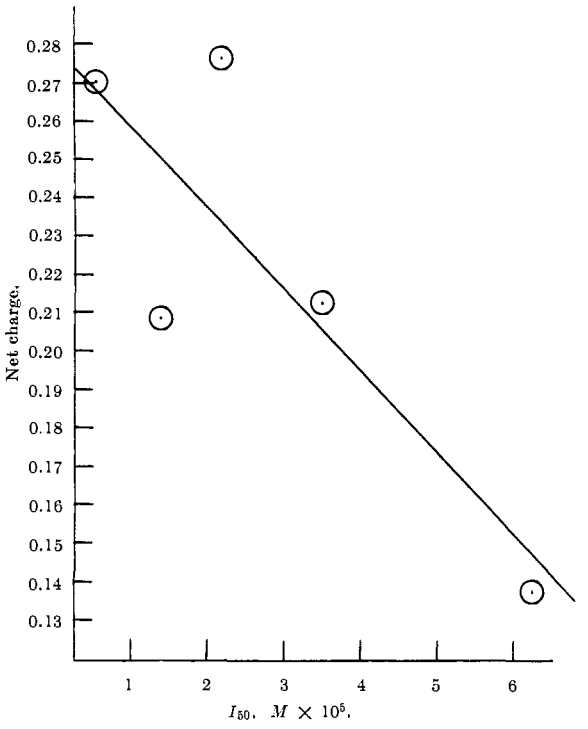
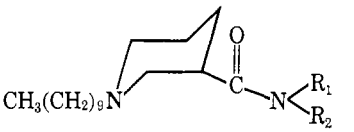


Figure 1.—Net charge at the amide nitrogen atom of 1-decyl-3-[(alkyl)- and 1-decyl-3-[(N,N-dialkyl)-substituted carbamoyl]-piperidines *vs.* the corresponding cholinesterase inhibition.

TABLE V

CHOLINESTERASE INHIBITORY PROPERTIES OF 1-DECYL-3-[(N-ALKYL)- AND 1-DECYL-3-[(N,N-DIALKYL)-SUBSTITUTED CARBAMOYL]PIPERIDINES



NR_1R_2	I_{50}, $M \times 10^5$[a]
NH_2	6.23[b]
$NHCH_3$	3.48[b]
NHC_2H_5	1.37[b]
$N(CH_3)_2$	2.17[b]
$N(C_2H_5)_2$	0.53[c]
$N(C_3H_7)_2$	0.105[d]

[a] Molarity of inhibitor effecting 50% inhibition. [b] See ref 7b. [c] See ref 7a. [d] J. G. Beasley, unpublished data.

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Application of Partition Coefficients, Electric Moments, Electronic Structures, and Free-Energy Relationships to the Interpretation of Cholinesterase Inhibition[1]

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Cholinesterase inhibition of some N-alkyl-substituted amides is interpreted in the light of their partition coefficients, electric dipole moments, electronic structures calculated from the Hückel molecular orbital method, and free-energy relationships. At least 75% of the observed cholinesterase inhibition can be accounted for from a linear relationship between log I_{50} and log (partition coefficient). The activities of seven mono(carbamoylpiperidino)decanes are explained in terms of electronic, stereochemical, and hydrogen-bonding factors. The inhibitory properties of several mono- and bis[3-(N,N-diethylcarbamoyl)piperidino]alkanes are discussed from considerations of the smooth curves obtained from plotting $1/I_{50}$ against n, the number of carbon atoms in the alkyl chain. It is believed that the mono derivatives have competing electronic and hydrophobic factors which contribute to the activity, while the inhibition of the bis compounds can be approximated nicely from the parabolic equation, $1/I_{50} = An^2 + Bn + C$. Linear free-energy relationships indicate that the inhibitors under study have similar binding modes. A model for the inhibitor–enzyme complex is proposed which has points of attachment (1) at the anionic site between the carboxyl group of the enzyme and the positively charged quaternary ring nitrogen of the inhibitor, and (2) at the esteratic site in the form of a quasi-ring formed from association of (a) the serine hydroxyl oxygen of the enzyme with the amide nitrogen of the inhibitor, and (b) the serine hydroxyl hydrogen of the enzyme with the amide oxygen.

The inhibitory effect upon isolated human plasma pseudocholinesterase (acylcholine acylhydrolase, EC 3.1.1.8) systems produced by series of substituted arylalkylaminopropionamides[2,3] and of piperidinecarboxamide derivatives[4,5] has been studied extensively. In continuing investigations designed to elucidate structure–activity relationships in these series, we have (1) measured dielectric properties,[6-8] (2) calculated electronic structures,[9] (3) applied regression analyses[10] to the structure–activity data, (4) evaluated surface-

(1) This research is being supported by the National Science Foundation (GB-2381/B-15989). Computer facilities were provided through U. S. Public Health Service Grant HE-09495.

(2) A. Lasslo, P. D. Waller, A. L. Meyer, and B. V. Rama Sastry, *J. Med. Pharm. Chem.*, **2**, 617 (1960).

(3) A. Lasslo, P. D. Waller, and G. J. Epperson, *ibid.*, **6**, 26 (1963).

(4) A. Lasslo, J. G. Beasley, G. G. Nelms, and G. J. Epperson, *ibid.*, **6**, 811 (1963).

(5) J. G. Beasley, R. P. Quintana, and G. G. Nelms, *ibid.*, **7**, 698 (1964).

(6) W. P. Purcell, *J. Phys. Chem.*, **68**, 2666 (1964).

(7) W. P. Purcell and J. A. Singer, *ibid.*, **69**, 691 (1965).

(8) W. P. Purcell, J. G. Beasley, and R. P. Quintana, *Biochim. Biophys. Acta*, **88**, 233 (1964).

(9) W. P. Purcell, *J. Med. Chem.*, **9**, 294 (1966).

(10) W. P. Purcell, *Biochim. Biophys. Acta*, **105**, 201 (1965).

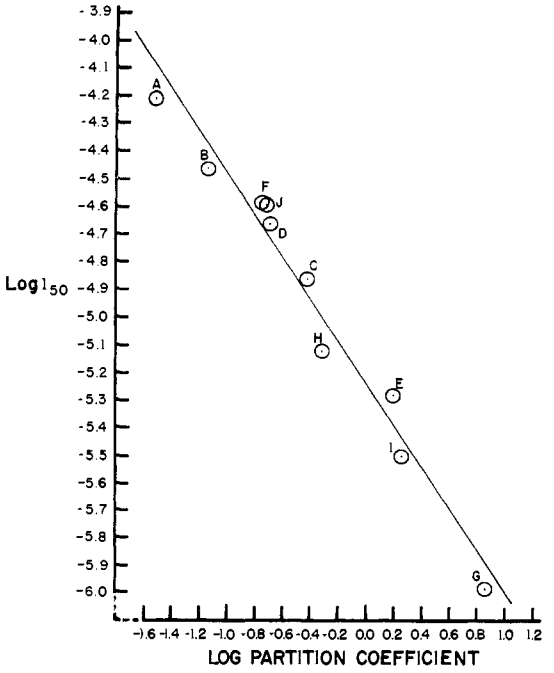


Figure 1.—Log I_{50} *vs.* log (partition coefficient) for some mono(carbamoylpiperidino)decanes: 1-decyl-3-carbamoylpiperidine, A; 1-decyl-3-(N-methylcarbamoyl)piperidine, B; 1-decyl-3-(N-ethylcarbamoyl)piperidine, C; 1-decyl-3-(N,N-dimethylcarbamoyl)piperidine, D; 1-decyl-3-(N,N-diethylcarbamoyl)piperidine, E; 1-decyl-4-(N,N-diethylcarbamoyl)piperidine, F; 1-decyl-3-(N,N-dipropylcarbamoyl)piperidine, G; 1-decyl-3-(pyrrolidinoformyl)piperidine, H; 1-decyl-3-(piperidinoformyl)piperidine, I; 1-decyl-3-(morpholinoformyl)piperidine, J. The data for Figure 1 are given in Table I.

active properties,[11,12] and (5) measured partition coefficients[13] of some of our cholinesterase inhibitors. In view of the increased emphasis on physicochemical considerations in examining the behavior of synthetic entities in biological systems,[14-16] we have attempted to approach our experimental observations with appropriate mathematical interpretations and correlations.

We have found (1) a linear relationship between log I_{50} and log partition coefficient which accounts for at least 75% of the observed activity (*i.e.*, the maximum deviation between observed I_{50} and calculated I_{50} is 25%); (2) a parabolic relationship between $1/I_{50}$ and n, the number of carbon atoms in the alkyl chain of several bis[3-(N,N-diethylcarbamoyl)piperidino]-alkanes; and (3) free-energy relationships that indicate similar modes of molecular binding to common enzyme sites for our inhibitors. Also we consider (1) electronic structure (as determined from electric dipole moment measurements and Hückel molecular orbital (HMO) calculations), (2) stereochemical factors, and (3) hydrogen-bonding possibilities in the interpretation of the inhibition of some mono(carbamoylpiperidino)-decanes.

Development and Derivation of Relationships. Cholinesterase Inhibition as a Function of Partition Coefficient.—Table I gives the activities and benzene–water partition coefficients of a series of mono(carbamoylpiperidino)decanes. A log–log plot (Figure 1) of these two parameters is linear, *i.e.*, the properties can be approximated rather closely by eq 1

$$I_{50} = cP^m \qquad (1)$$

where c is the antilog of the least-squares intercept of log I_{50} *vs.* log P. P is the partition coefficient, and m is the slope of the least-squares line (Figure 1).

Table II gives the observed (Table I) and calculated (eq 1) I_{50} values, and also the values of π calculated from Hansch's definition[17]

$$\pi = \log P_X - \log P_H \qquad (2)$$

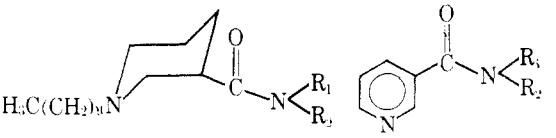
where P_X is the partition coefficient of a derivative and P_H is that of the parent compound.

Cholinesterase Inhibition and Polarity.—In Figure 2a we have plotted the inhibition against the amide group moment[18] from the data in Table I. In the adjacent figure (Figure 2b) the inhibition is plotted (using the same scale as Figure 2a) against the net electronic charge at the amide nitrogen atom.

Cholinesterase Inhibition and Alkyl Chain Length.—Previously,[4] we reported graphic relationships between log I_{50} and log n, the number of carbon atoms in the alkyl chain, for several mono- and bis[3-(N,N-diethylcarbamoyl)piperidino]alkanes. Upon closer examination of the data, we found a rather smooth curve when $1/I_{50}$ was plotted against n, the number of carbon atoms in the alkyl chain. In fact, for the bis derivatives, the points fit the equation for a parabola fairly well; Table

TABLE I

CHOLINESTERASE INHIBITION, PARTITION COEFFICIENTS, NET AMIDE NITROGEN CHARGES, AND AMIDE GROUP MOMENTS OF SOME N-ALKYL-SUBSTITUTED AMIDES



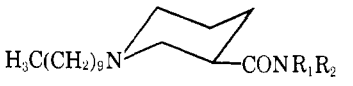
NR_1R_2	I_{50},[a] $M \times 10^5$	Partition coefficient[b] (benzene–water)	Net charge at amide nitrogen[c]	Net charge at amide nitrogen[c]	Amide group moment (μ_g), D.[d]
NH_2	6.23[e]	0.03	+0.1375	+0.1375	3.57
$NHCH_3$	3.48[e]	0.07	+0.2126	+0.2126	4.20
NHC_2H_5	1.371[e]	0.38	+0.2088	+0.2088	4.23
$N(CH_3)_2$	2.15[e]	0.20	+0.2763	+0.2764	4.87
$N(C_2H_5)_2$	0.527[f]	1.58	+0.2700	+0.2702	5.05
$N(C_2H_5)_2$[g]	2.155[e]	0.17			
$N(C_3H_7)_2$	0.105[h]	7.26[i]			5.12
	0.766[e]	0.48			
	0.308[e]	1.80			
	2.35[e]	0.09			

[a] I_{50} is the molarity of compound effecting 50% cholinesterase inhibition. [b] See ref 13. [c] See ref 9. [d] See ref 6. [e] See ref 5. [f] See ref 4. [g] The amide substituent is in position 4 of the ring. [h] J. G. Beasley, unpublished data. [i] See ref 42.

(11) R. P. Quintana, *J. Pharm. Sci.*, **53**, 1221 (1964).
(12) R. P. Quintana, *ibid.*, **54**, 573 (1965).
(13) R. P. Quintana, *ibid.*, **54**, 462 (1965).
(14) W. D. M. Paton, *Proc. Roy. Soc.* (London), **B154**, 21 (1961).
(15) E. J. Ariëns and A. M. Simonis, *J. Pharm. Pharmacol.*, **16**, 137 (1964); E. J. Ariëns and A. M. Simonis, *ibid.*, **16**, 289 (1964).
(16) B. Belleau, *J. Med. Chem.*, **7**, 776 (1964).
(17) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).
(18) This is the aromatic amide group moment; the values for the net charge at the amide nitrogen (Table I) show them to be almost identical for aliphatic and aromatic analogs, thus indicating that the amide group moments also would be similar and justifying the use of the aromatic group moments, since the trends should be the same in either system.

TABLE II

COMPARISON OF OBSERVED AND CALCULATED CHOLINESTERASE INHIBITION BY SOME MONO(CARBAMOYLPIPERIDINO)DECANES AND THE CORRESPONDING VALUES FOR HANSCH'S SUBSTITUENT CONSTANT, π, CALCULATED FROM BENZENE-WATER PARTITION COEFFICIENTS



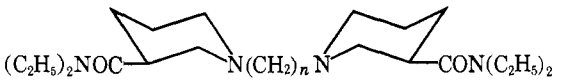
NR_1R_2	I_{50}, $M \times 10^5$ Obsd	Calcd[a]	π[b]
NH_2	6.23	7.93	0.0
$NHCH_3$	3.48	4.20	0.368
NHC_2H_5	1.371	1.18	1.103
$N(CH_3)_2$	2.17	1.91	0.824
$N(C_2H_5)_2$	0.527	0.404	1.722
$N(C_2H_5)_2$[c]	2.65	2.16	0.753
$N(C_3H_7)_2$	0.105	0.129	2.384
	0.766	0.974	1.213
	0.318	0.365	1.781
	2.57	1.98	0.802

[a] Calculated from $I_{50} = cP^m$. [b] $\pi = \log P_X - \log P_H$, where P_X is the partition coefficient of a derivative and P_H is that of the parent compound.[17] [c] The amide substituent is in position 4 of the ring.

III gives the observed values and those calculated from this relationship. The multiple correlation coefficient R is significant at the 99% confidence level.

TABLE III

INHIBITION CALCULATED[a] FROM $1/I_{50} = An^2 + Bn + C$ FOR SOME BIS(CARBAMOYLPIPERIDINO)ALKANES



n	I_{50}, $M \times 10^5$ Obsd[b]	Calcd
2	17.5	20.3
3	99.3	39.8
4	42.0	50.5
5	27.1	29.8
6	15.0	15.1
10	2.59	2.59

[a] A, B, and C are the parabola constants. [b] See ref 4.

Similar Binding Modes among Carbamoylpiperidinoalkane Cholinesterase Inhibitors.—Linear free-energy relationships have been used in detecting similar modes of molecular binding to common receptors for three series of N-substituted phenylpiperidine analgesics.[19] We have applied this method to our series of carbamoylpiperidinoalkanes by plotting log I_{50} of members of one series *vs.* log I_{50} of identically substituted members of another series. The slopes of the lines are reported in Table IV.

Linearity of the log–log plots and a slope equal to unity suggest similar binding modes. Our data (Table IV), however, were obtained (with exception of series II *vs.* series V) from series having only two members and, therefore, the two points defined the slopes without a test for linearity. Nevertheless, we found linearity for the one example having three points, series II *vs.* V, and consider it significant that the slopes from analyses of series I through V are virtually unity. Comparison of series VI with the other series gives slopes differing from unity by a magnitude greater than the experimental error (0.07).

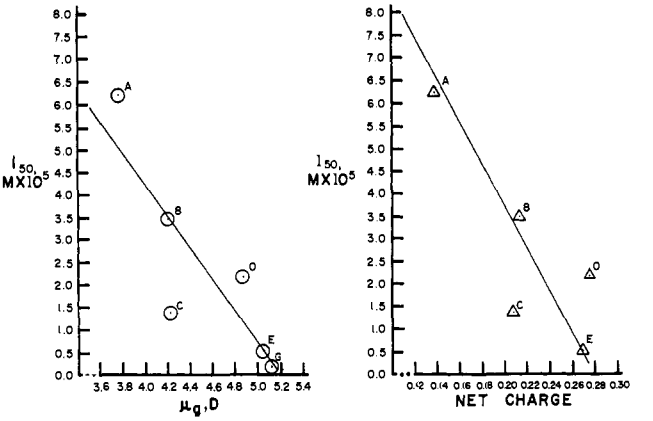


Figure 2.—Cholinesterase inhibition of some mono(carbamoylpiperidino)decanes *vs.* amide group moment of nicotinamides, identically substituted at the amide nitrogen (a, circles), and cholinesterase inhibition of some mono(carbamoylpiperidino)-decanes *vs.* net electronic charge at the amide nitrogen (b, triangles): 1-decyl-3-carbamoylpiperidine, A; 1-decyl-3-(N-methylcarbamoyl)piperidine, B; 1-decyl-3-(N-ethylcarbamoyl)piperidine, C; 1-decyl-3-(N,N-dimethylcarbamoyl)piperidine, D; 1-decyl-3-(N,N-diethylcarbamoyl)piperidine, E; 1-decyl-3-(N,N-dipropylcarbamoyl)piperidine, G. The data for Figure 2 are given in Table I.

Discussion

Clearly, many factors determine a molecule's ability to inhibit cholinesterase, and we interpret our results in this light. Nevertheless, it is remarkable that the single parameter, partition coefficient, accounts for better than 75% of the observed inhibition (Table II). It is also worthy of notice that π increases, without exception, with increasing inhibitory potency (Table II), which is comparable to the findings of Hansch, *et al.*,[20] that localization of substituted benzeneboronic acids in the brain of mice can be rationalized in terms of a single parameter obtained from octanol–water partition coefficients.

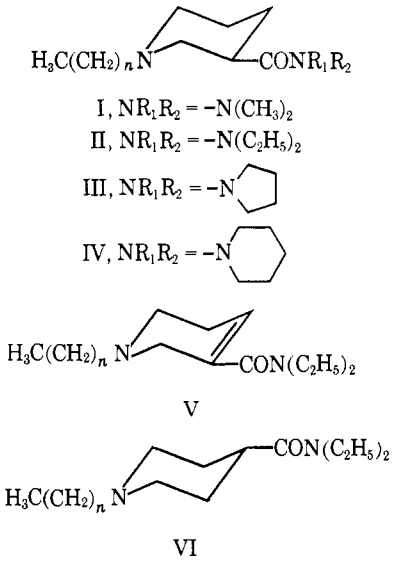
Another parameter which should be considered in interpreting cholinesterase inhibition is the electronic structure of the inhibitor. We studied this factor through electric moment measurements[6] and HMO calculations[9] (Table I, Figure 2a and 2b). As might be expected, the significant variation in electron density for the mono(carbamoylpiperidino)decanes was found to be at the amide group atoms. Although variation in the net charge at the carbonyl carbon[9,21] or carbonyl oxygen[9] was insignificant for our series of cholinesterase inhibitors, we observed a marked decrease in electron density at the amide nitrogen as anticholinesterase activity increases (Table I, Figure 2b). Along this line, and in view of the fact that the net charge comes from theoretical calculations using semiempirical param-

(19) P. S. Portoghese, *J. Pharm. Sci.*, **54**, 1077 (1965); *cf.*, P. S. Portoghese, *J. Med. Chem.*, **8**, 609 (1965).

(20) C. Hansch, A. R. Steward, and J. Iwasa, *Mol. Pharmacol.*, **1**, 87 (1965).

(21) F. Bergmann, I. B. Wilson, and D. Nachmansohn, *J. Biol. Chem.*, **186**, 693 (1950).

TABLE IV
SLOPES OF LOG–LOG PLOTS OF I_{50} VALUES FOR VARIOUS CARBAMOYLPIPERIDINOALKANES



Carbamoylpiperidinoalkane series Abscissa	Ordinate	Slope, log I_{50}[a] *vs.* log I_{50}
I	II	1.01
I	III	1.04
I	V	1.06
II	III	1.03
II	V	1.02[b]
III	V	1.02
IV	I	1.01
IV	II	1.03
IV	III	1.05
IV	V	1.08
VI	I	1.14
VI	II	1.16
VI	III	1.18
VI	IV	1.13
VI	V	1.22

[a] Values of I_{50} were obtained from ref 4 and 5. For all series evaluated, $n = 1$ and 9; in the plot of series II *vs.* V, the compounds in which $n = 0$ were also included. [b] The slope was determined by the method of least squares; the standard deviation of the points is 0.04.

eters,[22] whereas the origin of the group moments is experimental, we find the similarity between Figure 2a and 2b particularly interesting. The lines in these figures are the least-squares lines and do not necessarily indicate a linear relationship. They are used here only as an aid in comparing the two figures. Figure 2b represents the same carbamoyl groups as Figure 2a with the exception that there is no dipropylcarbamoyl group in Figure 2b. Notice, specifically, that the monoethyl derivative (C) has points to the left and below, and the dimethylcarbamoyl group (D) has points to the right and above the lines in both Figure 2a and 2b. That is, the ethyl derivative is more active than the dimethyl compound, but has a less positive amide nitrogen and a smaller amide group moment; the relative deviations (Figure 2a and 2b) from the least-squares line is strikingly similar.

(22) See, for example, (a) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Publishers, Inc., New York, N. Y., 1963, pp 104–115; (b) A. Streitwieser, Jr., "Molecular Orbital Theory for Organic Chemists," John Wiley and Sons, Inc., New York, N. Y., 1961, pp 33–134.

An *a priori* examination of the amide group, as alkyl substitution is varied, leads to a consideration of three important properties contributing to cholinesterase inhibition. Differences in electronic structure were mentioned above; differences in alkyl group size (*e.g.*, hydrophobic properties are influenced by the bulk) and the possibility of hydrogen bonding at the amide hydrogen could affect inhibition as well. If we assume, therefore, that these properties (*i.e.*, electronic structure, stereochemical[23] factors, and the presence of hydrogen-bonding sites[24]) are those operative in the changing inhibitory potency of substituted mono(carbamoylpiperidino)decanes, a semiempirical approach to the estimation of the direction and magnitude of the influence upon cholinesterase inhibition attributable to these effects may be undertaken, employing the accurately determined I_{50} values and the amide group moments for this series of closely related compounds.

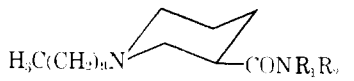
Cholinesterase inhibition has been compared with amide group moment in Figure 2a; deviations from this approximately linear relationship may reflect factors other than electronic. As a first approximation, one can ignore these other factors and consider the electronic effect only in the empirical relationship where k

$$1/I_{50} = kP(\text{amide group}) \qquad (3)$$

is a proportionality constant and P(amide group) is the orientation polarization[25] of the amide group.

The values of $1/I_{50}$, P(amide group), and k (eq 3) for the mono(carbamoylpiperidino)decanes are given in Table V where it can be seen that eq 3 does not adequately describe the relationship between inhibition and amide group polarization, since k is not constant. Therefore, one may conclude that (1) there is some other equation attributing the inhibition to electronic factors only, or (2) other factors are reflected in the differences in k. Of these alternatives, the second seems more reasonable since, for example, one cannot rationalize the large difference in activity and the small

TABLE V
VALUES OF $1/I_{50}$, P(AMIDE GROUP), AND k FROM $1/I_{50} = kP$(AMIDE GROUP) FOR SOME MONO(CARBAMOYLPIPERIDINO)DECANES



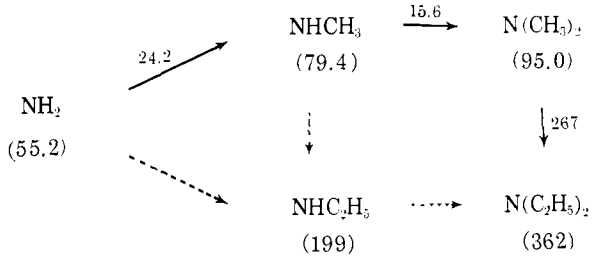
NR_1R_2	$1/I_{50}$, $M^{-1} \times 10^{-6}$	P(amide group), cm³	k, M^{-1} cm^{-3}
NH_2	0.1605	291	55.2
$NHCH_3$	0.2874	362	79.4
NHC_2H_5	0.7299	366	199
$N(CH_3)_2$	0.4608	485	95.0
$N(C_2H_5)_2$	1.887	521	362
$N(C_3H_7)_2$	9.524	536	1777

(23) We mean stereochemical to include size, conformation, steric hindrance, molecular flexibility, and conformational fit at the enzyme surface.

(24) Note that J. J. Fiscber and O. Jardetzky [*J. Am. Chem. Soc.*, **87**, 3237 (1965)] consider electrostatic binding, potential hydrogen bonding, and hydrophobic bonding in their study of penicillin binding to serum albumin.

(25) $\mu = 0.01281\ (10^{-18})\ (P_M T)^{1/2}$ (C. P. Smyth, "Dielectric Behavior and Structure," McGraw-Hill Book Company, Inc., New York, N. Y., 1955, pp 14, 221). Since I_{50} should decrease with an increase in the force binding the inhibitor to the enzyme, and since we are studying factors which directly affect this binding force, we have chosen the reciprocal of I_{50} as the parameter for comparison (eq 3).

moment difference between the monomethyl and monoethyl derivatives (Table I) in terms of electronic effects alone. We shall assume, therefore, that the differences in k, Δk, represent factors other than electronic. Then, the changes in inhibition resulting from variation in the amide function can be interpreted in terms of the electronic (polarization) factor, and a combination of stereochemical and hydrogen bonding (Δk) factors. The values for k (parentheses) and Δk (numbers by arrows) can be represented schematically as shown below. It should be emphasized that the



values of k incorporate the electronic factors, and hence the Δk values should reflect only factors other than electronic.

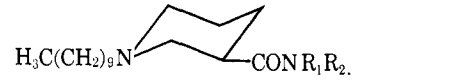
The Δk between the diethyl- and dimethyl-substituted inhibitors is 267; therefore, one may assign a value of 267/2 to the substitution of one ethyl for one methyl group. This value should reflect the stereochemical factor of the ethyl group as compared to the methyl group, and would therefore include contributions from hydrophobic forces. The value, 134, would not include hydrogen-bonding effects since there has been no change in the number of hydrogen-bonding sites and should not include electronic factors since the polarization has been incorporated into the calculation of k.

The large difference (24.2 − 15.6) between Δk, $NH_2 \rightarrow NHCH_3$, and Δk, $NHCH_3 \rightarrow N(CH_3)_2$, clearly indicates nonequivalent results from supposedly equivalent substitutions, *i.e.*, a methyl group for a hydrogen. This substitution involves (1) the replacement of a larger group for a smaller one (stereochemical factor), and (2) the loss of a hydrogen bonding site. Assuming that the size effect is negligible and that the loss of the hydrogen atom accounts for the difference in k between the monomethyl- and dimethyl-substituted derivatives,[26] and that the first methyl substitution (monomethyl for unsubstituted) gives a Δk which includes both stereochemical and hydrogen-bonding factors, a Δk value of 15.6 can be assigned to the loss of either hydrogen-bonding site and 8.6 (24.2 − 15.6) to the stereochemical factor of the first methyl substitution.

We have suggested that the Δk values represent stereochemical (acting mostly through hydrophobic forces) and hydrogen-bonding factors (both of which should greatly influence benzene–water partition coefficients) with no relation to electronic factors. Hansch and Fujita[27] have indicated the value of using a substituent constant, π, derived from logarithms of octanol–water partition coefficients, in estimating the lipophilic–lipophobic character of organic molecules. Further, it was found[17] that although π varies for a substituent depending upon its electronic environment, the variation is small. The similarity between the factors represented by Δk and the factors evaluated by π led to consideration of a plot of log (partition coefficient), Table I, against Δk for our mono(carbamoylpiperidino)decanes. The resulting curve was smooth, and the data fit the equation for a parabola fairly well (Table VI). We consider the relationship between

TABLE VI

SUM OF Δk FOR AMIDE SUBSTITUENTS AND CORRESPONDING PARTITION COEFFICIENTS FOR SOME MONO(CARBAMOYLPIPERIDINO)DECANES



NR_1R_2	Δk sum, M^{-1} cm^{-3}	Partition coefficient (benzene–water) Obsd	Calcd[a]
NH_2	−31.2	0.03 ± 0.01	0.05
$NHCH_3$	−7.0	0.07 ± 0.01	0.07
NHC_2H_5	118	0.38 ± 0.03	0.36
$N(CH_3)_2$	17.2[b]	0.20 ± 0.01	0.10
$N(C_2H_5)_2$	268	1.58 ± 0.03	1.85
$N(C_3H_7)_2$	1415[c]	7.26 ± 0.40	7.22

[a] Calculated from $\log P = A(\Delta k)^2 + B(\Delta k) + C$. [b] The Δk for the dimethyl derivative was assumed to be twice the value for CH_3 (first), *i.e.*, 2 × 8.6, since the highly specific spatial arrangement presumably responsible for the ineffectiveness of the second methyl in the enzyme–inhibitor complex is lacking in the simple partitioning between benzene and water. [c] Calculated from the k for the dipropyl inhibitor minus the k for the diethyl inhibitor.

Δk and log (partition coefficient) real and significant (the multiple correlation coefficient R is significant at the 99% confidence level) and find this parabolic fit interesting in the light of the statement that "the movement of organic compounds into tissue is parabolically rather than linearly dependent on π or log P."[20]

As a test of the validity of the Δk assignment, consider the replacement of a hydrogen with an ethyl group in the derivatives 1-decyl-3-carbamoylpiperidine and 1-decyl-3-(N-ethylcarbamoyl)piperidine. Adding 15.6 for the loss of a hydrogen-bonding site, 8.6 for the substitution of a methyl for a hydrogen, and 134 for the substitution of an ethyl for a methyl eq 3 becomes $1/I_{50} = (55.2 + 15.6 + 8.6 + 134)(366)$, which gives a calculated I_{50} of 1.28×10^{-5} M; this agrees well with the observed value, 1.37×10^{-5} M (the activity of this derivative was virtually impossible to rationalize from electronic factors only, Figure 2a).

Thus, Δk values can be used in quantitatively evaluating the effect upon inhibition of the combined stereochemical and hydrogen-bonding factors associated with some amide substituents in our 1-decyl-3-carbamoylpiperidines. The ranking per group is: C_2H_5, 134; CH_3 (first), 8.6; CH_3 (second), 0; H, −15.6.[28]

The relative importance of electronic effects as compared with stereochemical and hydrogen-bonding factors may be estimated by calculating a theoretical $1/I_{50}$ for the unsubstituted derivative, without the effect upon inhibition of the two hydrogen atoms. Equation 3 becomes $1/I_{50} = [(55.2) - 2(-15.6)](291) = 2.51 \times 10^4$. Further, if 291 cm^3 (polarization)

(26) See, for example, I. B. Wilson, *J. Biol. Chem.*, **197**, 215 (1952).
(27) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).
(28) The Δk value for a hydrogen substituent is considered negative since the Δk for the loss of a hydrogen bonding site is positive.

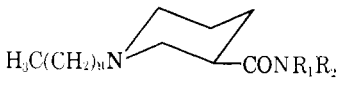
corresponds to $1/I_{50}$ of 2.51×10^4 M^{-1}, 1 cm³ contributes 86.4 M^{-1} to $1/I_{50}$. Then the expected activity from electronic factors only can be estimated using eq 4.

$$1/I_{50} = P(\text{amide group}) \times 86.4 \quad (4)$$

Comparing the observed activity with that calculated from eq 4, the percentage of the activity contributed by amide group polarization (Table VII) can be calculated.

TABLE VII

PERCENT ACTIVITY CONTRIBUTED BY AMIDE GROUP POLARIZATION OF SOME MONO(CARBAMOYLPIPERIDINO)-DECANES AND SUM OF Δk FOR AMIDE SUBSTITUENTS



NR_1R_2	% activity contributed by polarization[a]	Δk sum, M^{-1} cm^{-3}
NH_2	157	−31.2
$NHCH_3$	109	−7.0
NHC_2H_5	43	118
$N(CH_3)_2$	91	8.6
$N(C_2H_5)_2$	24	268
$N(C_3H_7)_2$	(5)	1415

[a] Comparing observed I_{50} values with those calculated from $1/I_{50} = P(\text{amide group}) \times 86.4$.

The 157% (Table VII) contributed by the unsubstituted amide group polarization, is greater than 100 because of the deleterious effect upon activity of the two hydrogen atoms (negative Δk[28]), *i.e.*, one would expect greater activity from electronic effects alone than is observed. From further inspection of Table VII, one can see that the relative importance of the polarization factor fits well with the Δk values for the alkyl-substituted amide groups. For the monomethylamide group, the 109% means that the polarization alone would produce more than the observed inhibition but the net effect of the other factors (small positive methyl stereochemical effect and somewhat larger negative hydrogen-bonding factor) decreases the inhibition slightly. The much smaller percentage, 43%, contributed by the monoethylamide group polarization is consistent with the large positive stereochemical factor of the ethyl substituent. The fact that most of the activity, 91%, of the dimethylamide group arises from the polarization reflects the small positive stereochemical contribution of the substituents. The small contribution, 24%, for the diethylamide group is consistent with the idea that the two ethyl groups contribute predominately *via* stereochemical effects rather than electronic.

Supporting evidence for this treatment can be found if one considers the cholinesterase inhibition of 1-decyl-3-(N,N-dipropylcarbamoyl)piperidine. The dipropylamide group moment, 5.12 D., is virtually the same as the moment for the diethylamide group moment, 5.05 D. (Table I) and, therefore, from electronic factors only, one would predict similar activities. From the discussion above, however, one would expect that the stereochemical factor (acting predominately through increased hydrophobic forces) would be very important, and that the dipropyl derivative would be a better inhibitor than the diethyl. This is indeed true; the diethyl compound has an I_{50} of 0.53×10^{-5} M compared with 0.105×10^{-5} M for the dipropyl derivative (Table I). In addition, the small contribution, 5% (Table IV), to activity from the polarization of the dipropylamide group illustrates dramatically the great importance of the size of the two propyl groups in their role in affecting cholinesterase inhibition.

Turning attention to the effect of alkyl chain length of our [3-(N,N-diethylcarbamoyl)piperidino]alkanes[1] upon activity, one might consider two important factors. First, the electron-releasing effect of the alkyl group would make the ring nitrogen less positive, thereby reducing the electrostatic attraction between it and the anionic site on the enzyme surface. Secondly, the hydrophobic force at the alkyl chain should push the inhibitor closer to the enzyme surface, thereby improving the opportunity for bonding between inhibitor and enzyme.[29] Both of these effects should increase with increasing chain length (n = number of carbons in alkyl chain), but the electronic one should level off rapidly with chain length while the hydrophobic forces should continue to increase as the chain lengthens. Qualitatively, this argument finds corroboration in the observed mono[3-(N,N-diethylcarbamoyl)piperidino]-alkane I_{50} values.[1] At $n = 1$, $I_{50} = 63.5 \times 10^{-5}$ M, while at $n = 2$, $I_{50} = 118.5 \times 10^{-5}$ M. This decrease in inhibitory potency can be ascribed to the electronic factor, which should be important when comparing one and two carbon atom chains, and the relatively unimportant hydrophobic effect. At $n = 3$, $I_{50} = 101.0 \times 10^{-5}$ M; this small increase in inhibition can be credited to the hydrophobic effect's being slightly more important than the electronic effect. As n is increased to 4, I_{50} becomes 78.0×10^{-5} M, indicating the increased importance of the hydrophobic effect. When the chain length increases beyond $n = 4$, the inhibition increases rather smoothly ($I_{50} = 26.1$, 8.13, and 0.527×10^{-5} M for $n = 5$, 6, and 10, respectively) indicating the relative importance of the hydrophobic effect compared with the electronic effect.

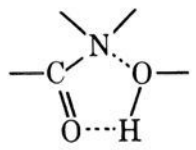
An interesting example of inhibition difference between positional isomers is found in the compounds 1-decyl-3-(N,N-diethylcarbamoyl)piperidine and 1-decyl-4-(N,N-diethylcarbamoyl)piperidine. The electronic structure difference between the isomers should be negligible, there are no hydrogen atoms on the amide nitrogen atom (no hydrogen-bonding possibilities), and the free-energy relationships (Table IV) indicate roughly similar modes of binding. Surprisingly, however, the 3 isomer is about five times more potent than the 4 isomer, having an I_{50} of 0.527×10^{-5} M compared with 2.65×10^{-5} M. Also, the partition coefficients (Table I) are quite different; the value for the 3 isomer is 1.58 and the 4 isomer is 0.17. Certainly, the greater hydrophobic character of the 3 isomer is primarily responsible for its being a more potent inhibitor.

Building upon (1) Nachmansohn and Wilson's[30] proposal of the presence of anionic and esteratic receptor sites on plasma cholinesterase and acetylcholinesterase,

(29) That hydrophobic forces should have an important effect upon enzyme inhibition is seen more clearly from Pullman's concept (ref 22a, p 372) of the enzyme surface as a nonpolar environment excluding aqueous solvent molecules from its immediate vicinity and B. Belleau and G. Lacasse's discussion [*J. Med. Chem.*, **7**, 768 (1964)] of the significance of the driving force for adsorption onto the enzyme originating in the hydrophobic interactions of nonpolar substituents of inhibitors.

(30) D. Nachmansohn and I. B. Wilson, *Advan. Enzymol.*, **12**, 259 (1951).

(2) later investigations[31-33] emphasizing the probable role of a serine moiety in the esteratic site of cholinesterases, and (3) the probable tetrapeptide sequence[34] of the active site(s) of cholinesterases, Gly-(Asp or Glu)-Ser-(Gly or Ala), we would like to propose the structural configuration illustrated in Figure 3 as a possible model for our inhibitor–enzyme complex. Our experimental results support the contention that both anionic and esteratic sites are present in plasma cholinesterase, and the tetrapeptide which we have selected contains functions which could serve in these capacities. Choosing 1-decyl-3-(N,N-diethylcarbamoyl)piperidine as a representative inhibitor,[35] we show points of attachment (1) at the anionic site between the carboxyl group[36] of the enzyme and the positively charged quaternary ring nitrogen of the inhibitor; and (2) at the esteratic site in the form of a quasi-ring formed from association of (a) the serine hydroxyl oxygen of the enzyme with the amide nitrogen of the inhibitor,[37] and (b) the serine hydroxyl hydrogen of the enzyme with the amide oxygen



The influence of other factors (*e.g.*, an imidazole ring and secondary protein structure) upon the serine moiety does not find contradiction in our proposed model (Figure 3) since these functions could also be operative here.

The model is consistent with the following observations: (1) inhibition increases as the hydrophobic forces (acting on both the amide alkyl groups and the alkyl chain at the ring nitrogen) increase, (2) inhibition decreases when hydrogen atoms are attached to the amide nitrogen of the inhibitor (the hydrogens are more hydrophilic and, therefore, would be attracted toward the aqueous medium or away from the enzyme surface[38]), (3) inhibition increases as the polarity of the amide group increases (the greater the polarity, the greater the electrostatic attraction between the group and the enzyme), and (4) inhibition increases as the electron density of the amide nitrogen decreases (thereby increasing the electrostatic attraction between this nitrogen and the serine hydroxyl oxygen).

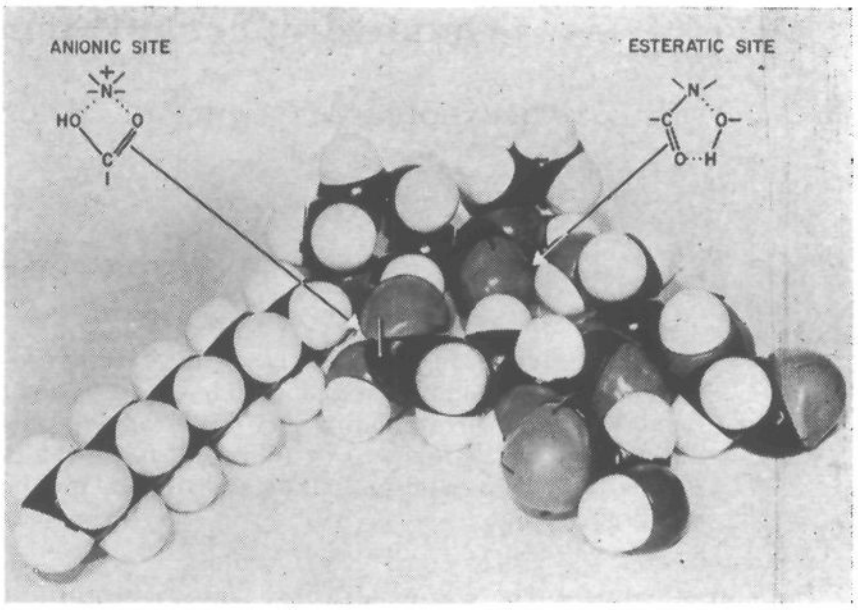


Figure 3.—Stuart–Briegleb model of proposed inhibitor–enzyme complex of 1-decyl-3-(N,N-diethylcarbamoyl)piperidine and the serine and glutamic acid moieties of cholinesterase.

Experimental Section

Materials.—The preparation and properties of our compounds, with the exception of the derivative described below, have been reported[5,39-41] previously. All of the compounds employed in our studies were of analytically pure grade or the equivalent.

1-Decyl-3-(N,N-dipropylcarbamoyl)piperidine Hydrobromide.—N,N-Dipropylnicotinamide[42] (79.5 g, 0.385 mole) and 1-bromodecane (216.1 g, 0.977 mole) were dissolved in 200 ml of anhydrous benzene, and the solution was refluxed for 53 hr. After the benzene was removed by distillation, the residual oily liquid was dissolved in aqueous ethanol. This solution was subjected to hydrogenation in the presence of a total of 2 g of platinum oxide at a maximum pressure of 3.16 kg/cm² (45 psi). When absorption of hydrogen ceased, solvent was removed by distillation *in vacuo*. The product (128.0 g, 76.7%) was recrystallized from ethyl acetate. The white crystals melted at 106° (cor).[43]

Anal.[44] Calcd for $C_{22}H_{45}BrN_2O$: C, 60.95; H, 10.46; Br, 18.43; N, 6.46. Found: C, 60.97; H, 10.59; Br, 18.24; N, 6.50.

Biochemical Evaluation.—Manometric determinations were carried out on a GME-Lardy RWB-3 Warburg instrument using a procedure described elsewhere.[5]

Partition Coefficients.—Benzene–water partition coefficients were determined using the method of Quintana.[13]

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(31) J. Thomas, *Australian J. Pharm.*, **42**, 572 (1961).

(32) R. M. Krupka and K. J. Laidler, *J. Am. Chem. Soc.*, **83**, 1458 (1961).

(33) I. B. Wilson, M. A. Hatch, and S. Ginsberg, *J. Biol. Chem.*, **235**, 2312 (1960).

(34) O. Svensmark, *Acta Physiol. Scand.*, **64**, *Suppl.* **245**, 9 (1965).

(35) Although we use a specific example, Figure 3, the linear free-energy relationships, Table IV, indicate that the inhibitors discussed here interact with the enzyme in a similar manner.

(36) We wish to make it clear that, although we have depicted a carboxyl group from an adjacent amino acid moiety as a possible anionic site, the group performing this function might well be located at some other position in the peptide chain depending upon the over-all conformation of the enzyme molecule.

(37) There would, of course, be electrostatic attraction between the carbonyl carbon of the amide group and the oxygen of the serine hydroxyl group.

(38) Measurements in our laboratories show that N-decylpiperidine hydrobromide is a more powerful cholinesterase inhibitor than 1-decyl-3-carbamoylpiperidine hydrobromide. This would seem to indicate that even though the latter compound possesses a group capable of binding at the esteratic site, the hydrophilicity of this group actually is greater than any enzyme-binding ability which might contribute to its inhibitory potency.

(39) A. Lasslo, W. M. Marine, and P. D. Waller, *J. Org. Chem.*, **21**, 958 (1956).

(40) A. Lasslo and P. D. Waller, *ibid.*, **22**, 837 (1957).

(41) R. P. Quintana and W. A. Shrader, *J. Pharm. Sci.*, **52**, 1186 (1963).

(42) W. P. Purcell and J. A. Singer, *J. Phys. Chem.*, **69**, 4097 (1965).

(43) Determined with a Swissco melting point apparatus filled with silicone fluid.

(44) Analyses were done by Drs. G. Weiler and F. B. Strauss, Oxford, England.