Table I
Effectiveness of Derivatives of N,N-Dimethylhydrazine against L1210 Leukemia in BDF_1 Mice

Compd	Dose,[a] mg/kg	Number of doses	Survivors	T/C, %[b]
1	400	1	6/6	100
2	400	9	6/6	110
3	400	1	0/6	
3	75	9	6/6	101

[a] Route of administration ip. [b] T/C is ratio of survival time of test animals to survival time of control animals.

Table II
Derivatives of N,N-Dimethylhydrazine

No.	Compd	Mp or bp (mm), °C	% yield	$n^{25}D$	d_{25}	Analyses
1	$CH_3C(=NN(CH_3)_2)CH_2COOC_2H_5$	85 (8.1)	76	1.4750	0.9655	MR 50.1[a]
2	$CH_3C(=NN(CH_3)_2)CH_2COOC(CH_3)_3$	90 (7.5)	65	1.4640	0.9352	MR 59.0[b]
3	1-naphthyl-$OCONHN(CH_3)_2$	136–137	58			C,[c] H, N
4	1-naphthyl-$CH_2CONHN(CH_3)_2$	139.5–140.5	24			C, H, N

[a] Calcd 48.7. [b] Calcd 57.7. [c] C: calcd, 67.81; found, 68.51.

Compds **1**, **2**, and **3** in Table II were evaluated against L1210 leukemia in BDF_1 mice.[2] Results are given in Table I.

Ethyl acetoacetate N,N-dimethylhydrazone (**1**) was tested against *Plasmodium berghei* in ICR/HA Swiss mice.[3] At 640 mg/kg the survival time of the mice was increased 1.8 days (mean survival time of controls was 6.2 days; mean survival time of treated animals was 8.0 days). Compd **1** showed some toxicity in this test which did not appear when it was tested against L1210 leukemia at a much higher total dosage.

Experimental Section

Ethyl Acetoacetate N,N-Dimethylhydrazone (1).—A mixt of 100.0 g (0.77 mole) of ethyl acetoacetate and 48.6 g (0.81 mole) of Me_2NNH_2 in 150 ml of EtOH was allowed to stand at room temp for 18 hr. At the end of this period the mixt was concd under vacuum and distd through a 30-cm vacuum-jacketed Vigreux column to give 100.3 g (75% yield) of 1, bp 85° (8.1 mm). This material was collected in 5 fractions, $n^{25}D$ 1.4747–1.4751.

***tert*-Butyl Acetoacetate N,N-Dimethylhydrazone (2).**—*tert*-Butyl acetoacetate (97.1 g, 0.61 mole) and Me_2NNH_2 (38.4 g, 0.64 mole) were dissolved in 150 ml of *tert*-BuOH and the mixt was allowed to stand at room temp for 18 hr. It was concd under vacuum and the residue was distd as above. The product (**2**) (79.2 g, 65%) was collected in 4 fractions, $n^{25}D$ 1.4639–1.4641.

α-Naphthyl N,N-Dimethylcarbazate (3).—A mixt of α-naphthol and $C_6H_5NMe_2$ was treated with $COCl_2$ to give α-naphthyl chloroformate in 79% yield as a nearly colorless liquid, bp 101.5–104.5° (0.5 mm), $n^{25}D$ 1.5959 [lit.[4] bp 117° (1 mm)]. This was treated with Me_2NNH_2 in Et_2O and the resultant solid on extn with EtOAc and recrystn from EtOH yielded **3** (58%), mp 136–137°.

α-Naphthylacetyl N,N-Dimethylhydrazide (4).—α-Naphthylacetyl chloride, bp 136° (1.5 mm), $n^{25}D$ 1.6209 [lit.[5] bp 175–76° (1.5 mm)] was prepd from α-naphthylacetic acid and $SOCl_2$. It was treated with Me_2NNH_2 in a mixt of Et_2O and Et_3N and after extn with C_6H_6 and concn, the recovered solid was recrystd from EtOH to give **4** as a white solid, mp 139.5–140.5°.

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Comparisons of Butyrylcholinesterase Inhibitory Potencies of Selected 3-Substituted-1-decylpiperidines with Their Electron Charge Densities†

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Beasley, *et al.*,[1] and Purcell, *et al.*,[2] have investigated the inhibitory potencies against butyrylcholinesterase

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(acylcholine acylhydrolase, EC 3.1.1.8) of 3-(1-alkyl-piperidyl)carboxamides (I) in which the R_1 substituent varied from a C_1 to a C_{10} chain and in which the structural variation at R_2 included the substituents NH_2, $NHCH_3$, NHC_2H_5, $N(CH_3)_2$, $N(CH_3)(C_2H_5)$, and $N(C_2H_5)_2$. Mathison, *et al.*,[3] have reported the synthesis and evaluation of the butyrylcholinesterase (BuChE) inhibitors II and III in which the carboxamide function in I is replaced by the acetamide moiety and by the urea moiety, resp. R varies in II and III as did R_1 in I. It was shown by Mathison, *et al.*,[3] that, although an increase in the alkyl chain length of the N^1 substituent increased the BuChE inhibitory potencies of I, II, and III, the effect of chain length was far less pronounced for series III than for series I and II.[3]

R_1N — COR_2 (I); RN — $NHCOCH_3$ (II); RN — $NHCON(CH_3)_2$ (III)

The experimental evidence presented makes it apparent that the dramatic change in inhibitory potency found when one compares the shorter alkyl chain derivatives of II with the corresponding shorter alkyl chain derivatives of III is associated with replacing the acetamide ($NHCOCH_3$) moiety with the urea ($NHCONMe_2$) moiety.[3] It was suggested by Mathison, *et al.*, that the urea moiety "provides a better "fit" in the vicinity of the esteratic site" of BuChE than either the acetamide moiety or the *N,N*-diethylcarboxamide moiety.[3] It is the purpose of this paper to report further interpretations of the differences in the contributions to activity of the urea, acetamide, and carboxamide moieties in I, II, and III.

In order to investigate the contribution of electronic charge densities to the activity of BuChE inhibitors, it was decided that, in accordance with Rogers and Cammarata's emphasis of the importance of the σ electron charge densities as well as the π electron charge densities in describing the partitioning phenomenon,[4] the total charge densities of the inhibitors I, II, and III should be calculated. It would then be possible to compare the BuChE inhibitor potencies with the net charges at various centers that have been proposed as important in the interaction of inhibitors with cholinesterases.[5]

Experimental Section

σ Electron charge distributions were determined using a computer program written by Dr. G. E. Bass of this laboratory, utilizing the method of Del Re.[6,7] The π electron charge distributions were determined using a computer program written by Dr. K. Sundaram, utilizing the Hückel method.[8] The total charge was obtained as a sum of the σ electron charge densities and π electron charge densities. The values used for the atomic parameter, $\delta_\mu{}^\circ$, and the bond parameters, $\gamma_{\mu\nu}$ and $_{\mu\nu}$, used in the Del Re method and the Coulomb and resonance parameters, δ_μ and $\eta_{\mu\nu}$, resp, used in the Hückel method are given in Table I. All calcns were carried out on the IBM Systems 1620[II] computer.

TABLE I

SEMIEMPIRICAL PARAMETERS USED IN MO CALCULATIONS

Parameters Employed in HMO Calculations[a]

μ	δ_μ	$\mu\nu$	$\eta_{\mu\nu}$
N:	Piperidyl 1.6	>CN<	0.8
C:	>C= 0.0	>C=O	2.0
O:	=O 0.7		

Parameters Employed in Del Re Calculations

μ	$\delta_\mu{}^0$			$\mu\nu$	$\epsilon_{\mu\nu}$	$\gamma_{\mu\nu}$
C:	Tetrahedron	0.07[b]	CC:	>C–C<	1.0	0.1[b]
N:	Aniline	0.24[a]	CC:	–(=)C—C<	1.0	0.1[b]
$\overset{+}{N}$:	Tetrahedron	0.31[b]	CN:	>C–N<	1.0	0.1[a,b]
O:	–O–	0.40[b]	$C\overset{+}{N}$:	>C–$\overset{+}{N}$<	1.33	0.1[b]
H:	–H	0.00[b]	CO:	>C–O–	0.95	0.1[b]
C:	>C=	0.12[b]	CH:	>C–H	1.0	0.3[b]
O:	=O	0.28[a]	HC:	H–C<	1.0	0.4[b]
			HN:	H–N<	0.45	0.4[b]
			$H\overset{+}{N}$:	H–$\overset{+}{N}$<	0.60	0.4[b]
			NH:	>N–H	0.45	0.3[b]
			N^+H:	>$\overset{+}{N}$–H	0.60	0.3[b]
			CO:	>C=O	0.70	0.1[a]

[a] From Berthod and Pullman, *J. Chim. Phys. Physicochim. Biol.*, **62**, 942 (1965). [b] From Del Re.[6]

Results and Discussion

Results of the molecular orbital calculations along with the BuChE inhibitory activities are given in Table II. Calculations were made on the molecules in which the piperidine N is protonated since this species is expected to predominate at pH 7.4, the pH maintained during the enzymatic evaluation.§ Some calculations

TABLE II

BUTYRYLCHOLINESTERASE INHIBITORY POTENCIES AND TOTAL CHARGE DENSITIES

$H_3C(CH_2)_9\overset{+}{N}(H)$ — R

Compd	R	$K_i{}^a \times 10^6$	I_{50},[b] $M \times 10^5$	$Q_N{}^c$	$Q_C{}^d$	$Q_O{}^e$
1	$NHCOCH_3$	9.38[f]		−0.2776	0.2821	−0.3168
2	$NHCON(CH_3)_2$	0.50[f]		−0.1158[g]	0.3365	−0.3675
3	$CON(C_2H_5)_2$	1.46[f]	0.53[h]	−0.1235	0.2726	−0.3186
4	$CONHCH_3$	11.32[f]	3.48[h]	−0.2747	0.2807	−0.3170
5	$CON(CH_3)_2$	2.96[f]	2.17[h]	−0.1185	0.2731	−0.3185
6	$CON(CH_3)(C_2H_5)$		0.98[h]	−0.1210	0.2728	−0.3186
7	CO-*N*-pyrrolidyl		0.77[h]	−0.1240	0.2725	−0.3186
8	CO-*N*-piperidyl		0.32[h]	−0.1240	0.2665	−0.3186
9	CO-*N*-morpholinyl		2.57[h]	−0.1200	0.2729	−0.3185

[a] K_i is the inhibitor dissociation constant as calcd by the method of Bergmann and Segal. [*Biochem. J.*, **58**, 692 (1954)]. [b] I_{50} is the molarity of compd effecting 50% inhibition. [c] Net charge on amide N. [d] Net charge on carbonyl C. [e] Net charge on carbonyl O. [f] J. G. Beasley, unpublished data. [g] Net charge on the dimethyl-substituted N of the urea moiety. [h] From ref 2b.

§ Quintana and Smithfield have determined the pK_a' for the piperidine N in a series of substituted 1-benzyl-3-(*N,N*-diethylcarbamoyl)piperidine hydrobromides in which the average pK_a' value is 7.55.[9] Since the substituents examined are classically considered as electron-withdrawing groups, one would expect the piperidine N to be less basic (have a lower pK_a') than in the compds considered here.

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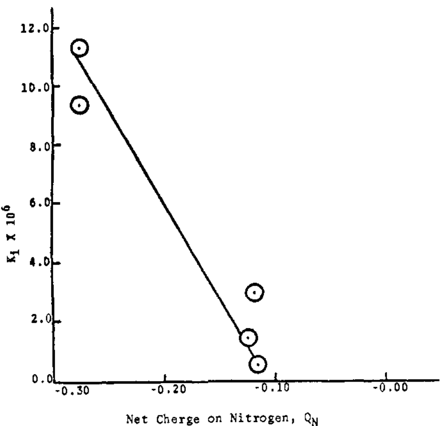

Figure 1.—Butyrylcholinesterase inhibition of some 3-substituted-1-decylpiperidines *vs.* net charge on the N of the substituent group. (Data points included are those for the compds for which K_I values have been determined, *i.e.*, **1–5**, Table II.)

were done on the nonprotonated species, however, to see if protonation of the piperidine nitrogen was "felt" by the substituent atoms at the 3 position of the piperidine ring. Using parameter values for a protonated piperidine nitrogen instead of a tertiary N did not change the charge densities on the atoms attached at the 3 position.

An explanation that has been proposed by Bergmann, *et al.*, for the cholinesterase inhibition of compds containing the RCO function is "that the inhibition is related to the effect of the substituting (R) group upon the electrophilic character of the carbonyl carbon."[10] With this in mind one would expect that an increase in the activity of the compds of series I, II, and III (**1–5**, Table II) would be reflected in an increase in the positive value for the net charge on the carbonyl C. This does not seem to be the case, however, as can be seen in Table II. Although the most active compd does have the most positive value as anticipated, the least active compds do not have the smallest positive charge on the carbonyl C. Thus, it would seem that while the electrophilic character of the carbonyl C is important for BuChE inhibition as illustrated by the most active compd (**2**, Table II), its contribution to BuChE inhibition is modified by the alkyl groups of the carboxamide function as seen in **3**, **4**, and **5**.

If one examines the total charge on the carbonyl O, Q_O, of **1–5** in Table II, it can be seen that the most active compd has the greatest negative charge. The total charge on the carbonyl O for the other compds has such small variation, however, that no conclusion can be drawn concerning a relationship between the total charge on the carbonyl O and activity.

Purcell has reported that, for a series of 1-decyl-3-[(*N*-alkyl)- and 1-decyl-3-[(*N*,*N*-dialkyl)substituted carbamoyl]piperidines, the BuChE inhibitory activity increased as the amide N became more positive.[11] The activity for the acetamide, urea, and substituted amides (**1–5**, Table II) increases as the N in the respective moieties (dimethyl-substituted N for **2**) becomes more positive (Figure 1). It is interesting to note that, whereas a part of the acetamide V shown by darker bonds is isosteric with the choline ester, acetylcholine (IV), and would thus be expected to "fit" the active site better than those compds which do not have the

IV V

ACh "backbone," it is less active than 2 of the carboxamide compds (**3** and **5**, Table II) in which the number of atoms separating the cationic N and the CO group is less than in the choline esters. This points out the dynamic nature of the interaction of inhibitor and enzyme; factors other than the proper positioning of certain atoms which are believed to be active-site-directing centers must also be of vital importance. Also, it is the charge density on the dimethyl-substituted N of **2** that seems to correlate with activity (Figure 1); this N is situated differently than either the N of the acetamide or the carboxamides. That the charge density on the differently situated (*i.e.*, regarding the number of bonds between the N and the piperidinium N) nitrogens is related to activity indicates that electronic charge densities may affect the manner in which the enzyme and inhibitor molecules fit together so that the piperidinium nitrogens and the nitrogens examined in Table II have similar distances between them.

A comparison was made of BuChE inhibitory activities and amide N charge densities for the series of carboxamide derivatives (**3–9**, Table II) in an attempt to delineate further the role of electrostatic charges in BuChE inhibition. Calcns show that there is very little variation in the charge distribution of the $CONH_2$ function while there is considerable variation in the BuChE inhibitory potencies, thus serving to illustrate that the hydrophobic character of the alkyl groups on the $CONH_2$ function is the controlling factor in the BuChE inhibition of these compds. These results are in agreement with those of Purcell, *et al.*, who attribute a major part of the activity of **3–9**, Table II, to relative hydrophobicities of the molecules.[2b]

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Chemistry of Cephalosporin Antibiotics. 25. 3-Cyanomethyl Cephem Nucleus

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Numerous structural manipulations have been carried out at the 3 position of the cephem nucleus. Modifica-