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THE SIZE OF HYDROXYL GROUPS IN SOLUTION AND THE CHANGES IN SIZE ASSOCIATED WITH THE IONIZATION OF PHENOLIC, CARBOXYLIC AND AMINO GROUPS IN PHENOLIC QUATERNARY AMMONIUM SALTS, NICOTINE AND SOME AMINO ACIDS: POSSIBLE IMPLICATIONS FOR DRUG-WATER AND DRUG-RECEPTOR INTERACTIONS

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1 Size in solution can be expressed either as the apparent molal volume at infinite dilution (ϕ_v^0) and the concentration parameter (j) or as the partial molal volume of the solute at infinite dilution ($\bar{V}_2^0$) and the concentration parameter for the solute or solvent (q_s or q_w). Although calculated differently, these are derived from the same results and are equivalent.

2 From measurements with phenolic quaternary ammonium salts, including compounds with high nicotine-like activity, the apparent size of the hydroxyl group in water is small and variable. Phenolic groups are slightly larger than alcoholic groups, which should be better hydrogen donors.

3 By measuring the volume change associated with ionisation it is possible to measure the size of charged groups such as phenate and carboxylate; these are much smaller than phenolic and carboxyl. Ammonium groups, however, are only slightly smaller than the corresponding amines.

4 The zwitterion forms of amino acids are associated with a minimum in volume but the volume changes increase with chain length from glycine to γ-aminobutyric acid. Groups separated by less than this distance interact in their effects on water.

5 Decreases in volume or unexpectedly small increments in apparent molal volume represent decreases in entropy which must be taken into account in drug-water-receptor interactions. Although they may be offset by enthalpy changes, they should favour binding because there is more scope for an increase in entropy. This might explain the association of the small apparent size in water of the hydroxyl group in many compounds with its effects on their affinity for receptors.

Introduction

There is general agreement that water plays an important part in drug-receptor interactions. Gill (1965) wrote 'In considering the possibility of hydrogen bonding between two different groups in an aqueous solution the relevant thermodynamic quantity is not the free energy of formation of a hydrogen bond *per se* but the difference in free energy between donor/water, acceptor/water and the donor/acceptor, water/water hydrogen bonds'. Belleau (1964; 1967; 1968), from studies of the affinity of compounds for acetylcholinesterase over a range of temperatures, described 'water as the determinant of thermodynamic transitions in the interaction of aliphatic chains with acetylcholinesterase and the cholinergic receptors' and considered it to be an important factor in his molecular perturbation theory of drug action. More recently, Franks (1978) has written 'That solvent interactions influence the conformational stability of proteins is now generally accepted', but present knowledge is limited to relatively simple proteins. The direct experimental study of the involvement of water in the interactions between drug and receptor awaits developments in receptor biochemistry.

However, part of the equation involves the interaction between the drug and water and it is the purpose of this paper to collect together some information about this. The size of a drug in solution has been estimated from its apparent molal volume which can be obtained from precise measurements of the densities of solutions of known composition (Barlow, Lowe, Pearson, Rendall & Thompson, 1971; Barlow & Franks, 1973). In the course of many measurements (Abramson, Barlow, Franks & Pearson, 1974; Barlow, 1978) it became apparent that the hydroxyl group has

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a very small apparent volume in water which varies considerably from drug to drug. Most of the compounds studied were antagonists of acetylcholine at muscarine-sensitive receptors and the hydroxyl group was important for affinity. The hydroxyl group is also important for the ganglion-stimulant activity of many phenylalkyltrimethylammonium salts, such as *m*-hydroxyphenylpropyltrimethylammonium and coryneine (Barlow, Bowman, Ison & McQueen, 1974) and cinobufotenine (Barlow & Burston, 1980), so measurements have now been made of the size of the hydroxyl group in a range of phenolic quaternary ammonium salts. The results from the density experiments were used to calculate partial molal volumes as well as apparent molal volumes. Apparent molal volumes assume that the volume of the solvent is constant and unaffected by the solute; partial molal volumes do not make this assumption and it was therefore of interest to compare the two.

An attempt was made to see whether the size, and the variation in size, of phenolic groups is different from that of alcoholic groups. It was expected that it might be, because the electron withdrawing effect of the aromatic ring should make a phenolic group less able to act as a hydrogen donor in bonding to water. In the extreme situation in which the phenolic group becomes completely ionized, the size would be expected to be very different indeed. A method was devised for studying this without isolating the phenate salts, by measuring the volume change on the addition of alkali. The method was first checked in experiments with acetic acid, for which the volume change was measured by King (1969) by pycnometric measurements with acetic acid and sodium acetate. It was applied not only to phenolic groups but also to the ionization of certain amines, pyridine, pyrrolidine, N-methylpyrrolidine and nicotine and to the ionization of amino and carboxyl groups in the amino acids glycine, β-alanine and γ-aminobutyric acid. The purpose of this work was to find out the size of these volume changes, about which little is known, and also the extent to which they may be additive. Nicotine and the amino acids contain two groups capable of interacting strongly with water and the results my show how far apart these must be before they act independently. The results might indicate, for instance, whether the hydration of γ-aminobutyric acid is substantially different from that of glycine.

The connection between entropy and volume is given by the Maxwell relation

$$\left(\frac{\partial S}{\partial V}\right)_T = \left(\frac{\partial P}{\partial T}\right)_V$$

and Hepler (1965), for example, has noted that there is a good linear relationship between entropy and volume changes for the ionization of many aqueous acids (at constant temperature). This linear relationship may not extend to other changes, such as replacement of H by OH, which may alter

$$\left(\frac{\partial P}{\partial T}\right)_V$$

but at least qualitatively decreases in volume, or unexpectedly small increments in volume, should indicate a decrease in entropy (increase in order).

For compounds which are substrates or agonists, a highly ordered complex may be necessary for subsequent events to occur easily and a decrease in entropy on binding may be the price which must be paid for this (Jencks, 1975). For compounds which are antagonists, and perhaps do not alter receptor structure, however, affinity may be favoured if the process of binding is associated with the breakup of interactions between solute and solvent and the binding process is associated with an increase in entropy. It is remarkable, for instance, that the increment in apparent molal volume for the hydroxyl group in some potent atropine-like compounds is very small indeed (Abramson *et al.*, 1974). The subsequent paper reports an estimate of the size and contribution to affinity of the hydroxyl group in atropine (Barlow & Ramtoola, 1980).

The actual extent of binding between drug and receptor of course depends on the enthalpy change as well as on the entropy change and it may be possible to relate these studies of apparent molal volume to work in which the entropy of adsorption has been assessed from estimates of the enthalpy of adsorption made from the effects of temperature on affinity (Barlow & Burston, 1979; Barlow, Birdsall & Hulme, 1979).

Methods

Density measurements

Solutions were made from weighed amounts (corrected to vacuum) of solute and solvent and their density was measured with an Anton Paar Precision Density Meter, DMA O2D. This was calibrated with solutions of sodium chloride, exactly as in previous work (Lowe, MacGilp & Pritchard, 1973; Barlow & Franks, 1973). The calibration was included in every set of experiments and was usually based on at least 8 measurements with water and two concentrations of sodium chloride, spanning the range of densities to be studied. The work was greatly facilitated by linking the Density Meter to an Anadex printer and a Palmer time-clock, so that readings were recorded automatically once every minute. Values were usually steady within 10 min of inserting a sample. The temperature

was 25.0°C, nominally kept within ±0.01°C by a Haake thermostat model FT supplied with cold water (17°C) from a Grant heater and cooler unit (FH15 and FC15). The biggest source of error appeared to be the tendency for the temperature to drift during the experiment because of the changes in room temperature which occurred over a period of 6 to 10 h. A temperature change of 0.01°C is likely to alter the density by about 0.000003 g/ml, which produced detectable changes in the instrument readings. Within an experiment the estimates of the density of a solution did not usually differ by more than 0.00001 g/ml.

Apparent molal volumes

These were calculated as before (Barlow, Lowe, Pearson, Rendall & Thompson, 1971) from the relation

$$\phi_v = \frac{1}{m}\left(\frac{1000 + mM}{d} - \frac{1000}{d_w}\right)$$

where m is the molality, M is the molecular weight, d is the density of the solution and d_w is the density of water. In previous work the relation between ϕ_v and molar concentration, c, has been assumed to be that derived by Redlich & Rosenfeld (1931; Millero, 1971):

$$\phi_v = \phi_v^0 + S_v c^{\frac{1}{2}} + jc$$

where S_v is determined by the physical properties of the solvent (and is 1.868 for water at 25°C); the constant j is determined by the solute. Values of $(\phi_v - 1.868c^{\frac{1}{2}})$ have therefore been fitted to c by the method of least-squares, weighted according to the concentration as in previous work: this gives estimates of ϕ_v^0 and j.

Partial molal volumes

In the calculation of apparent molal volumes it is assumed that the volume occupied by the solvent remains constant; the volume estimated is the space which the solute appears to occupy in the solution. It is likely that the solute alters the volume occupied by the solvent so the values of molality and density have also been used to calculate the partial molal volumes of both solute and solvent (Moelwyn-Hughes, 1961).

If each ml of solution contains n_1 solvent molecules of size v_1 and density d_1, and also n_2 solute molecules of size v_2 and density d_2,

$$n_1v_1 + n_2v_2 = 1$$

and the density

$$\begin{aligned} d &= n_1v_1d_1 + n_2v_2d_2 \\ &= (1 - n_2v_2)d_1 + n_2v_2d_2 \\ &= d_1 + n_2v_2(d_2 - d_1) \end{aligned}$$

The fraction of solute present by weight

$$= \frac{n_2v_2d_2}{d}$$

so the concentration of solute in g/ml, $c_g = n_2v_2d_2$ and

$$d = d_1 + c_g\left(\frac{d_2 - d_1}{d_2}\right).$$

The graph of d against c_g should therefore be a straight line and the density of the solute,

$$d_2 = \frac{d_1}{1 - m},$$

where m is the slope. The partial molal volume of the solvent is

$$\frac{M_1}{d_1}$$

and that of the solute is

$$\frac{M_2}{d_2}$$

where M_1 and M_2 are the respective molecular weights.

If the solute and solvent molecules interact, the molal volumes vary with concentration and the graph of d against c_g is not a straight line. In the range of concentrations tested it can be approximately represented by the parabola

$$d = A + B_1c_g + B_2c_g^2$$

and a least-squares fit has been used to obtain estimates of A, B_1 and B_2. For a particular value of c_g the slope is given by $B_1 + 2B_2c_g$ and this can be used to calculate the partial molal volumes of solute and solvent for that particular concentration. Estimates can be made of the value for the solute at infinite dilution ($\bar{V}_2^0$) and for the change in partial molal volume with respect to concentration (q_s and q_w). The effects on water appear very small when expressed in this way but because of the amount of water present the total effect is comparable with that seen with the solute.

Measurements with alkali or acid

A weighed amount, w_1, of solute was dissolved in a weighed amount, w_2, of NaOH (or HCl) of known molarity, whose density, d_2, was measured as well as the density, d, of the solution. All weights were corrected to vacuum. The fraction of solute, x, which had reacted was calculated from the weights and the molarity and density of the NaOH. For the amounts taken, the change in volume when the solute is added to the solvent is

$$\frac{w_1 + w_2}{d} - \left(\frac{w_1}{d_1} + \frac{w_2}{d_2}\right)$$

where d_1 is the density of the solute. For solid solutes this was estimated from the buoyancy of the material in organic liquids (usually *n*-propyl bromide and 1,2,4-trichlorobenzene and mixtures of these). For 1 mole of solute and 1 kg of solvent the change

$$\Delta V = \frac{M}{w_1}\left(\frac{w_1 + w_2}{d} - \frac{w_1}{d_1} - \frac{w_2}{d_2}\right)$$

where M is the molecular weight.

This change was also calculated for the experiments with solute and water. With most of the compounds $x = 0$ in these conditions but with some of them appreciable ionization can occur. The value of x for a concentration, c, of an acid, HA, can be calculated from the ionization constant, K_a.

$$HA = H^+ + A^-,$$

$$K_a = \frac{[H^+][A^-]}{[HA]} = \frac{x^2c}{1 - x}$$

so for small values,

$$x \doteq \sqrt{\frac{K_a}{c}}.$$

For 0.1 M acetic acid ($pK_a = 4.76$ at 25°C),

$$\log x = \tfrac{1}{2}(-4.76 + 1.0)$$

and $x = 0.013$.

For

$$B + H_2O = BH^+ + OH^-,$$

$$K = \frac{[BH^+][OH^-]}{[B]} = \frac{K_w}{K_a} = \frac{x^2c}{1 - x};$$

so for small values

$$x \doteq \sqrt{\frac{K_w}{K_a c}}.$$

For 0.1 M N-methylpyrrolidine, ($pK_a = 10.46$ at 25°C), $\log x = \tfrac{1}{2}(-14.0 + 10.46 + 1.0)$ and $x = 0.054$. For 0.1 M pyrrolidine, however,

$$\log x = \tfrac{1}{2}(-14.0 + 11.27 + 1.0)$$

and $x = 0.136$. If the approximation is not made x can be calculated from the quadratic equation

$$cx^2 + zx - z = 0,$$

where

$$z = \frac{K_w}{K_a};$$

the exact value is 0.127.

The values of ΔV were plotted against x and for the reaction $B + H^+ = BH^+$ they indicate the electrostrictive effect associated with the positive charge. For the reaction $HA + OH^- = A^- + H_2O$ it is necessary to deduct the increase in volume due to the formation of water (18.07 cm^3/mol) and also to the disappearance of the hydroxyl ions. Millero (1971) gives a value of -4.04 cm^3/mol as the apparent molal volume for the hydroxyl ion so the total expected increase for the reaction is 22.11 cm^3/mol (Dunn, Stokes & Hepler, 1965).

Compounds

Most of the compounds were samples whose melting-point and analyses were given by Barlow, Thompson & Scott (1969) or by Barlow & Burston (1980). *o*-Hydroxybenzyltrimethylammonium bromide had m.p. 184.3–185.0°C, found Br^-, 32.41%; *m*-hydroxybenzyltrimethylammonium bromide had m.p. 195.8–196.5°C, found Br^-, 32.46%; $C_{10}H_{16}ONBr$ requires Br^-, 32.46%. *p*-Methoxyphenethyltrimethylammonium bromide had m.p. 215.7–216.2°C, found Br^-, 29.15%; *m*-methoxyphenethyltrimethylammonium bromide had m.p. 188.0–189.0°C, found Br^- 28.98%; $C_{12}H_{20}ONBr$ requires Br^-, 29.14%. 3:4-Dimethoxyphenethyltrimethylammonium bromide had m.p. 232.6–233.2°C, found Br^- 26.25%; $C_{13}H_{22}O_2NBr$ requires Br^-, 26.26%. 2-(Indol-3-yl)ethyltrimethylammonium bromide had m.p. 196.5–197.0°C, found Br^- 28.32%; $C_{13}H_{19}N_2Br$ requires Br^-, 28.21%. 2-(5-Methoxyindol-3-yl)-ethyltrimethylammonium bromide had m.p. 171.8–172.4°C, found Br^-, 24.48%; $C_{14}H_{21}ON_2Br$, H_2O requires Br^-, 24.12%. Melting-points were recorded with a Mettler FP5 instrument connected to a potentiometric recorder. The rate of heating was 0.2°/min; analyses were gravimetric with samples of 50–150 mg.

Acetic acid, pyridine, pyrrolidine, N-methylpyrrolidine and nicotine were all redistilled before use. Glycine was Analar grade (B.D.H.); β-alanine and γ-aminobutyric acid were obtained from Sigma and dried (70°C) before use.

Calculations

As in previous work (Barlow & Franks, 1973), the densities of solutions were calculated from the readings obtained with the Density Meter with a Fortran programme written in double precision. The least-squares fit to a parabola was made with a programme written in Focal on a PDP 8/L machine or with a programme in Basic on a Commodore PET 2001.

Results

Phenolic quaternary ammonium salts: comparison of methods

The results of the survey of phenolic quaternary ammonium salts are summarized in Table 1. In the range of concentrations studied the changes in ϕ_v are small and the estimate of ϕ_v^0 should be precise; the standard error of the estimate was usually less than 0.1 cm^3/mol. There is a bigger variation in estimates obtained in different sets of experiments but even so the estimates of ϕ_v^0 are reproducible to about 0.2 cm^3/mol. Figure 1 shows results for phenethyltrimethylammonium bromide, and for the *p*-hydroxy- and *p*-methoxy-compounds. For the phenyl- and *p*-hydroxyphenyl-compounds these are less than expected from Debye-Hückel theory, whereas for the *p*-methoxy-compound they exceed the expected value. This is indicated by the value of j, which is negative for the former and positive for the latter. Although j cannot be estimated with much accuracy there are trends in its size and sign and there is a striking association of j with q_s, the change in the partial molal volume of the solute with respect to concentration. For the values of j and q_s in Table 1 A and B the correlation coefficient is 0.86. As would be expected there is a strong negative correlation between q_s and q_w ($r = -0.97$).

The values of j and q_s depend on the nature of the anion, as can be seen by comparing the bromides and iodides of the hydroxyphenethyl compounds. The *m*- and *p*-hydroxyphenethyltrimethylammonium iodides have large values of j and q_s and are not very soluble. Possibly the associated decrease in q_w indicates that the solute decreases the entropy of the water. This seems also consistent with the large values of j and q_s found with *m*- and *p*-methoxyphenethyltrimethylammonium bromides and with 4-methoxyindolylethyltrimethylammonium bromide where the hydrophobic effect of the methyl group should also decrease the entropy of the water.

The two methods of expressing the results, as apparent molal volumes or as partial molal volumes, appear to give the same information. Interactions with water can be expressed as j, the measure of departure from Debye-Hückel predictions, as q_s, the change in the partial molal volume of solute with respect to concentrations, or as q_w, (though this is numerically much smaller). Estimates of ϕ_v^0 and $\bar{V}_2^0$ differ by less than 1 cm^3/mol and, provided there is some indication of j, there seems no particular reason for abandoning the use of apparent molal volumes in favour of partial molal volumes.

Size of hydroxyl groups

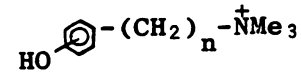
The increments in ϕ_v^0 for the replacement of hydrogen by hydroxyl are shown in Table 2, which also shows the effects of replacing hydrogen by methoxyl and —CH_2— by —CO—. From the results obtained with phenolic hydroxyethyltrimethylammonium compounds it seems that the alcoholic group is smaller than the phenolic group. For the 8 estimates of the increment for a phenolic group the range was from 2.3 to 4.3 cm^3/mol and the average was 3.4 (s.d. = 0.8); for the 4 estimates of the increment for an alcoholic group the range was from 1.6 to 3.2 and the average was 2.5 (s.d. = 0.8). If the other instances in which the increment for an alcoholic group has been estimated (Abramson *et al.*, 1974; Barlow, 1978) are included the overall average is 1.5 cm^3/mol (s.d. = 1.2, 13 estimates). These results are consistent with the idea that the phenolic hydroxyl is a weaker hydrogen donor than the alcoholic hydroxyl group.

Size of carbonyl groups

The negative increment associated with the replacement of methylene by carbonyl indicates the change

Table 1 Apparent molal volumes (ϕ_v^0) and partial molal volumes ($\bar{V}_2$); 25°C

(A)

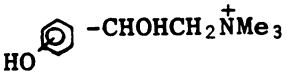


n = 1	*conc.* (mM)	ϕ_v^0	*j*	$\bar{V}_2^0$	q_s	q_w	*n*
o-HO	30–145	190.05	6.3	190.61	15	−0.014	10
m-HO	30–134	190.45	2.2	190.47	14	−0.015	9
p-HO	35–177	191.19	−0.4	191.65	3.0	−0.008	9
H*	29–166 (Br^-)	176.25	−4.3	176.48	−2.0	+0.004	18

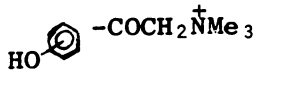
(*continued*

Table 1—*continued*

	conc. (mM)	ϕ_v^0	*j*	$\bar{V}_2^0$	q_s	q_w	*n*
n = 2							
o-HO	15–60	207.34	−7.5	207.21	5.8	−0.003	8
m-HO	18–47	207.36	14.8	208.04	22	−0.010	7
p-HO	20–108	205.92	14.2	206.20	35	−0.028	18
3:4(HO)$_2$-	13–57	207.09	0.0	207.81	−7.1	0.003	15
H*	26–221 (Br^-)	192.02	−5.0	192.35	−4.7	0.011	13

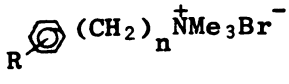


m-HO	46–73 (Br^-)	197.91	−0.7	198.31	2.3	−0.001	7
p-HO	31–65 (Br^-)	197.65	2.6	197.88	14	−0.009	8
3:4(HO)$_2$	43–170 (Cl^-)	191.81	−1.0	192.44	0.0	−0.003	10
H	36–75	205.14	18.6	205.58	40.4	−0.028	6

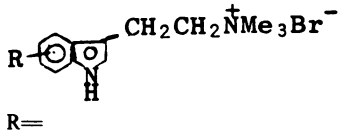


m-HO	52–132 (Br^-)	191.00	−4.8	191.77	−12	0.008	11
p-HO	47–156 (Br^-)	191.15	−3.4	191.34	0.0	0.001	10
3:4(HO)$_2$	40–164 (Cl^-)	185.10	−5.0	185.47	−5	0.008	9
H	50–142 (Br^-)	188.22	−2.5	188.65	−0.9	0.001	11

(B)



n = 1							
o-HO	25–88	180.19	−8.0	180.07	−29.8	0.02	9
m-HO	23–98	178.34	3.2	178.71	−16.8	0.01	8
n = 2							
m-HO	28–153	194.68	−4.0	195.03	−3.0	0.004	12
p-HO	33–133	195.09	−4.7	195.23	−1.8	0.003	16
3:4-(HO)$_2$	23–161	195.49	−1.5	195.96	0.3	−0.002	28
m-MeO	28–73	211.64	9.6	211.60	31.9	−0.021	14
p-MeO	20–75	212.16	11.4	212.64	23.3	−0.016	12
3:4-(MeO)$_2$	24–70	234.86	−9.2	234.61	0.1	0.003	13



R=							
H	28–114	214.24	−3.2	214.24	2.9	−0.002	18
5-HO	32–143	217.17	−5.9	217.57	−7.2	0.009	15
5-MeO	25–89	242.59	20.3	243.42	35.9	−0.031	12

Values of ϕ_v^0 and j are calculated from the equation: $\phi_v = \phi_v^0 + 1.868c^{\frac{1}{2}} + jc$, where c is the molar concentration, and assume that there is no change in the partial volume of water. Values of $\bar{V}_2$ are calculated for the solute allowing for changes in the partial volume of water though the value for water at infinite dilution of solute was assumed to be 18.068 in all instances. Changes in the partial volume of solute with concentration was indicated by q_s and in the partial volume of water by q_w. The range of concentrations tested is indicated; *n* is the number of estimates, usually from 3 to 5 concentrations. The asterisk indicates that Barlow *et al.* (1971) recorded $\phi_v^0 = 176.0$ for benzyltrimethylammonium bromide (by pycnometry) and Barlow & Franks (1973) recorded $\phi_v^0 = 191.9$ for phenethyltrimethylammonium bromide. In part A the compounds are iodides, except where indicated; the increment for conversion from chloride to iodide was 18.39 cm^3/mol and from bromide to iodide it was 11.51 cm^3/mol. In part B the compounds are all bromides.

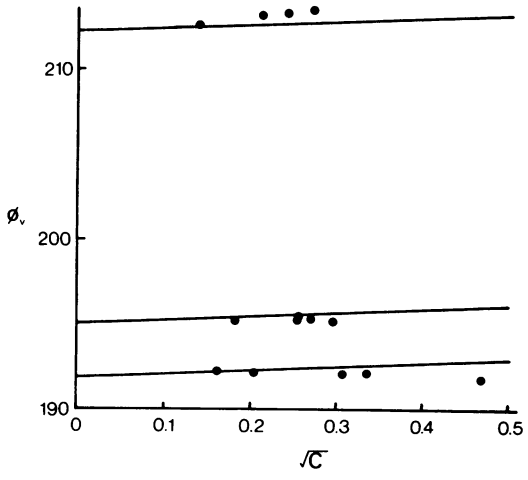


Figure 1 Relation between apparent molal volume, ϕ_v and $c^{\frac{1}{2}}$ for phenethyltrimethylammonium bromide (bottom), the *p*-hydroxy compound (middle) and the *p*-methoxy compound (top). The line shows the prediction from Debye-Hückel theory, $\phi_v = \phi_v^0 + 1.868c^{\frac{1}{2}}$, where ϕ_v^0 has been calculated from the least-squares fit to the equation including the term jc; the values of j are −5.0, −4.7 and 11.4 (Table 1B).

to be expected from a group which can act as a hydrogen acceptor or where the partial charge may have an effect. Other examples of the effect are afforded by comparing 4-keto-*n*-pentyltrimethylammonium iodide with its *n*-pentyl analogue, for which the increment in ϕ_v^0 is −4.3 cm³/mol; for the triethyl compounds it is −2.1 cm³/mol (Barlow, 1974).

Size of phenate groups and a check on the method

If the phenolic group is actually ionized, the negative charge would be expected to have an electrostrictive effect and the size should be very different from that of the unionised phenolic group. The method for assessing this without isolating the phenate salts was tested with acetic acid and the results are summarized in Table 3 and illustrated in Figure 2. These indicate that the volume change for the ionisation of acetic acid is −11.54 cm³/mol, which agrees well with the value, −11.50, obtained by King (1969) from pycnometric experiments with solutions of acetic acid and sodium acetate. The transition from liquid acetic acid to aqueous acetic acid to aqueous acetate is associated with changes in volume from 57.5 to 51.9 to 40.4 cm³/mol.

Measurements were made with some phenolic compounds which were selected so as to see how far apart positively and negatively charged groups must be if they are to act independently in their effects on water. The results are shown in Table 4A. The densities of the solids are known less accurately than those of the liquids and errors will affect the values of ΔV. They do not, however, affect the change in ΔV with the extent of ionisation because the term involving d_1 is constant:

$$\Delta V = \frac{M}{d}\left(\frac{w_1 + w_2}{w_1}\right) - \frac{M}{d_2}\left(\frac{w_2}{w_1}\right) - \frac{M}{d_1}.$$

Table 2 Increments in apparent molal volume at infinite dilution ($\Delta\phi_v^0$; 25°C) for the replacement of hydrogen by hydroxyl, hydrogen by methoxyl, or of —CH_2— by —CO—

Benzyl compounds	Phenolic OH		*2-Hydroxyphenethyl compounds* Phenolic OH	alcoholic OH
o	2.3			
m	2.7		4.3	2.1
p	3.4		4.0	3.2
3:4-$(HO)_2$-			5.1	3.1
H				1.6
Phenethyl compounds		H → MeO—	*Phenacyl compounds*	—CH_2— → —CO—
o	3.8			
m	3.8 (2.7)	19.6	2.8	−4.8
p	2.4 (3.1)	20.1	2.9	−3.3
3:4-$(HO)_2$	3.6 (3.5)	42.8	3.8	−3.6
Indolylethyl compounds				
5-HO-	2.9	28.3		

Where necessary it has been assumed that the increment for replacing iodide by bromide is −11.5 cm³ mol⁻¹ and the increment for replacing chloride by bromide is 6.88 cm³/mol (Millero, 1971). Values in parentheses are for bromides listed in part B of Table 1.

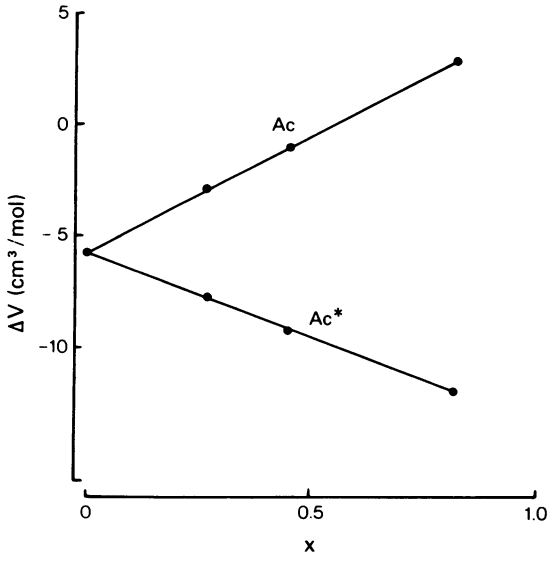


Figure 2 The change in volume on solution, ΔV, plotted against the fraction ionized, x. The line Ac shows the actual change for acetic acid; Ac* shows the net changes (ΔV*) after allowance for the amount of hydroxyl lost and the amount of water formed.

The equation shown as the first entry in Table 4A indicates that the process of solution of the solid is accompanied by an increase in volume (5.56 cm^3/mol). With molecular weight of 246.5 and a density of 1.41 the solid has a V_m of 174.6 so the equation gives ϕ_v^0 = 180.1 and the value in Table 1B is 180.19. The change accompanying ionization is −14.30 and ϕ_v^0 for the phenate ion, $^-OC_6H_4CH_2NMe_3Br^-$, is 180.19 − 14.30 = 165.89 cm^3/mol. The decrease in size is bigger than for the carboxyl group in acetic acid but the change is appreciably smaller for *o*-hydroxybenzyltrimethylammonium bromide than for *m*- or *p*-phenethyltrimethylammonium compounds (Figure 3). It appears that in the *o*-compound the groups interacting with water, onium and phenolic, are near enough to affect each other. An interaction between groups can be seen also with coryneine, in which the presence of one phenolic group reduces the increment for ionization of the other, whereas the presence of —O^- increases the increment for the second phenolic groupl

Size of ammonium groups

Evidence for interactions between groups can be seen, too, in the experiments involving the addition of a proton to amines (Table 4B). The increment for the ionization of methylpyrrolidine is −3.1 cm^3/mol and for pyridine it is −4.3 cm^3/mol but in nicotine the increments are −4.0 and −7.6 cm^3/mol respectively (Figure 4a and 4b). Kauzmann, Bodansky & Rasper (1962) have described the 'reinforcing effect' on electrostriction seen when two negatively charged groups are close together in divalent anions, such as succinate, and this appears to be an example of similar effects in cations.

The most striking feature of the results with the amines is the very small change associated with the loss or addition of a proton (Figure 4a). The volume decreases considerably when the amine dissolves in water (compare V_m and the value of ΔV when x = 0 in Table 4B) and there is only small further decrease

Table 3 Density and ionization of acetic acid

Molality	*Solvent*	*Density*	*n*	*x*	*ΔV*	ϕ_v
0.1188	water	0.99802	3	0.012	−5.59	51.96
0.2525	water	0.99910	3	0.008	−5.62	51.92
0.3783	water	1.00011	3	0.007	−5.62	51.93
						ΔV*
0.3654	0.1 M NaOH	1.00335	3	0.274	−2.69	−8.75
0.2196	0.1 M NaOH	1.00223	4	0.455	−0.89	−10.95
0.1217	0.1 M NaOH	1.00143	4	0.822	+3.09	−15.08

$$\Delta V = 10.55x - 5.61 \quad (4)$$
$$\Delta V^* = -11.54x - 5.62 \quad (4)$$

Estimates of the density are the mean of *n* values which did not usually differ by more than 0.00001; x is the degree of ionization; ϕ_v is the apparent molal volume; ΔV is the change in volume (in cm^3/mol) for the addition of 1 mol of acetic acid to 1 kg solvent and ΔV* is the change after allowing for the water formed and the OH^- ions removed. The density of the glacial acetic acid was estimated to be 1.04367 and its molal volume, V_m = 57.54; the density of 0.1 M NaOH was 1.00151. The equations show the least-squares fit for the number of values of x shown in parentheses.

Table 4 Volume changes for the ionisation of some amines, phenols and amino acids

A	*o*-$HOC_6H_4CH_2\dot{N}Me_3\ Br^-$		$\Delta V^* = -14.30x + 5.56$ (6)	$d_1 = 1.41$
	m-$HOC_6H_4CH_2\dot{N}Me_3\ Br^-$		$\Delta V^* = -13.76x + 2.98$ (6)	1.40
	m-$HOC_6H_4CH_2CH_2\dot{N}Me_3\ Br^-$		$\Delta V^* = -17.69x + 2.83$ (5)	1.35
	p-$HOC_6H_4CH_2CH_2\dot{N}Me_3\ Br^-$		$\Delta V^* = -17.62x + 5.01$ (9)	1.37
	3:4-$(HO)_2C_6H_3CH_2CH_2\dot{N}Me_3\ Br^-$		$\Delta V^* = -14.63x + 1.79$ (6) for x = 0 to 1	1.42
			$= -18.52x + 5.80$ (2) for x = 1 to 2	
B	Pyridine		$\Delta V = -4.34x - 3.64$ (6)	
	m = 0.119	$\phi_v = 77.46$		$V_m = 80.89$
	0.251	77.32		
	0.302	77.30		
	Pyrrolidine		$\Delta V = -1.99x - 6.28$ (5)	
	m = 0.069	$\phi_v = 72.74$		$V_m = 82.51$
	0.142	74.12		
	0.255	74.82		
	0.282	74.94		
	N-Methylpyrrolidine		$\Delta V = -3.10x - 10.39$ (6)	
	m = 0.101	$\phi_v = 95.44$		$V_m = 105.87$
	0.201	95.46		
	0.225	95.46		
	Nicotine		$\Delta V = -4.00x - 8.59$ (6) for x = 0 to 1	
	m = 0.038	$\phi_v = 152.48$	$= -7.63x - 5.21$ (4) for x = 1 to 2	
	0.068	152.43		
	0.111	152.49		
	0.125	152.46		$V_m = 161.09$
C	ΔV is the change for $—COOH \rightarrow —COO^-$; ΔV^* is the change for $—\dot{N}H_3 \rightarrow —NH_2$			
	H_2NCH_2COOH		$\Delta V = -6.35x - 3.88$ (5)	$d_1 = 1.607$
			$\Delta V^* = 2.00x - 3.92$ (5)	
	m = 0.103	$\phi_v = 42.63$		
	0.186	43.04		
	0.234	43.15		
	0.282	43.26	$\phi_v = 40.57 + 8.54c^{\frac{1}{2}} - 6.55c$	
			$= 41.72 + 2.99c^{\frac{1}{2}}$	
	$H_2NCH_2CH_2COOH$		$\Delta V = -7.81x - 5.31$ (6)	
			$\Delta V^* = 2.49x - 5.31$ (5)	$d_1 = 1.411$
	m = 0.045	$\phi_v = 57.81$		
	0.105	58.13		
	0.166	58.27		
	0.276	58.44	$\phi_v = 56.99 + 4.66c^{\frac{1}{2}} - 3.61c$	
			$= 57.42 + 2.03c^{\frac{1}{2}}$	
	$H_2NCH_2CH_2CH_2COOH$		$\Delta V = -10.33x - 12.63$ (5)	
			$\Delta V^* = 3.94x - 12.66$ (5)	$d_1 = 1.213$
	m = 0.048	$\phi_v = 72.35$		
	0.101	72.70		
	0.147	72.80		
	0.220	73.00	$\phi_v = 71.36 + 5.50c^{\frac{1}{2}} - 4.28c$	
			$= 71.82 + 2.57c^{\frac{1}{2}}$	

The coefficients in the equation relating ΔV (or ΔV^*) and x were calculated by the method of least squares for the numbers of values of x shown in parentheses. For the amino acids the fit was weighted according to $1/c^2$ (see text). For the amines and amino acids values of molality, m, and apparent molal volume, ϕ_v, are also shown, together with the molal volume, V_m, for the pure liquids and the estimate of the densities of the solids, d_1. The value for glycine is taken from Cohn, McMeekin, Edsall & Weare (1934), who also recorded 1.231 for α-aminobutyric acid. Equations show the least-squares fit of ϕ_v to $c^{\frac{1}{2}}$ and c and also to $c^{\frac{1}{2}}$ alone.

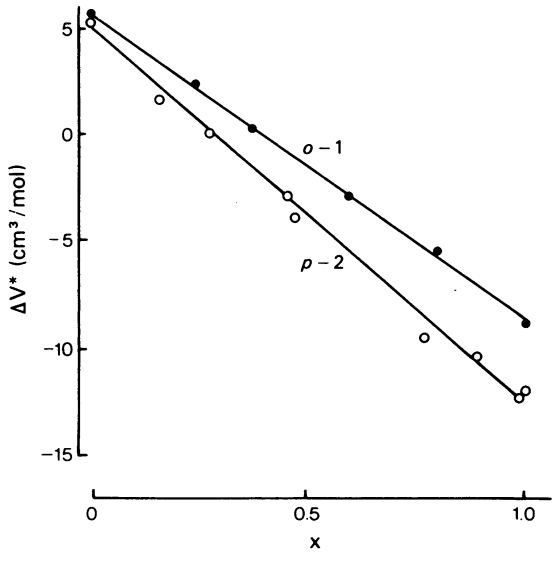


Figure 3 The net change in volume on solution, ΔV^*, after allowance for the amount of hydroxyl lost and the amount of water formed, plotted against the fraction ionized, x. Results are shown for *p*-hydroxyphenethyltrimethylammonium bromide (*p*-2) and for *o*-hydroxybenzyltrimethylammonium bromide (*o*-1). The slope is appreciably reduced when the hydroxyl and quaternary groups are closer together.

in volume on addition of a proton. A check on the results can be made with the values for pyridine. Hamann & Lim (1954) obtained $\bar{V}^0 = 90.5$ cm³/mol for pyridine chloride; from $\phi_v = 77.4$ for pyridine in Table 4B, −4.3 as the increment for conversion to pyridinium and 18.1 as the increment used by Hamann and Lim for Cl^-, the value calculated from the present results is 91.2 cm³/mol.

Size of amino acids

With the amino acids (Table 4C) there is a much bigger change in ϕ_v with concentration than that observed with the phenolic quaternary ammonium salts or with the amines (the large range with pyrrolidine is because there is a significant amount of ion present, see methods). According to Daniel & Cohn (1936) changes in ϕ_v for several amino acids are proportional to the molar concentration, c, in the range 0.25 to 3 M but with the lower concentrations studied here this is not so. The least squares-fit to the equation $\phi_v = \phi_v^0 + Ac^{\frac{1}{2}} + Bc$ indicates a marked dependence on $c^{\frac{1}{2}}$. The graph of ϕ_v against $c^{\frac{1}{2}}$ (Figure 5) is probably not a straight line, however; the lines for all three compounds show similar trends suggesting curvature. This introduces considerably uncertainty into the estimates of ϕ_v^0. For glycine the value is 41.72 from the equation involving only $c^{\frac{1}{2}}$ but it is 40.57 from the equation with a term for c included. The

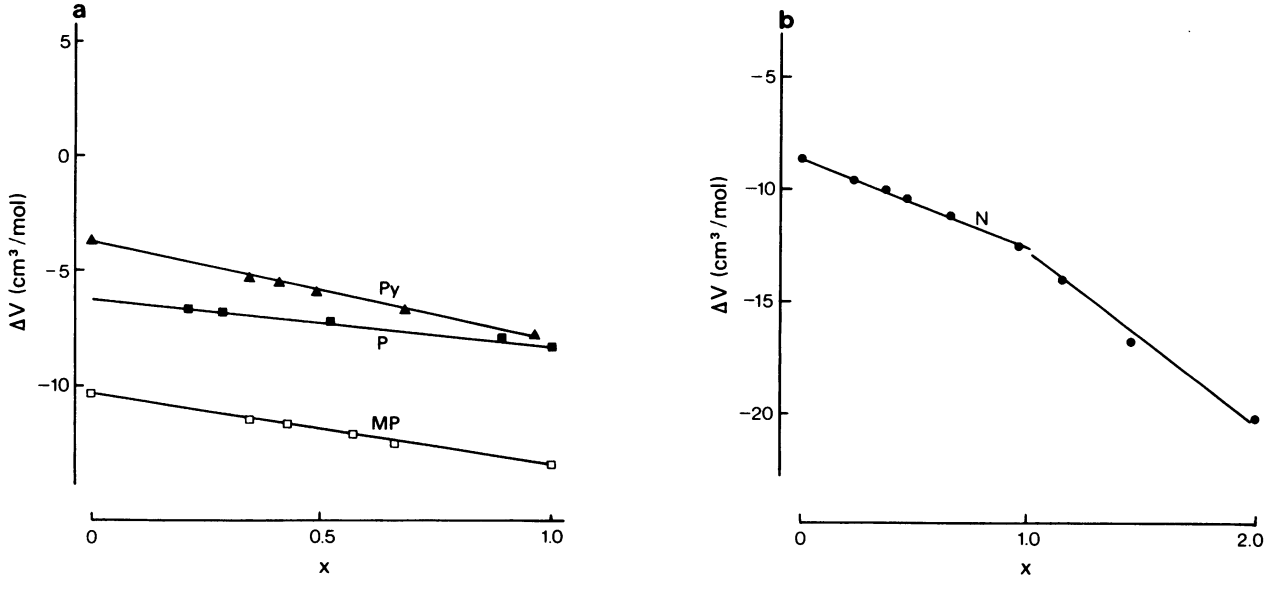


Figure 4 The change in volume on solution, ΔV, plotted against the fraction ionized, x. (a) shows results for pyridine (Py), pyrrolidine (P), and N-methylpyrrolidine (MP); (b) shows results for nicotine (N). Note the increased effect associated with the ionisation of the pyridine ring with the pyrrolidine ring already ionized (x = 1 to 2).

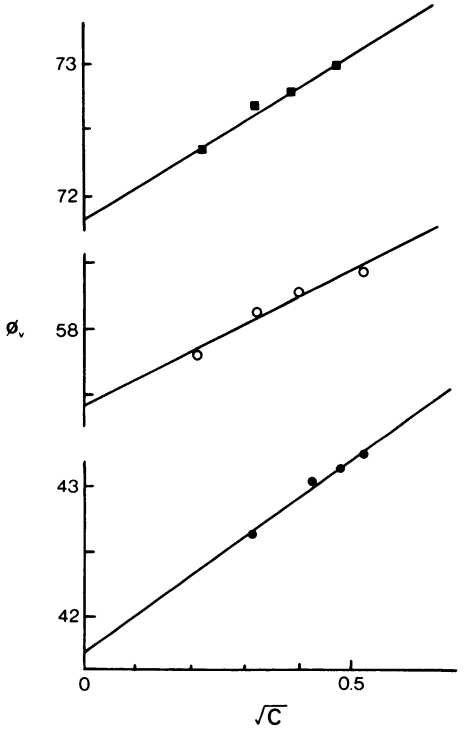


Figure 5 Relation between apparent molal volume, ϕ_v, and $c^{\ddagger}$ for amino acids. The lines are the least-squares fit for the equation $\phi_v = \phi_v^0 + bc^{\ddagger}$, with the values shown in Table 4C. Note that the values for the lowest concentration do not really fit a straight line and indicate curvature.

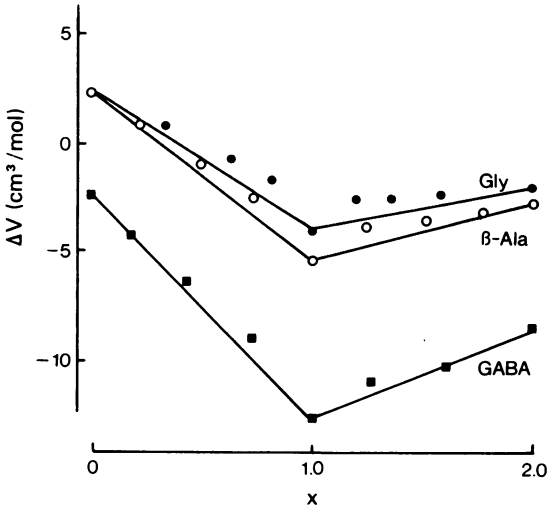


Figure 6 The change in volume on solution, ΔV, plotted against the extent of proton loss, x, from amino acids: (●) glycine (Gly); (○) β-alanine (β-Ala); (■) γ-aminobutyric acid (GABA). For x = 0 to 1 the change represents the ionisation of carboxyl and is the net change (ΔV*) after correction for the loss of hydroxyl and for the water formed. The lines are the least-squares fit, weighted according to $1/c^2$ go allow for the effects of concentration. The value for x = 1, corresponding to the zwitterion, was calculated from ϕ_v^0 (see text).

discrepancies are less (0.5 cm³/mol) with the other amino acids.

Allowance for the effects of concentration must be made when studying the relation between ΔV and x (Figure 6). The concentrations were very different for different values of x: to obtain values of $x > 0.5$ or <1.5 more amino acid was taken and smaller volumes of acid or alkali. The values of ΔV or ΔV* were therefore weighted by $1/c^2$ when being fitted to x, which accounts for the bicuspid nature of the middle portion of the graphs, though the value at x = 1 corresponds to ϕ_v^0 for the neutral amino acid. The slopes of the lines are largely determined by the results with pure amino acid and with 1 equivalent of acid or alkali. In spite of the complications from the effects of concentration, the change in volume, −6.34 cm³/mol, associated with the ionization of the carboxyl group in glycine, $H_3\overset{+}{N}CH_2COOH \rightarrow H_3\overset{+}{N}CH_2COO^-$, agrees reasonably with the estimate, −6.80 cm³/mol, obtained by King (1969). Kauzmann *et al.* (1962) obtained −6.4 for the carboxyl group in glycine and −7.5 for the carboxyl group in β-alanine, compared with −7.81 in this work.

The results in Figure 6 show that the decrease in size associated with the zwitterionic charges is much less in glycine than in γ-aminobutyric acid, with the changes for β-alanine close to that for glycine. This can be expressed numerically as the electrostriction, E, which was estimated by Edsall (1943) by comparing the values of ϕ_v for amino acids and uncharged analogues. The amino acids have large zwitterion constants so there was no need to correct his values of ϕ_v for the presence of the uncharged species $(H_2N(CH_2)_nCOOH)$. By comparing glycine (ϕ_v = 43.5) and glycollamide ($HOCH_2CONH_2$: ϕ_v = 56.2), E = 12.7 cm³/mol. By comparing β-alanine (ϕ_v = 58.9) and lactamide (ϕ_v = 73.8), E = 14.9 cm³/mol. From the results in Table 4C the ionization of the carboxyl group in glycine decreases the volume by 6.35 cm³/mol and the ionization of the ammonium group decreases it by 2.00 cm³/mol, so E = 8.35; for β-alanine E = 10.3 and for γ-aminobutyric acid E = 14.3. It is possible to calculate the value for ε-aminocaproic acid from results obtained by Daniel & Cohn (1936) who found ϕ_v = 104.35 for the acid, 135.4 for the hydrochloride and 106.0 for the sodium salt of the acid.

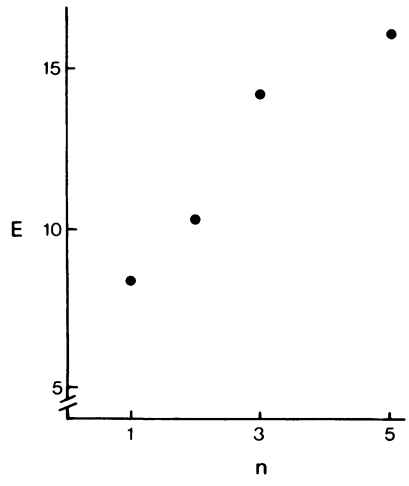


Figure 7 The effect of chain length on the electrostriction, E, of amino acids, $H_2N(CH_2)_nCOOH$. The values of E have been calculated from the results in Table 4C.

Accordingly $\Delta V = -13.25$, $\Delta V^* = 2.86$ and $E = 16.1$ cm^3/mol. It is now possible, therefore, to show the effects of charge separation on electrostriction in these compounds with experimental estimates (Figure 7), rather than with values based on comparisons with uncharged compounds which may not be valid.

Discussion

Increments in apparent molal volume can be considered simply descriptively; certain groups 'disappear' into the solvent. This work has amply confirmed that there is a big variation in the extent to which hydroxyl groups do this and in part it may depend on the ability of the hydroxyl group to act as a hydrogen donor as well as a hydrogen acceptor. The variation in the size of the hydroxyl group in solution is in strong contrast to what is found in the liquid state, assessed from increments in molal volume (V_m). For hydroxyl groups in *n*-pentyldiethylamine the size of the group ranged from 11.0 cm^3/mol in the 5-position to 16.9 cm^3/mol in the 3-position and could be related to its likely effect on how well the molecules could pack together (Barlow, 1978). In analogous trimethylammonium and triethylammonium salts the increments in ϕ_v^0 were in the range 0.1 to 2.2 cm^3/mol. In aqueous solution interactions between groups such as hydroxyl and quaternary ammonium clearly involve the surrounding water molecules. The distance between groups at which they act independently probably varies from one type of molecule to another but in the series of amino acids studied there are interactions between amino groups, carboxyl groups and water until the groups are at least as far apart as in γ-aminobutyric acid. With shorter chains the groups share effects on water. If they were hydrophobic they could be described as sharing an umbrella but these particular groups are hydrophilic.

The minimum in volume seen with the zwitterionic forms of the amino acids suggests that these are associated also with a minimum in entropy. The compounds nevertheless have large zwitterion constants so should have large negative enthalpies of formation and the zwitterion constants should be reduced by raising the temperature. Edsall (1943) has, in fact, calculated that the zwitterion constants for α-amino acids are approximately halved for a rise of 10°C. In the interaction of an amino acid with a receptor, then, there should be scope for a considerable increase in entropy and this should be true for any drug whose hydration is associated with decreased entropy. From considerations solely of entropy, therefore, groups which 'disappear' into the solvent might be capable of more energetic interactions with receptors than those which do not interact with water to the same extent.

The energetics of binding to enzymes have been reviewed by Jencks (1975) and in many situations the organized nature of the complex means that there is a decrease in entropy on binding, which is offset by a large negative enthalpy of binding. For the binding of antagonists to muscarine-sensitive acetylcholine receptors it appears that for most drugs studied there is an increase in entropy (Barlow, Berry, Glenton, Nikolaou & Soh, 1976; Barlow & Burston, 1979; Barlow *et al.*, 1979), though there is some evidence that with (−) forms of hyoscine and hyoscyamine the entropy of binding is negative (this was found with experiments involving intact preparations but not with those with isolated receptors).

An increase in entropy may indicate that the binding process is accompanied by a breakup of water structure, such as might occur if the binding involves hydrophobic parts of the receptor. Whatever the situation, however, interactions between drug and water in the bulk phase which result in a decrease in entropy should potentially favour the entropy component of binding. There is no reason to believe that this must be linked to the enthalpy of binding, though it appears to be; for the results obtained by Barlow *et al.* (1976) the correlation coefficient between $T\Delta S$ and ΔH is 0.99, for the results of Barlow & Burston (1979) it is 0.98 and for those of Barlow *et al.* (1979) it is 0.94. This may be because the range of values of ΔG is small, from about −8 to −14 kcal/mol (and $\Delta H - T\Delta S = \Delta G$).

Whatever the link, if any, between the entropy and enthalpy of binding, the occurrence of abnormally low increments in apparent molal volume may indicate high binding potential, though the converse does

not necessarily follow, compounds may have high affinity without existing in the bulk phase in a highly ordered state. It also does not follow that abnormally low increments in apparent molal volume must be associated with high affinity, because some of the compounds considered, such as phenyl*cyclo*hexylglycolloylcholine and atropine methiodide (see following paper), contain an asymmetric carbon atom and only one enantiomer is highly active. Presumably the binding of the other enantiomer is not associated with extensive loss of hydration. Nevertheless, although increments in apparent molal volume cannot be correlated strictly with binding, they are important because they indicate drug-water interactions which form part of the process: drug + water + receptor $\rightleftharpoons$ drug-water-receptor complex.

For the results of Hepler (1965) the slope of

$$\left(\frac{\partial \Delta S}{\partial \Delta V}\right)_T$$

is 1 cal deg^{-1} cm^{-3}, so an interaction involving a disappearance of 1 cm^3 mol^{-1} at 298°K should indicate a decrease in $T\Delta S$ of 0.3 kcal(1.2 kJ)mol^{-1} and the complete disappearance of a hydroxyl group (16 cm^3 mol^{-1}) should indicate a decrease of nearly 5 kcal mol^{-1}. Even if these figures are only roughly correct they suggest that the break up of drug-water interactions could make a considerable contribution to drug-receptor binding.

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