SOME BIOLOGICAL APPLICATIONS OF REGULAR SOLUTION THEORY

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ABSTRACT

There is firm experimental and theoretical justification for expecting that solution theories, in this case regular solution theory, can be applied in a semi-empirical manner to questions of biological interest. This background is outlined and one of the possible models resulting from the use of regular solution theory is applied to the analysis of erythrocyte haemolysis. Suggestive evidence arising from correlations of *n*-octanol–water partition coefficients with parameters appropriate to this approach tends to indicate that regular solution theory may apply to a wider variety of biological systems than has previously been thought.

INTRODUCTION

Solubility-related phenomena are pervasive throughout the pharmaceutical and pharmacological literature. Among the areas where aspects of solution behaviour arise are (a) in the design of liquid dosage forms; (b) in considerations of the influence of carrier vehicles on the biological absorption or response of a drug entity; (c) in accounting for the penetrability of molecular species into or through biological tissues; and, (d) in rationalizing physicochemical influences on relative biological responses. As a more specialized component of the latter category one might also include certain factors, such as hydrophobic bonding, which relate to drug–biomacromolecule interactions. Underscoring literature accounts within each of these areas is the frequency with which simple oil–water partition coefficients are found to parallel test results obtained with biological systems. Hansch and his associates[1–3] have extensively exploited the ubiquitous occurrence of this correlation and have established statistical analyses that make use of *n*-octanol–water partition coefficients as a useful tool in the design of drug molecules or in the study of enzyme reactions. Less frequently have efforts been made to apply a theory of solution, such as regular solution theory[4, 5],

to these same types of systems. Such work as has been reported has been sporadic[6–10] although in some instances[11–19] promising results have been obtained with commerical pharmaceuticals.

There are a variety of reasons why one might prefer to make use of empirical or extrathermodynamic relations involving partition coefficients rather than relations based on a solution theory in the study of pharmaceutical or pharmacological systems: (a) empirical parameters ordinarily provide better 'fits' with biological data than do theoretically-based indexes[20]; (b) partition coefficients are approximately additive–constitutive, thus enabling the estimation of values appropriate to additional compounds[21]; (c) there are few theoretical constraints associated with the application of partition coefficients to interpretations of biological data as distribution processes involving an organic or lipophilic phase and an aqueous phase are linearly interrelated[22].

On the other hand, several valid reasons can also be given for desiring to take a more fundamentally based approach: (a) seemingly disparate experimental observations such as carrier vehicle influences on biological activity, isotonicity, and passive membrane transport are readily understood as involving a common physicochemical component; (b) the 'distribution system' is the biological assay object itself; thus, adjustable parameters within a theoretical framework have significance in relation to the actual test system; (c) based on a theoretical rationale it is sometimes possible to design independent types of assay for substantiation of a mechanistic hypothesis; (d) disagreement with theoretically-based relationships frequently are readily interpretable as providing information which is either intrinsic to the chemical nature of the compounds under consideration or is specific to biological processes; (e) in the realm of drug design, there is a potential for estimating optimum solubility characteristics without the necessity for an extensive set of test compounds and there is also afforded a possible route to the design of tissue-specific agents.

As should be evident from this brief introduction, the possible scope of application of a solution theory, in this instance regular solution theory, to biological systems is extremely broad. What we hope to do by this presentation is to report on our very preliminary findings which tend to indicate that regular solution theory might have a much more promising future in application to biological systems than has previously been thought. We do not claim our present interpretations to be immutable. Rather we anticipate that clarifications and refinements will be made in establishing a modification of regular solution theory that is appropriate to questions of biological interest.

HISTORICAL FOUNDATIONS

Virtually all efforts to relate biological activity with physically-based indexes stem from observations of a relationship between narcotic potency and water solubility[23]. Later, independent investigations led Overton[24] and Meyer[25] to the finding of a correlation between narcotic potency and partition coefficient from which it was surmised that the relative effectiveness of anaesthetic compounds was due to their distribution between biological lipids and water. This observation was extended to the penetration of

molecular species through membranes by Collander[26] and has reached fruition with the work of Hansch and his coworkers[1–3].

Meyer had a son, and it is from this Meyer's efforts that a basis for quantitative work with biological systems is derived. He showed[27] that despite the apparent trend in narcotic potency with partition coefficient, the equilibrium concentration of the drug in the organic phase of the *model* distribution system was effectively a constant. From this it was concluded that narcosis commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of cells. It was also noted that this specific concentration depends on the nature of the animal or cell but is independent of the narcotic selected. More recent work by Roth and Seeman[28]

Table 1. Comparison of biological effects with partition coefficients and equilibrium concentrations in lipid.

A. Paralysis of tadpoles by narcotic agents[27]

Substance	Partition coefficient (oleyl alcohol–water)	Narcotic concentration (mol l^{-1} of water)	Oleyl alcohol conc. (mol l^{-1})
Ethanol	0.10	0.33	0.033
1-Propanol	0.35	0.11	0.038
1-Butanol	0.65	0.03	0.020
Valeramide	0.30	0.07	0.021
Antipyrine	0.30	0.07	0.021
Aminopyrine	1.30	0.03	0.039
Barbital	1.38	0.03	0.041
Benzamide	2.5	0.013	0.033
Salicylamide	5.9	0.0033	0.021
Phenobarbital	5.9	0.008	0.048
Thymol	950	0.000047	0.045

B. Antihaemolytic effect of narcotics with erythrocyte membrane[28, 29]

Substance	Partition coefficient (membrane–0.4% NaCl)	Equiprotective conc. (mol l^{-1} of water)	Membrane conc. (mol kg^{-1} membrane)
Benzyl alcohol	4.0	0.022	0.089
Butanol	1.5	0.042	0.062
Pentanol	3.6	0.0145	0.053
Hexanol	13.0	0.00175	0.023
Heptanol	39.6	0.000618	0.025
Octanol	151.8	0.000235	0.036
Nonanol	582	0.0000410	0.024
Decanol	1226	0.00001	0.012
Chlorpromazine	1600	0.0000081	0.013
Morphine	7.1	0.0052	0.037
Phenol	8.5	0.0078	0.066
4-MeO-phenol	5.4	0.0060	0.033
4-F-phenol	13.1	0.0030	0.039
3-Me_2N-phenol	8.0	0.0028	0.022
4-CO_2Me-phenol	18.1	0.0024	0.044
3-NO_2-phenol	20	0·0022	0.045
4-Me-phenol	17.7	0·0014	0.025
Chloroform	18.0	0.0105	0.189
Ether	1.3	0.12	0.156
Ethanol	0.14	0.85	0.119

on the distribution of phenol anaesthetics with erythrocyte membrane has verified this conclusion, but acidic compounds that are ionized and have a negative charge under the conditions of the assay are tentatively exceptions[29]. *Table 1* compares the results of Meyer with those of Roth and Seeman to illustrate the basis for Meyer's hypothesis.

A physical rationale for Meyer's hypothesis based on thermodynamic principles was subsequently put forward by Ferguson[30] and it is this rationale which provides a foundation for applying solution theories to the interpretation of biological data. Ferguson points out that under equilibrium conditions the chemical potential for a compound in the assay medium, μ_a, should equal the chemical potential of the compound in the biophase, μ_b, irrespective of the nature of the biophase. Necessarily, then,

$$\mu_a = \mu_b^0 + RT \ln a_b \tag{1}$$

where μ_b^0 is a reference chemical potential for the drug in some standard state, and a_b is the thermodynamic activity of the drug under the experimental conditions. For perfect solutions $a_b \approx C$, the concentration measure, and hence the concentration of a substance accumulated in a common biophase is expected to be a constant and this value should be independent of the nature of the drug. If this rationale is correct, then the converse of equation 1 should also hold,

$$\mu_b = \mu_a^0 + RT \ln a_a \tag{2}$$

That is, drug activities assayed using a specific biological test system and measured to an equivalent endpoint in terms of molar concentrations or partial pressures should provide a constant value when these concentration measures are converted to thermodynamic activities. This has been found to be the case for a variety of biological assays where the test compounds are either in solution[30–34] or in a gaseous state[30, 35, 36]. *Table 2* presents an

Table 2. Toxic partial pressures and thermodynamic activities of some gaseous substances[36]

Substance	B.P., °C	Toxic partial pressure, p_t	LD_{50} (g mol $\times 10^{-6}$ l^{-1})	Thermodynamic activity, a_a
Nitrogen	−195.8	340 atm	12000000	0.28
Oxygen	−183.0	13 atm	530000	0.02
Methane	−161.5	180 atm	8800000	0.53
Argon	−185.7	92 atm	3900000	0.10
Ethylene	−103.9	15.5 atm	700000	0.31
Nitrous oxide	−89.5	14.5 atm	640000	0.36
Ethane	−88.3	11.5 atm	520000	0.37
Ethyl chloride	12.2	330 mmHg	17000	0.29
Chloroform	61.3	40 mmHg	2100	0.20
Ethylene dichloride	83.6	10 mmHg	1000	0.24
sym-Tetrachloroethane	146.3	1.7 mmHg	90	0.24

example of this type of relationship. In this case a modification of Raoult's Law is used to estimate the thermodynamic activities

$$p_t \approx p^0 a_a \tag{3}$$

where p_t is the toxic partial pressure and p^0 is the vapour pressure of the pure liquid. With solutions the thermodynamic activities are estimated[30] as the ratio S_t/S_0 of the molar concentration of the test solution S_t to the solubility S_0 of the drug.

MODELS FOR THE ANALYSIS OF BIOLOGICAL ACTIVITIES

From Ferguson's rationale it is evident that there are two alternative but equivalent expressions that can serve as a basis for the analysis of drug response A. These are (a) the distribution model suggested by Overton and Meyer

$$A = \ln (a_b/a_a) = -(\mu_b^0 - \mu_a^0)/RT \tag{4}$$

and (b) the phase saturation model advanced by Meyer's son

$$A = \mu^0 + RT \ln a \tag{5}$$

The subscript is omitted from equation 5 as this relation may apply to either phase.

Under the assumption that solubility-related phenomena account primarily for variations in many types of drug responses, equations 4 and 5 may be used to introduce parameterizations appropriate to a solution theory. This is usually done by including an appropriate expression for the thermodynamic activity coefficient γ.

Taking equation 4 as a starting point, and choosing a standard state such that $\mu_b^0 = \mu_a^0$ (e.g., pure drug), it is readily shown that

$$A = \log (S_b/S_a) = \log \gamma_a - \log \gamma_b \tag{6}$$

where S_b and S_a are molar concentrations and γ_b and γ_a are thermodynamic activity coefficients for the drug in the biophase and the assay medium, respectively. For the same choice of standard state, equation 5 would become

$$A = \log \gamma_b + \log S_b \tag{7}$$

in which S_b can be taken to be constant for a particular type of biophase.

FORMULATION IN TERMS OF REGULAR SOLUTION THEORY

A regular solution may be defined phenomenologically as having a positive heat of mixing, ΔH^M, and an entropy of mixing given by $\Delta S^M = -R \ln X_1$, where X_1 is the mole fraction of solvent. For such solutions, Hildebrand and Scott[4,5] have shown that deviations from Raoult's Law can be accounted for in many instances by expressing the thermodynamic activity coefficient as

$$\log \gamma_2 = \frac{\Phi_1^2 V_2}{2.303\, RT} (\delta_1 - \delta_2)^2 \tag{8}$$

in which V_2 is the molal volume of solute, Φ_1 is the volume fraction of solvent, and δ_1, δ_2 are the 'internal pressures' of the solvent and solute respectively. The 'internal pressure' or 'solubility parameter' δ is given strictly by the

ratio of the energy of vaporization to the molar volume of a pure substance

$$\delta = (\Delta E_v/V)^{\frac{1}{2}} = (\Delta E_v V)^{\frac{1}{2}}/V = F/V \tag{9}$$

but in many practical applications it may be estimated by making use of the approximate additive–constitutive nature of the molar attraction constant F[37,38] or by calculation from molecular polarizability[39]. Swelling, solubility and related measurements may also be made in order to estimate δ[4].

In dilute solutions $\Phi_1 \approx 1$ and if this condition applies to each of the distribution phases of a biological test system the substitution of equation 8 into equations 6 and 7 yields, respectively, the relationships

$$2.303\,RT\,A = (V_a\delta_p^2 - V_b\delta_n^2) - 2(V_a\delta_p - V_b\delta_n)\delta + (V_a - V_b)\delta^2 \tag{10}$$

$$2.303\,RT\,A = V_b\delta_n^2 - 2V_b\delta_n\delta + V_b\delta^2 + 2.303\,RT\log S_b \tag{11}$$

where δ is the solubility parameter for drug and δ_p, δ_n are *apparent* solubility parameters for the phases of the assay system. Multicomponent phases have their solubility parameter expressed in terms of the solubility parameters for the individual components by the relationship[5]

$$\delta_{app} = \sum \Phi_i\delta_i \tag{12}$$

so that the apparent solubility parameters in equations 10 and 11 may be interpreted in terms of the components making up the assay medium or the biophases. V_a and V_b are molal volumes appropriate to the drug in each phase. To an additional degree of approximation these may be considered essentially constant for a given series of drug molecules. Hence, equations 10 and 11 could be used, if desired, as model equations in a regression analysis such as is done by Hansch and his associates[1–3]. In a later section this type of approach is followed.

One advantage of having interpreted Ferguson's relationships in terms of regular solution theory is that a parabolic relationship between drug activity and drug solubility is a natural consequence of the theory. Such parabolic relationships are frequently observed and with extrathermodynamic types of approaches[1–3] had to be taken into account by virtue of a statistical expedient, i.e., the essentially arbitrary introduction of higher-powered (squared) terms into the model equation. With biological systems a variety of alternative possibilities exist that could also lead to a parabolic curve, but for the present it will be assumed that solubility alone leads to this type of curve.

A second advantage, pointed out by Mullins[6], is that optimum solubility in a particular phase is achieved when the δ values for the drug and the biophase are equal or at a minimum value. Hence, presuming δ values for certain tissues have been established empirically or by some other means one could hope to localize a drug predominantly in one tissue by attempting to match the δ value of the drug with that of the tissue. The additive–constitutive nature of molar attraction constants F and molar volumes, at least for molecules that are not strongly self-associated due to hydrogen bonding or to their charge characteristics[37–39], allow this approach to be of some value in drug design. A possible shortcoming to this concept, however, is that δ values tend to vary over a relatively narrow range (4.0 to 23) and as a

consequence discrimination between tissues would most readily be possible if the tissues involved differed greatly in their constitution.

Certainly approaching the study of biological activities by the application of regular solution theory is not above severe criticism, especially if a chemically and biologically rigorous theoretical framework is desired. In attempting to apply this theory it is perhaps appropriate to take a dual perspective, the choice depending on the use that is to be made of the method. If the intention is to test the approach in terms of rigorous foundations, it would be best to consider only relatively nonpolar substances and their action on the most simple of tissues for which δ values may be determined by swelling, vapour pressure, or osmotic pressure measurements. The contribution made by the Flory–Huggins[40–43] size correction, due to the difference in molar volumes of drug and biophase components, might also be determined. This correction could be included in equations 10 and 11 as an additional set of terms given by

$$-RT\left[\ln \Phi_2 + \Phi_1\left(1 - \frac{V_2}{V_1}\right)\right] \tag{12}$$

From a drug design standpoint, however, a more flexible yet readily applied procedure is most desirable. In this case the molar volumes and solubility parameters in equations 10, 11 and 12 may be considered to be adjustable, the separate relationships providing an empirical framework within which to work. This latter view is adopted in this article.

An alternative criticism might take note that Ferguson's development applies only to an equilibrium situation and hence should not be carried over into kinetic situations, e.g., membrane penetration. That this criticism is invalid is easily demonstrated from a consideration of Fick's Law for diffusion through thin membranes, which is the usual model used in biological work. The steady-state rate of penetration (dQ/dt) of a drug through a membrane is most simply represented by the relation

$$\frac{dQ}{dt} = \frac{DM}{\Delta x} KS = kS \tag{13}$$

where K is the partition coefficient for distribution of a compound between the membrane and the applied vehicle, D is the effective average diffusivity of the drug in the membrane, M is the effective cross-sectional area through which the diffusion flux passes, and Δx is the thickness of the membrane. An alternative way of writing equation 13 is

$$\frac{dQ}{dt} = \frac{DM}{\Delta x} \frac{a_a}{\gamma_b} \tag{14}$$

in which a_a is the thermodynamic activity of the drug in its vehicle and γ_b is the effective activity coefficient of the agent in the membrane. Thus, taking the logarithm of an observed penetration constant leads to a modification of equation 10 in which the quantity $\log (DM/\Delta x)$ is a part of the intercept. Alternatively, since biological activities are usually taken after a fixed time interval, they may be taken as analogous to an instantaneous rate.

Hence, taking the logarithm of the biological response measure corresponds to $\log (dQ/dt)_t$ and again equations 10 and 11 are seen to apply.

Since, in taking a regular solution theory approach to analyzing biological data, attention is focused on the activity coefficient γ, it is appropriate to ask what the range in activity coefficients might be as it is transferred from one medium to another. What this range could be for biological tissues is as yet an open question, but extremely wide variations in the activity coefficient have been reported by Higuchi[44] for Sarin, a nerve gas, in differing solvents. *Table 3* reproduces these values to show the variation that has been observed.

Table 3. Limiting activity coefficients of Sarin in solvents[44].

Substance	γ
Perfluorotributylamine	66.6
Hexadecane	15.6
Water	14
Tributylamine	10.4
Tetralin	4.3
2-Pyrrolidone	2.8
Diethylene glycol	2.4
Carbon tetrachloride	2.4
Phenyl ether	2.38
Diisooctyl adipate	1.84
Methyl salicylate	1.74
N-Methylacetamide	1.44
Dibutyl phthalate	1.42
Butyrolactone	1.31
Isoamyl alcohol	1.07
Ethyl lactate	0.536
Benzyl alcohol	0.446
m-Cresol	0.044

APPLICATION TO STUDIES OF ERYTHROCYTE HAEMOLYSIS

A simple membrane for which an extensive literature exists and which has been the subject of a recent extrathermodynamic study[45] is the erthrocyte membrane. This system was used by Roth and Seeman[28,29] in verifying the Meyer hypothesis and as a consequence should be capable of analysis in terms of regular solution theory. We show here that (a) data correlating linearly with *n*-octanol:water partition coefficients sometimes show a definite parabolic trend when plotted against solubility parameters. More frequently, when such comparisons are possible, there is general agreement between the type of curve obtained by either approach, i.e., linearity in one case also is found with the other or quadratic behaviour with one is also found with the other. (b) Identical conclusions are obtained taking either approach regarding the similarity in the nature of the erythrocyte membrane in differing animal species. (c) For the erythrocyte membrane, equation 11, and not equation 10, is the more suitable basis for interpretation, since the

coefficients to the derived regression equations are related in a more consistent manner with equation 10 than with equation 11. (d) The apparent δ value for erythrocyte membrane seems to be about 8.08, which can be contrasted with an apparent δ value of around 10.5 reported[6,46] for nerve membrane.

Solubility parameters calculated[47] for the compounds compiled by Hansch and Glave[45] when plotted with their haemolytic concentrations as the dependent variable tended to provide curves paralleling the type of equation reported from regression analysis. *Figures 1a* and *1b* show this

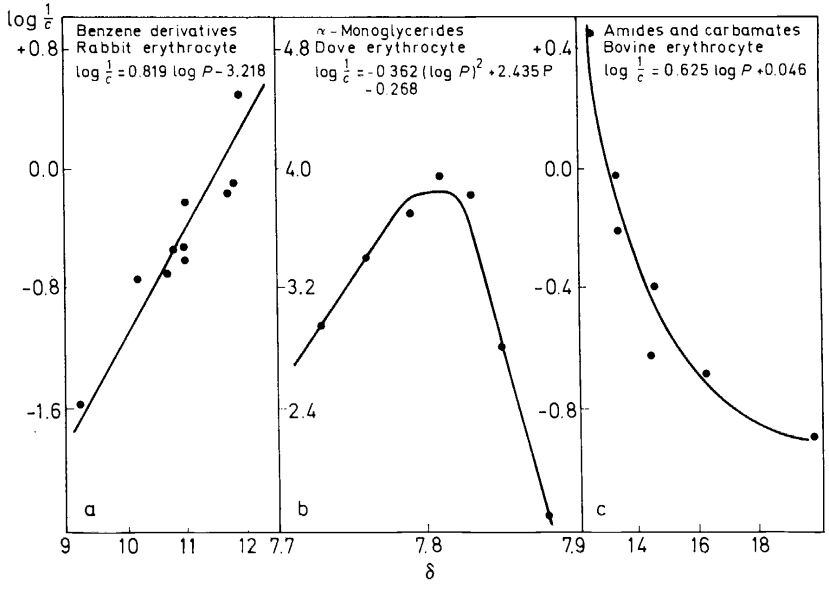

Figure 1. Comparison of haemolytic activities correlated with partition coefficients and plotted against the solubility parameter δ.

correspondence for substituted benzenes tested against rabbit erythrocyte and α-monoglycerides tested against dove erythrocyte. In some instances, however, as for amides and carbamates tested against bovine erythrocyte (*Figure 1c*), curvature in the solubility parameter plot is noted while the equation involving partition coefficients was reported to be linear. While nothing substantive can be made of this observation, the discrepancy between the two types of correlative approaches might be interpreted as indicating that a distinction between a simple partitioning model and a saturation model could be made using erythrocyte membrane data.

As may have been expected, not all the data compiled by Hansch and Glave[45] led to smooth curves when δ values were used as a measure of solubility. The reasons for this are not entirely clear, although it should be pointed out that for some sets of data there were uncertainties as to whether good approximations to F and V were used in calculating the δ values, while

Table 4. Regression equations for erythrocyte membrane haemolysis derived for the model $\log (1/C) = a + b\delta + c\delta^2$

Erythrocyte source	Type of compound	a	b	c	Temperature, °C	δ_n or δ^{opt}
Human	$ROSO_3^- Na^+$	1482	−408.1	27.99	24	7.28
	$RNMe_3^+ Br^-$	3726	−997.4	66.66	37	7.48
Bovine	ROH	718.4	−162.4	9.163	21	8.85
	RCO_2R	210.6	−45.20	2.423	21	9.32
Rabbit	ROH	650.9	−146.8	8.272	18	8.87
	$PhCH_2NR_3^+ Cl^-$	−8497	2152	−136.2	37	7.90
Dog	RCO_2H	1343	−362.4	24.48	25	7.40
	$RNH_3^+ Cl^-$	3679	−968.5	63·74	30	7.59
	N-R-pyridinium I^-	3385	−890.9	58.62	30	7.59
Sheep	$RCHBrCO_2^- K^+$	2465	−644.4	42.15	37	7.64
	$RNMe_3^+ Br^-$	−3692	913.6	−56.41	37	8.09
	$ROSO_3^- Na^+$	−5687	1432	−90.15	37	7.94
	$PhCH_2NMe_2R^+ Cl^-$	−2297	565.8	−34.73	37	8.13
Dove	RCO_2H	−4624	1170	−73.99	38	7.90
	α-R-monoglycerides	−15806	4058	−260.4	37	7.79
	N-R-piperidine Cl^-	−3014	754.5	−47.13	37	8.00
	N-R-pyridinium Br^-	−7436	1870	−117.5	37	7.95

with other sets it seemed possible to group compounds into subsets based on chemical structure. Of course another possibility is that it is hoping for too much to have regular solution theory apply to all the compounds. Those sets of data which provided smooth curves of a definite quadratic nature were thus selected for regression analysis making use of an orthogonal polynomials routine for the curve fit. *Table 4* summarizes the equations that have been derived in this manner[47].

It will be noted from *Table 4* that the signs to the coefficients are reversed in some instances but not in others. This may be recognized to depend on whether the compounds function in a disruptive or a protective manner towards erythrocytes. Since one action is the reverse of the other, the equations referring to, say, antihaemolytics should be multiplied through by -1. The parabola is thus defined always to be concave with respect to the x-axis, and the coefficients can then be compared in the same relative manner.

The question now arising is whether the equations represented in *Table 4* are best interpreted in terms of a distribution model (equation 10) or a saturation model (equation 11). By equating corresponding terms of equation 10 with the coefficients of a relationship from *Table 4*, making what seemed to be reasonable assumptions for the δ value of water ($\delta = 23$[4] and 16[48,49]) and the molar volumes for the compounds in the membrane (taken to be the same as the pure compound), and solving the simultaneous equations that were obtained, it was not possible to arrive at a δ value for the membrane that was positive in sign. It was thus concluded that a distribution model for erythrocyte haemolysis is most probably invalid.

The corresponding analysis involving equation 11 was approached in a different manner. Since the equilibrium concentration of drug in the erythrocyte membrane is expected[28,29] to be low it was presumed that the term involving S_b in equation 11 could be neglected. Differentiation of equation 11 with respect to the δ value for drug shows that the maximum haemolytic effect occurs at an optimum solubility parameter value δ^{opt} and this value is the same as that for the membrane δ_n. To determine δ^{opt} from the regression coefficients, it is thus necessary to use the relationship

$$\delta^{opt} = b/2c \tag{15}$$

However, from inspection of equation 11 it can be noted that the square root of the regression coefficient ratio a/c should provide an alternative estimate of the δ value for the membrane

$$\delta_n = (a/c)^{\frac{1}{2}} \tag{16}$$

Interpretation of the regression coefficients found in *Table 4* according to equations 15 and 16 leads to excellent agreement between the calculated values for δ^{opt} and δ_n. Membrane apparent solubility parameters appropriate to each data set are listed in *Table 4*. These average to 8.08 which should be contrasted with a value of about 10.5 reported[6,46] for nerve membrane. It may thus be stated that erythrocyte haemolysis involves a saturation-type process and not a distribution-type process. This conclusion is fully compatible with the saturation-like sigmoid curves frequently observed in plots of percentage haemolysis *versus* drug concentration, as shown for example by *Figure 2*. A similar extension to this type of experiment is not

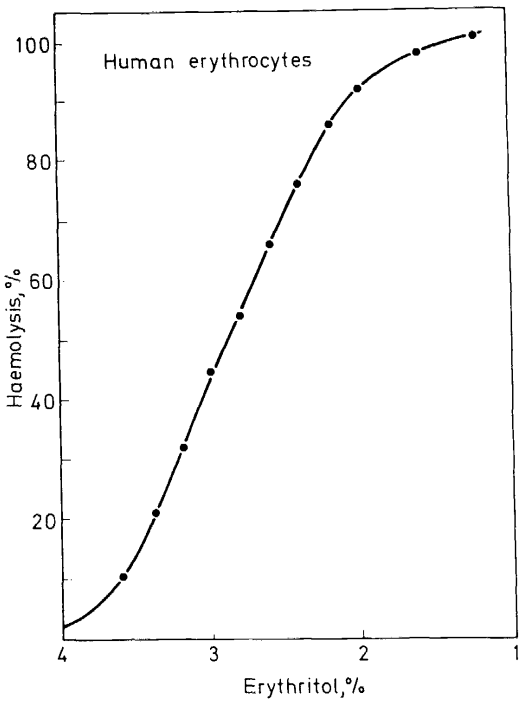

Figure 2. Typical percentage haemolysis *versus* dose of drug curve. (P. Zanowiak, Ph.D. Thesis, University of Florida, Gainseville, Florida, 1959)

indicated in any way from the results of an extrathermodynamic type of approach.

Having found regular solution theory to apply to a number of studies of erythrocyte haemolysis, it is pertinent to inquire into the possible reasons for this apparent success. At first sight, the compounds shown in *Table 4* would not appear suitable for study by regular solution theory, since they are either ionic or contain a functionality capable of hydrogen bonding. A plausible rationale for the findings takes note of the behaviour of surface active agents at an organic medium–aqueous medium interface. Surface active agents possess a polar or charged moiety attached to an alkyl group and, at an organic–aqueous interface, the polar groups tend to be associated with the aqueous phase while the nonpolar groups tend to be in the organic phase. For erythrocyte haemolysis, the polar components of the compounds found in *Table 4* would be associated with the aqueous assay medium while the nonpolar groups would be contained in the lipophilic interior of the membrane. Presuming the haemolytic action of the compounds to be a consequence of disruption of the interior organization of the membrane by the nonpolar substituent, it would thus be reasonable to expect regular solution theory to be applicable to erythrocyte haemolysis. The polar group no doubt also contributes to disruption of the membrane, but this

functionality is maintained constant with each series of compounds and whatever contribution made by it relative to the nonpolar fragment may be considered relatively invariant in passing from one compound to another.

CONCLUSIONS

A number of differing types of biological activities, such as narcotic potency, enzymatic activities, and protein binding, have been correlated against electronic polarizability[50–54] or, its magnetic equivalent, diamagnetic susceptibility[55]. It has recently been shown[39] that these electronic indexes can be used to calculate molar attraction constants, and hence δ values, in a semi-empirical manner for nonpolar and slightly polar molecules. The correlations of biological activity with electronic polarizability may thus be said to have a basis in regular solution theory[10]. Of much more significance, however, is the attempt by Davis[56] to calculate the extrathermodynamic lipophilic parameter π using solubility parameters and molar volumes. An encouraging degree of agreement was obtained between the calculated and observed values, prompting us[57] to seek a correlation between partition coefficients and electronic polarizability. It can be stated that at least for the *n*-octanol–water distribution system a surprisingly good correlation between partition coefficient and electronic polarizability is found[57], the correlation extending over 72 compounds. Two lines are found, one for the compounds with electronegative atoms (N, O) and the other for compounds that are ordinarily considered relatively nonpolar. The two lines suggest two types of partitioning processes which are potentially explicable in terms of molal volume differences for a compound contained in two differing phases[10]. The latter correlation thus suggests that a large proportion of the relationships between biological activities and partition coefficients[1–3] may be profitably reinvestigated in terms appropriate to regular solution theory. The results of our[55, 57] investigations along these lines will be reported in the near future.

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