the plane of the ring, as found from crystal data (14). This assumption may not be correct in solution or the gas phase, but this will have no effect on the rotational profiles computed since these groups lie too far from the rotating part of the molecule to exert any influence on the preferred conformation. The side chain C—C bond was rotated through 360 degrees in 60-degree increments. Although these increments may be considered somewhat large, it is expected that any gross changes in the rotational profile will be obvious even with such a large mesh.

The calculated conformational energies for norepinephrine, epinephrine, *N*-ethylnorepinephrine, and isoproterenol are listed in Table 2. We have previously reported the results for norepinephrine (12). Each adrenergic agonist has an energy minimum with the benzene ring and amine *trans* to each other (IIa), and secondary minima in the two *gauche* arrangements (IIb) of approximately equal energy increment above the minimum. Larsen (15)



(IIa) (IIb)

has recently proposed an explanation of the trends in adrenergic activity of the catecholamine series Ia–d involving the influence of the R group on the conformational preference of the side chain. His scheme begins with the enzymatic elimination of the benzylic hydroxyl of the catecholamine to form an aziridine ring (IIIa) and a quinone (IIIb) in equilibrium.



Subsequent reaction of intermediate IIIa or IIIb with the *alpha* or *beta* receptor, respectively, leads to the characteristic

TABLE 2
Comparison of calculated barrier heights separating IIa and IIb

R	E(II-a) − E(IIb)	E(barrier)
	kcal/mole	*kcal/mole*
H	3.46	5.58
CH_3	3.71	5.14
CH_3CH_2	3.22	5.43
$(CH_3)_2CH$	3.52	5.33

response. The ability of the aziridine (IIIa) or the quinone (IIIb) to predominate in the equilibrium determines whether the molecule is an *alpha* or *beta* agonist. The relative concentration of IIIa or IIIb is thus dependent on the nature of R, according to Larsen.

The nature of this R-group dependence was based on the reasoning that, for an aziridine ring to form IIIa, the molecule must be able to rotate from its preferred conformation, IIa, based on nuclear magnetic resonance (16) and molecular orbital studies (12), to a less favored conformation aligning the amino and hydroxyl groups in a *trans* arrangement (IIb). These *trans* groups would then be in a favorable relationship for rearward displacement and aziridine ring formation.

The barrier to this conformational change is, according to Larsen, dependent upon the bulk of the R substituent, which he assumes increases from Ia to Id in the catecholamine series, I, and thus decreases the proportion of conformer IIb and therefore of IIIa.

Several arguments may be raised against the proposed mechanism of Larsen. Although it is ingenious from a chemical point of view, it requires ultimate covalent bond formation with both *alpha* and *beta* receptor features. This is not consistent with the highly reversible nature of adrenergic action, as shown by the complete extractability of catecholamines from tissue (17) and the ease with which blocking agents reverse catecholamine effects.

The postulated influence of the R group on the conformation of the side chain is amenable to verification by our calculations. Our calculations on the series of catecholamines Ia–d indicated an identical conformational preference corresponding to IIa. The calculated energies of the lowest barriers between IIa and IIb are shown in Table 2. In this table is also shown the calculated energy difference between conformers IIa and IIb. It appears from Table 2 that the barrier between conformers IIa and IIb is of uniform magnitude throughout the series. Furthermore, the calculated energy difference between conformers is uniform throughout the series, indicating a comparable preference for IIa throughout. From these calculations, we postulate that the influence of the R group on the nitrogen in the catecholamine series, I, is probably not steric and that an explanation for the specific activity ratio of *alpha* and *beta* agonists invoking such an influence is not supported.

CHARGE DISTRIBUTIONS

Another explanation for the trends of activity observed in the series may involve a varying electronic effect of the R group on the onium nitrogen and hydrogens (18). To test this possibility we have again resorted to molecular orbital calculations on model compounds simulating the environment of the onium group in the series of catecholamines, I. Since extended Hückel theory is inadequate for the description of charge distributions, these calculations were carried out using the CNDO formulation of semi-empirical molecular orbital theory developed by Pople and Segal (19). This method is known to give adequate charge distributions, since electron repulsion effects are included, which prevents the large buildup of charge at electronegative centers found in extended Hückel theory. We have also carried out self-consistent field studies *ab initio* on several small molecules containing the onium group in order to assess the accuracy of the trends predicted by CNDO. The latter calculations were carried out with a modest set of Gaussians (seven *s*-orbitals and three *p*-orbitals for each of the x, y, and z directions centered at the carbon and nitrogen nuclei, and three *s*-orbitals centered at each proton). These orbitals were contracted to a minimal set using results obtained pre-

viously (20) for hydrocarbon molecules and from a calculation on the methylammonium compound using an uncontracted nitrogen set. The program required for the calculations *ab initio* was written by one of us (J. H.) and has been utilized extensively in previous computations on internal rotation and conformational studies in hydrocarbons (20–23).

It should be emphasized before discussing the charge distributions that the absolute values of charge densities are not of importance here, but rather the relative magnitudes and changes in charge distribution as one passes from molecule to molecule. It is not of concern that crude calculations *ab initio* or CNDO results for charge densities are not likely to be highly accurate in the absolute sense. The changes in charge distribution should be relevant, however.

Figure 1 illustrates the charge distribution for five onium compounds as computed

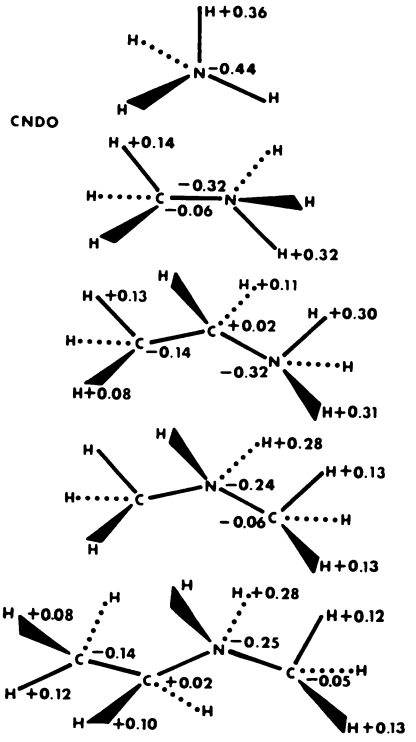


FIG. 1. *Charge distribution in some quaternary amines as computed from ab initio and CNDO wave functions*

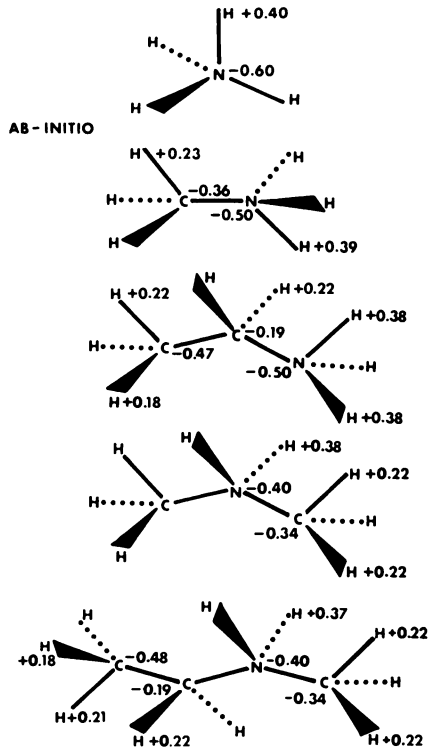


FIG. 1 (*Continued*)

by the CNDO and *ab initio* self-consistent field studies. The numbers are gross charges at each center as found from a Mulliken population analysis (24). It is apparent that the same general trends in charge distribution are noted in both the *ab initio* and CNDO calculations, the major difference between the two methods being that CNDO appears to smooth out the charge distribution somewhat more than is found from a self-consistent field wave function. Both methods predict certain features. First, the positive charge of these onium compounds is not localized in a small region of space which would be energetically unfavorable. Rather, the positive charge is distributed throughout the molecule on the hydrogen nuclei, with the carbons and nitrogens being negatively charged or essentially neutral. Second, the charges on the nitrogen atoms appear to be a function only of the number of bonded alkyl groups, not of the identity of these groups. This is also true for the hydrogen nuclei of the

onium group. A third observation is that the charge density in alkyl groups bonded to the nitrogen appears to be essentially independent of the number or type of other alkyl groups present. Small differences of ± 0.01 do occur, and it is possible that more refined calculations might indicate a somewhat larger variation, but it is probable on the basis of the present results that any such variations will be very small. It should also be noted that a change in charge density of ± 0.02–0.03 electron at a given center will have no effect whatsoever on our conclusion. An additivity principle based on these observations suggests that the charge distribution in the onium compounds can be computed by adding the charges on bonded alkyl groups found from calculations on $R-NH_3^+$ type molecules, and distributing the remaining charge among the nitrogen and bonded hydrogen nuclei according to the trends noted in Fig. 1.

Figure 2 illustrates the charge distribution in a number of compounds of the type $CH_3CH_2NHR^+$, which are models for the onium group in the adrenergic agonists Ia-d. These charge distributions were calculated by means of the CNDO method. It is seen that in the series R = CH_3, C_2H_5, $(CH_3)_2CH$ there is apparently very little change in the charge distribution at the onium group, so that a consideration of this distribution as an explanation of adrenergic activity trends does not appear to be reasonable for either *alpha* or *beta* receptors. This conclusion is supported by the finding that the largest change in the charge distribution in the onium group occurs in passing from norepinephrine to epinephrine, and that both these molecules have *alpha* adrenergic activity. The subsequent loss of *alpha* activity in progressing from epinephrine to the ethyl-substituted compound to isoproterenol is accompanied by little further computed change in the charge distribution in the onium group. Similarly, the very large enhancement of *beta* activity in progressing through the same substituents is not reasonably a function of this charge distribution. Since the constancy in onium group charges is also noted in calculations *ab initio*, it would

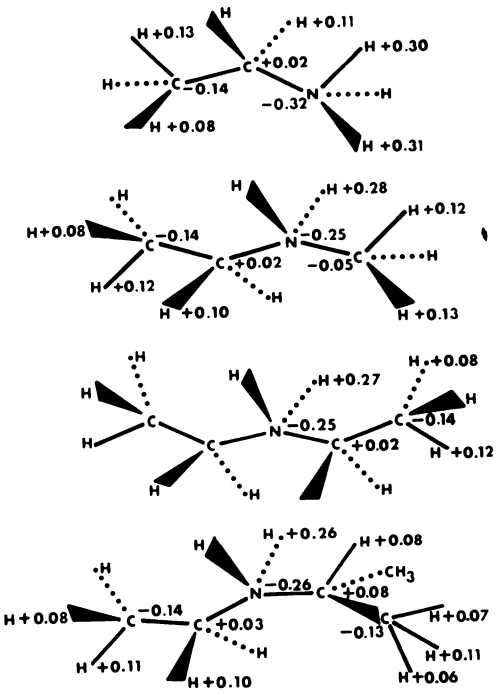


FIG. 2. *Charge distribution in some model adrenergic agents computed from CNDO wave functions*

appear that one must search further for a reasonable explanation of adrenergic activity.

ALKYL GROUP BINDING HYPOTHESIS

The theoretical considerations reported here suggest that the effect of various *N*-alkyl groups on the efficacy of *alpha* or *beta* adrenergic agents is probably due to interaction of these alkyl groups with specific features of the receptor. It would therefore appear reasonable to examine existing data on adrenergic agonists, in view of this tentative conclusion, and to speculate on the nature of the receptor site at which interaction with the onium group takes place.

Potent *alpha* adrenergic activity is confined to norepinephrine, epinephrine, and, much less so, to *N*-ethylnorepinephrine. Any further increase in the size of the alkyl group leads to a marked decrease in *alpha* activity and to an increase in *alpha* antagonism. Furthermore, rather large

bulky groups, especially those with a terminal phenyl ring, such as $C(CH_3)_2$—$CH_2(C_6H_5)$, are associated with marked *alpha* antagonist activity (25, 26), suggesting that they may be rather strongly bound to the receptor but incapable of producing an *alpha* adrenergic response. These observations suggest that there may be two binding mechanisms involving the onium group and its substituents. The binding associated with the *alpha* adrenergic response is probably the formation of a hydrogen bond between an onium proton and a negatively charged receptor moiety. It is postulated that this negative site is surrounded by bulky groups and consequently lies in a region of high steric hindrance. Increasing the size of the R group then results in an unfavorable repulsive interaction with the receptor, leading perhaps to a twisting of the onium group or to the inability to approach closely so that hydrogen bond formation becomes energetically less favorable. It is possible that binding of a fairly large R group to a secondary receptor site through dispersion or other long-range forces may occur, leading to stronger binding and increased antagonism as the polarizability of the group increases.

A consideration of the *beta* adrenergic receptor is somewhat more complicated, since it is apparent from published data that there can be a wide range of response to a given drug molecule by different tissues with *beta* receptors. Some general patterns, however, appear to persist in most cases. The activity patterns for unbranched alkyl substituents is generally ethyl > *n*-pentyl > *n*-propyl > *n*-butyl > methyl > H (27). Branched alkyl groups show more variability according to the receptor in question, the general order usually being isopropyl ≅ *tert*-butyl > *sec*-butyl ≫ isobutyl. The *cyclo*-hexyl group generally is associated with rather weak response, and the activity of the *cyclo*-pentyl compound is variable and highly dependent on the tissue. The average over-all order of activity is isopropyl ≅ *tert*-butyl > ethyl > *sec*-butyl > *n*-propyl ≅ *n*-pentyl > *n*-butyl > methyl > *cyclo*-hexyl > H, with *cyclo*-pentyl too variable to classify.

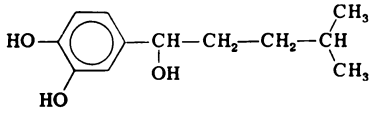
The marked increase in efficacy in the series R = H, methyl, ethyl, isopropyl is suggestive of interaction of the alkyl group and a receptor feature by long-range forces, most notably dispersion binding. The additivity of dispersion forces, coupled with the experimental data, indicates that this receptor feature is possibly an isopropyl-like moiety which lies essentially parallel to the onium alkyl group. We consider it to be highly significant that all the potent *beta* adrenergic agonists except *N*-ethylnorepinephrine present the same isopropyl-like feature to the receptor in their preferred conformation. The ethyl group also presents this feature, except for the branched methyl group.

The decrease in *beta* activity in passing from the ethyl to the *n*-propyl-substituted molecule appears to be indicative of the onset of steric hindrance. It is possible that the *n*-butyl and *n*-pentyl groups do not engage the receptor in a *trans* conformation, but rather in a *gauche* conformation. There is experimentally (28) a difference of only about 0.5–0.7 kcal/mole between the free energies of the *gauche* and *trans* conformations in butane or pentane, so that significant amounts of *gauche* conformers will be present at body temperature. The greater efficacy of the *n*-pentyl-substituted compound suggests that this hypothesis is reasonable, and that the twisted alkyl group lies in a position favorable to further dispersion binding. Examination of molecular models indicates that this suggestion of optimum chain length and onset of steric hindrance also accounts for the large difference between *N*-cyclopentylnorepinephrine and *N*-cyclohexylnorepinephrine. The cyclohexyl ring is simply too bulky to approach the receptor in an optimal manner for biological response.

Finally, the isobutyl-substituted compound is unique in the relative positioning of methyl and methylene groups within the series of molecules considered. The low activity suggests that the molecule cannot engage the receptor in a manner which avoids steric interference with receptor features.

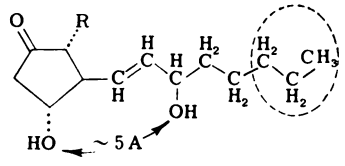
The role of the NH_2^+ moiety in *beta*

adrenergic activity does not clearly emerge from these considerations. It can be concluded that if it engages the receptor, it does so in a quantitatively constant manner. It is also possible that it may not be involved at all, leaving us to conclude that a molecule such as IV may be an active *beta* adrenergic agonist.

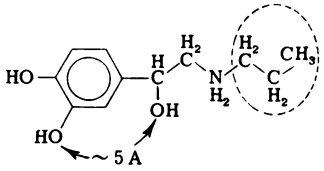


(IV)

Prostaglandin E_1 mimics certain actions of *beta* adrenergic agonists. It relaxes tracheal muscle (29) and isolated vascular strips (30), and increases myocardial contractile force (31). An intuitively reasonable but not proven conformation of prostaglandin E_1(V), in which one of the chains is all *trans* and the other is designated as R, is compared with *N*-*n*-propylnorepinephrine (VI) below.



(V)



(VI)

Models of V and VI demonstrate similar relationships between the ring and side chain hydroxyl groups and reveal similar alkyl areas of the molecules, marked by dashed lines. It is these alkyl regions that we suggest may interact with adrenergic receptors through dispersion forces and explain the *beta* adrenergic-like action of prostaglandin E_1. This similarity of possible conformations and actions tends to support our hypothesis that it is the *N*-alkyl moiety and not the nitrogen of *beta* adrenergic agents that is important for activity.

After this manuscript was written it was brought to our attention that the *dl* form of compound IV had recently been synthesized and reported to have modest *beta* activity (32). The real extent of this activity of the appropriate isomeric form of compound IV may be greater than was reported, since the racemic mixture tested was said to be poorly soluble in aqueous solutions and this may have prevented adequate concentrations from reaching the receptor site.

ACKNOWLEDGMENTS

We wish to acknowledge the assistance and use of the computer facilities of the Battelle Memorial Institute and the Ohio State University.

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