The variability of the amount delivered per actuation could be due to inadequate shaking of the aerosol container. The method of collection of the labeled microspheres could also cause the valve stem to be blocked, thus detrimentally affecting the results.

The cascade impactor, which was used to delineate the particle size distribution profile, showed that the majority of microspheres had sizes ranging from 2 to 8 μm. The size profile seen in Table III lists microspheres with sizes greater than the reported prepared size of 3 to 8 μm, indicating aggregation of the smaller particles into heavier larger clusters. These aggregates were deposited onto the glass slides of the first two stages of the impactor, as dictated by the aerodynamic parameters of this particle-size analyzer. Again, the radiotracer method proved superior to the classical weighing-by-difference procedure for quantitation of the amount of particulate matter present on each slide after only one actuation of the aerosol.

The deposition of the microspheres onto the components of the valve constituted the final portion of this study. Untreated valves permitted the release of ~5–10% of the total radioactivity. By pretreating the valves with albumin solutions, the adherence of the iodinated albumin microspheres to the plastic parts of the valve and the metal spring was significantly decreased. The delivery of the activity from the treated canister, however, was found not to exceed 50% of the total dose. These results indicate that much more work must be done before microspheres utilized as a drug delivery system can be released effectively from present aerosol canisters.

CONCLUSIONS

The advantages of radiotracer methodology described in the study of aerosols are multifold: (*a*) single doses of an aerosol delivered from a canister can be analyzed, rather than multiple doses as required in previous studies, (*b*) short-lived isotopes can be disposed of easily after the passing of an appropriate decay period, and (*c*) by proper choice of the radionuclide label, rapid and accurate data may be obtained both in *in vivo* and *in vitro* studies. Since aerosols are designed to deliver a metered amount of drug to the patient, the elucidation of the size of the dose and its deposition in the patient is an important consideration. The technique outlined here should prove to be useful in answering these problems.

REFERENCES

(1) J. Halik, V. Lenger, V. Klimet, and P. Voboril, *J. Hyg. Epidemiol. Microbiol. Immunol.*, **24**, 405 (1980).

(2) S. P. Newman, D. Pavia, F. Moren, N. F. Sheahan, and S. W. Clarke, *Thorax*, **36**, 52 (1981).

(3) D. L. Swift, *Am. Rev. Respir. Dis.*, **122**, 71 (1980).

(4) F. Moren, *Int. J. Pharm.*, **8**, 1 (1981).

(5) R. B. Schlesinger, B. Naumann, and L. Chen, *Health Phys.*, **42**, 73 (1982).

(6) S. P. Newman, F. Moren, D. Pavia, F. Little, and S. W. Clarke, *Am. Rev. Respir. Dis.*, **124**, 317 (1981).

(7) S. P. Newman, D. Pavia, and S. W. Clarke, *Chest*, **80**, 909 (1981).

(8) A. Cutie, J. Burger, C. Clawans, D. Dolinsky, W. Feinstein, B. Gupta, A. Grueneberg, and J. Sciarra, *J. Pharm. Sci.*, **70**, 1085 (1981).

(9) P. J. Davies, K. K. Amin, and G. A. Mott, *Drug Dev. Ind. Pharm.*, **6**, 653 (1980).

(10) M. K. Halbert, M. K. Mazumder, and R. L. Bond, *Food Cosmet. Toxicol.*, **19**, 85 (1981).

(11) C. S. Garrard, T. R. Gerrity, J. D. Schreiner, and D. B. Yeates, *Arch. Environ. Health*, **36**, 184 (1981).

(12) J. E. Agnew, D. Pavia, and S. W. Clarke, *Eur. J. Respir. Dis.*, **62**, 239 (1981).

(13) T. Isawa, T. Teshima, T. Hirano, E. Ebina, and K. Konno, *Tokoho J. Exp. Med.*, **134**, 245 (1981).

(14) G. B. Saha, "Fundamentals of Nuclear Pharmacy," Springer-Verlag, New York, N.Y., 1979, p. 85.

(15) P. J. McConahey and F. J. Dixon, *Int. Arch. Allergy*, **29**, 185 (1966).

(16) F. C. Greenwood, W. M. Hunter, and J. S. Glover, *Biochem. J.*, **89**, 114 (1963).

(17) W. L. Hughes, Jr. and R. Straessle, *J. Am. Chem. Soc.*, **72**, 452 (1950).

Effects of Solvent Medium on Solubility III: Hydrophilic–Lipophilic Character Exhibited by Some Functional Groups Having Oxygen or Nitrogen in Ethanol–Water

RUBEN H. MANZO ˣ, AMELIA A. AHUMADA, and ERNESTINA LUNA

Received August 12, 1981, from the *Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Sucursal 16, CC 61, 5016 Córdoba, Argentina.* Accepted for publication June 16, 1983.

Abstract □ Solubility data of nine derivatives of the structure p-X_1—C_6H_4—X_2 in ethanol-water at 25°C are reported. The correlation of such sets of data by $\log S_y = P_y \cdot \log S_x + C$ yields the medium effect parameter P_y for a variety of functional groups possessing either oxygen or nitrogen. P_y accounts for the hydrophilic-lipophilic character exhibited by each group.

Keyphrases □ Solubility—effects of solvent medium, hydrophilic-lipophilic character, ethanol-water □ Solvent medium—effects on solubility, hydrophilic-lipophilic character, ethanol-water □ Hydrophilic-lipophilic character—effects of solvent medium, solubility, ethanol-water

The existence of linear relationships between the logarithms of the solubility equilibria of structurally related crystalline compounds, as they vary with changes in solvent or solvent composition has been shown previously (1, 2). Such linear free energy relationships (LFER) are conveniently expressed by:

$$\log S_y = P_y \cdot \log S_x + C \qquad \text{(Eq. 1)}$$

where S_y and S_x are sets of solubility data of compounds A_y and A_x, respectively. By taking a proper solvent condition as reference, Eq. 1 becomes:

$$\log S_y - \log S_y^0 = P_y(\log S_x - \log S_x^0) \qquad \text{(Eq. 2)}$$

or

$$\Delta\log S_y = P_y \cdot \Delta\log S_x \qquad \text{(Eq. 3)}$$

The medium effect parameter P_y depends on the structural difference between A_y and A_x on the one side and on the effects of the solvent change on the solubility of the substrate series (*i.e.*, on the sign of Δlog S) on the other. To illustrate this point, Fig. 1 shows a typical LFER plot of a pair of p-aminobenzoic esters which pass through a solubility maximum when they move from the first to the last solvent condition. There, as the solvent system increases in lipophilicity, the solubility

0022-3549/84/0800-1094$01.00/0

Table I—Derivatives of p-X_1—C_6H_4—X_2 Used in the Solubility Determinations

Compound	X_1	X_2	mp, °C Experimental[a]	mp, °C Literature
I	H_2N—	—$COOCH_3$	113-114.5	114 (8)
II	H_2N—	—$CONHCH_3$	181-181.5	180 (9)
III	CH_3CONH—	—H	112-113	113-115 (10)
IV	CH_3CONH—	—OH	169-171	169-170.5 (10)
V	CH_3CONH—	—$COOCH_3$	128.5-129.5	130 (6)
VI	$(CH_3)_2N$—	—$COOCH_3$	100-101	102 (6)
VII	HO—	—$COOCH_3$	131	131 (10)
VIII	CH_3COO—	—$COOCH_3$	80-80.5	—
IX	CH_3O—	—$COOCH_3$	47.5-48.5	48-48.5 (11)

[a] Uncorrected.

of the propyl ester A_y increases at a faster rate (part B, ethanol-water) and decreases more slowly (part A, ethanol-cyclohexane) than that of the methyl ester A_x, as a result of its greater lipophilic portion.

In this study, the purpose has been to use this methodology to get information about the hydrophilic-lipophilic properties of a variety of functional groups possessing either oxygen or nitrogen. These were selected because the information available, mainly obtained from partition coefficient (PC) measurements, regards the high sensitivity of the properties of these atoms to the structural environment (3, 4). Hence, a set of oxygen and nitrogen analogues was obtained by the appropriate variation of the basic structure p-X_1 —C_6H_4—X_2 as indicated in Table I, and their solubility equilibria were measured in hydroxylic mixtures. In such systems, the specific solute-solvent interactions through hydrogen bridges are recognized to play a major role among the factors that determine the solubility behavior of the substrates.

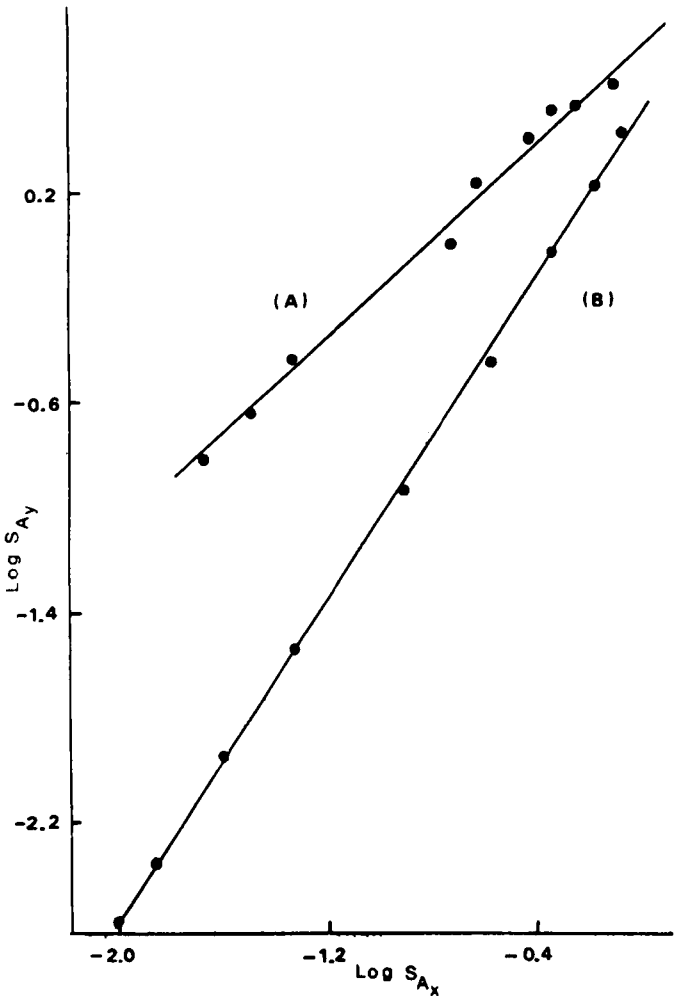


Figure 1—*LFER plot of propyl ester (A_y) and methyl ester (A_x) of* p-*hydroxybenzoic acid in ethanol-water (B) and ethanol-cyclohexane (A); data taken from Ref. 2.*

EXPERIMENTAL SECTION

Substrates—The calculated and literature melting points (mp) of all derivatives are reported in Table I. The following compounds were commercial products, purified by recrystallization: I[1], III[2], IV[2], and VII[2]. Compounds II, V, VI, VIII, and IX were prepared by adequate structural variation of the available derivatives through known techniques (5-7). All products were characterized by their melting points. In addition, for II and VIII, it was considered appropriate to report ^{1}H-NMR[3] and MS[4] data.

Solvents—Mixtures were prepared by mixing exactly measured volumes of absolute ethanol and water, *i.e.*, ethanol-water (40:60).

Solubility Determination and Correlation Procedure—The method employed for the solubility measurements was reported previously (1, 2). For all compounds, a spectrophotometric determination of substrate concentration at λ_{max} was performed. From the values of Table II, the corresponding molalities were calculated, and their logarithms were used in the correlations. The linear regression parameters reported in Table III were calculated by the least-squares method.

RESULTS AND DISCUSSION

Table II reports the solubility of I-IX in ethanol-water mixtures at 25°C. At low solubility conditions, small changes in solvent composition produce significant solubility variations; on the other hand, the lower the substrate

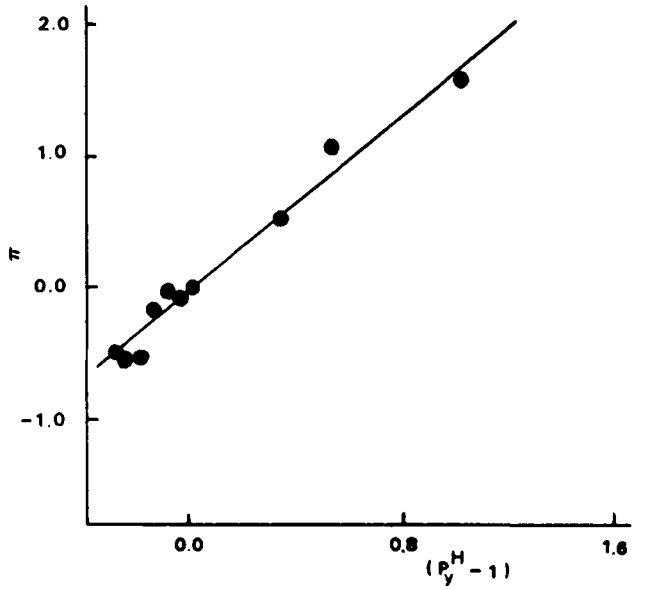


Figure 2—*Plot of the lipophilic constant π (octanol-water) against the medium effect parameter (P_y^H − 1) in ethanol-water for different functional groups.*

[1] R. Hellman.
[2] Aldrich.
[3] Varian T 60.
[4] Finnigan 3300F-100.

Table II—Solubility in Ethanol–Water at 25°C

Solvent Composition		Solubility, mg/g_{soln}								
Ethanol	Water	I	II	III[a]	IV	V	VI	VII	VIII	IX
00.0	100	1.45	8.64	6.38	15.1	1.35	0.112	2.89	0.621	0.753
10.0	90.0	2.05	13.2	9.64	25.6	2.39	0.168	3.81	0.889	1.19
20.0	80.0	3.70	17.9	14.4	35.7	4.63	0.338	6.73	1.25	2.19
30.0	70.0	6.94	26.1	36.3	62.8	12.0	0.866	20.5	2.73	5.82
40.0	60.0	18.3	39.4	52.6	113	19.9	2.85	49.3	6.63	17.6
50.0	50.0	38.0	55.3	76.5	160	40.0	8.79	123	15.7	—
60.0	40.0	63.5	72.4	141	203	116	17.0	196	28.0	—
70.0	30.0	89.6	80.5	206	247	158	29.8	265	45.9	—

[a] Solubility of III in ethanol-water has been reported early (12). Present results are in good agreement with those.

Table III—Correlation of Solubility Data According to Eq. 1 [a]

Compounds Correlated	Range	n	Slope	Intercept	r	δ_y	δ_{sl}
VI/I	0-70	8	1.334	−0.542	0.999	0.029	0.016
IV/III	0-70	8	0.851	0.188	0.992	0.066	0.043
V/IV	0-70	8	1.559	−0.681	0.989	0.125	0.095
VIII/VII	0-70	8	0.870	−1.014	0.997	0.062	0.028
V/I	0-70	8	1.107	0.138	0.992	0.108	0.058
V/III	0-70	8	1.347	−0.377	0.997	0.066	0.043
II/I	0-70	8	0.520	−0.104	0.994	0.043	0.023
V/VIII	0-70	8	1.079	0.647	0.991	0.111	0.058
VI/IX	0-40	5	1.024	−0.815	0.999	0.017	0.016
VIII/IX	0-40	5	0.746	−0.758	0.999	0.025	0.023
IX/VII	0-40	5	1.050	−0.497	0.997	0.049	0.047
I/VII	0-70	8	0.855	−0.520	0.998	0.044	0.018

[a] r is the regression coefficient, δ_{sl} is the standard deviation of the slope, and δ_y is the standard deviation of the points from the regression line in the y direction.

concentration, the lower the probability of particular solute–solute interactions. Therefore, to correlate solubility data by Eq. 1, those values corresponding to lower solubilities are preferred to the ones obtained under conditions of higher solubility—near the maximum solubility. A composition variation covering 0–70% of ethanolic content was considered appropriate.

Going from water to ethanol through a series of ethanol-water mixtures, the increase of the ethanolic content increases the lipophilic character of the mixture and raises the solubility of all the substrates studied [(+)$\Delta\log S$]. For a given correlation, a medium effect parameter P_y higher or lower than the unity means that A_y is respectively more lipophilic or hydrophilic than A_x.

Table III reports the results obtained by the correlation according to Eq. 1 when adequately selected pairs of compounds were matched together. As can be seen, good linear correlations were obtained in all cases affording the corresponding P_y parameters.

To get a uniform set of P_y values, the hydrogen atom attached to the aromatic ring was taken as the reference functional group ($P_{yH} = 1$). When direct calculation was not possible, the following procedure was used:

$$P_y = \Delta\log S_{Ar-Y}/\Delta\log S_{Ar-X} \qquad \text{(Eq. 4)}$$

and

$$P_x = \Delta\log S_{Ar-X}/\Delta\log S_{Ar-H} \qquad \text{(Eq. 5)}$$

Table IV—Hydrophilic–Lipophilic Character of Different Functional Groups Attached to the Benzene Ring

Functional Group	$(P_y^H - 1)$	π
$-CONHCH_3$	−0.33	
$-NH_2$	−0.28	−0.49[a]
CH_3COO-	−0.26	−0.58[b]
CH_3CONH-	−0.20	−0.56[b]
$-OH$	−0.15	−0.16[a]
CH_3O-	−0.09	−0.03[b]
$(CH_3)_2N-$	−0.05	−0.08[b]
$-H$	0.00	0.00
$-COOCH_3$	0.33	0.49[c]
$-CH_2-CH_2-$[d]	0.53	1.08
$-COO(CH_2)_2CH_3$	1.04	1.57[c]

[a] Calculated from log PC (octanol-water) of I (1.63) and VII (1.96), respectively, using as reference log PC of benzoic acid methyl ester (2.12); all data from Ref. 3. [b] Values from Ref. 13. [c] Calculated respectively from log PC of VII or log PC of *p*-hydroxybenzoic acid propyl ester using as reference log PC of phenol; all data from Ref. 3. [d] Concerned with the extension of the alkyl chain. The random value of P_y arises from propyl and methyl esters of both *p*-hydroxy- and *p*-aminobenzoic acids (2), while π is the difference between $\pi_{-COO(CH_2)_2CH_3}$ and π_{-COOCH_3}.

then

$$P_y^H = P_y \cdot P_x = \Delta\log S_{Ar-Y}/\Delta\log S_{Ar-H} \qquad \text{(Eq. 6)}$$

Table IV contains the calculated hydrophilic-lipophilic character of each functional group, which is expressed as $(P_y^H - 1)$. There, a negative value means higher hydrophilic character, while a positive value indicates higher lipophilic character than hydrogen.

Therefore, the inspection of the figures in Table IV reveals the higher hydrophilicity of the aromatic primary amino group with respect to the phenolic hydroxy group, while the respective acetyl derivatives exhibit a reverse order. However, the comparison of the aryl carboxy derivatives (*i.e.*, I and II) again shows a remarkably higher hydrophilic character of the nitrogenated derivative. On the other hand, the methoxy group appears to be more hydrophilic than the fully methylated tertiary amino.

The use of $(P_y^H - 1)$ is a convenient way to express the hydrophilic-lipophilic properties of the functional groups since, as it has been previously stated (1), it is directly related to the expected PC parameter π'_y by:

$$P_y - 1 = \frac{\Delta\log S_y - \Delta\log S_x}{\Delta\log S_x} = \frac{\pi'_y}{\Delta\log S_x} \qquad \text{(Eq. 7)}$$

π'_y has also been regarded as a lipophilic variable (1) whose meaning extends the concept of the lipophilic constant π (3) and hydrophobic constant (14) which arise from partition coefficient measurements.

In addition, Table IV also contains literature values of π for the octanol-water partitioning system, which also consists of two hydroxylic solvents. Because inspection of the columns suggests a parallel variation of both sets of figures, it was considered of interest to match them together in a plot. Therefore, Fig. 2 shows the agreement between the results obtained from medium effects on solubility in ethanol-water mixtures and those obtained from octanol-water partition coefficients.

The results quoted show how LFER applied to solubility equilibria measurements yields reliable information about the hydrophilic-lipophilic properties of different functional groups. It appears that this simple methodology would be a useful and versatile tool to extend our present knowledge of solubility behavior to a wide variety of solvent systems. In addition, the use of Eqs. 2–7 as a means of predicting medium effects on solubility appears to be attractive for the pharmaceutical sciences.

REFERENCES

(1) R. H. Manzo, *J. Pharm. Pharmacol.*, **34**, 486 (1982).

(2) R. H. Manzo, *An. Assoc. Quim. Argentina*, **71**, 47 (1983).

(3) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).

(4) S. S. Davis, T. Higuchi, and J. H. Rytting, *Adv. Pharm. Sci.*, **4**, 104 (1974).
(5) "Vogel's Textbook of Practical Organic Chemistry," 4th ed., Longman, London and New York, 1978.
(6) P. Grammaticakis, *Bull. Soc. Chim. Fr.*, **1951**, 220.
(7) K. A. Connors, "A Textbook of Pharmaceutical Analysis," 2nd ed., Wiley, New York, N.Y., 1975, pp 455-459.
(8) "Handbook of Chemistry and Physics," 56th ed., CRC Press, 1975-1976.
(9) H. Wenker, *J. Am. Chem. Soc.*, **60**, 1081 (1938).
(10) "The Merck Index," 8th ed., Merck & Co. Inc, Rahway, N.J., 1968.
(11) T. C. Corby and P. H. Elworthy, *J. Pharm. Pharmacol.*, **23**, Suppl., 39 S (1971).
(12) E. J. Cohn, *Chem. Rev.*, **19**, 241 (1936).
(13) S. M. Sicardi, "Farmacologia Molecular," Centro Editor Argentino, 1981.
(14) R. F. Rekker and H. M. de Kort, *Eur. J. Med. Chem.-Chim. Ther.*, **14**, 479 (1979).

ACKNOWLEDGMENTS

The authors thank the Consejo de Investigaciones Cientlficas y Technológicas de la Provincia de Córdoba for financial support (Grant 064/82).

Acute Intravenous Infusion of Disodium Dihydrogen (1-Hydroxyethylidene)diphosphonate: Mechanism of Toxicity

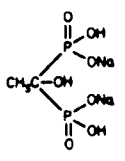
MARION D. FRANCIS ˣ **and CANDICE L. SLOUGH**

Received September 20, 1982, from the *Miami Valley Laboratories, 2N142, Proctor & Gamble Co., Cincinnati, OH 45247.* Accepted for publication July 26, 1983.

Abstract □ The acute intravenous toxicity of disodium dihydrogen (1-hydroxyethylidene)diphosphonate (etidronate disodium; I) and the mechanism of this toxic response have been investigated in 40 beagle dogs. The intravenous toxicity of I is dependent on the total dose administered and the length of the infusion interval. The toxicity of I is directly related to the ability of the drug to bind or complex with the circulating calcium in the blood. Maximum depressions in ionized calcium coincide in time with peak blood levels of I, and at lethal doses electrocardiographic changes indicative of hypocalcemia are observed. For a 2-min infusion of 2 mg of I/kg, no effect is observed on ionized calcium levels, and the electrocardiogram remains normal. At doses of 16 and 32 mg/kg, coincident with an immediate fall in ionized calcium levels, there is a transient rise in total calcium and a fall in phosphorus levels. The ionized calcium level rises, and total calcium level falls and stabilizes at baseline levels within 30 min after the infusion. However, the phosphorus level rises and exceeds the baseline value, reaching 3-4 times normal by 72 h after the infusion. With proven lethal doses of I (60 mg/kg infused over 2 min) and the simultaneous infusion of an ionized calcium salt such as calcium gluconate (20 mg of Ca^{2+}/kg), electrocardiograms remain normal and death is prevented. Thus, an effective antidote in the event of an overdose or too rapid an infusion of I can be employed to prevent acute toxic effects.

Keyphrases □ Etidronate disodium—hypercalcemia, intravenous toxicity, dogs □ Hypercalcemia—treatment with etidronate disodium and calcium gluconate, toxicity, dogs □ Calcium gluconate—hypercalcemia, use with etidronate disodium, dogs

Disodium dihydrogen (1-hydroxyethylidene)diphosphonate (etidronate disodium; I) is a substance which has both therapeutic (1-4) and diagnostic (5, 6) uses in metabolic bone disease. Clinically, the ability of orally administered drug[1] to reduce elevated bone turnover prompted speculation that the drug might be used intravenously to control other disease processes such as hypercalcemia secondary to malignancy or hyperparathyroidism. Studies to examine physiological and pharmacological responses to varying intravenous doses of diphosphonates were therefore undertaken.



I

The mechanism of intravenous toxicity of I might be expected to be similar to that of ethylenediaminetetraacetic acid (II) and polyphosphate compounds (III) because of the chelating properties (7-10) of each of these compounds. However, I differs from II and III in several respects, which could markedly alter its effect on calcium homeostasis.

At physiological pH, I is a less-effective simple chelator of calcium than II and, unlike II, forms polynuclear complexes (11) which can reach molecular weights of ≥20,000. There is some evidence for this formation *in vivo*, but their biological activity is unknown. In addition, I is cleared from the blood both by adsorption on the hydroxyapatite of bone (12) and by renal clearance, whereas II is cleared only by renal excretion. There are also differences in the p*K* values of I, II, and III. Furthermore, although III are adsorbed on hydroxyapatite like I, III are rapidly hydrolyzed *in vivo* to the natural orthophosphate metabolites (HPO_4^{2-}, $H_2PO_4^-$), while I is not metabolized (13). Based on the above differences, the present study was undertaken to establish a broader safety profile for I by clearly defining the acute intravenous toxicity of I and demonstrating the relationship between acute intravenous toxicity, serum ionized and total calcium levels, and diphosphonate blood levels. Subsequent to these studies, intravenously administered I and another diphosphonate (dichloromethylenediphosphonate) have been shown to effectively reduce hypercalcemia associated with malignant disease and hyperparathyroidism in humans (14-18).

EXPERIMENTAL SECTION

Purebred adult male and female beagle dogs weighing 5.7-13.9 kg were used in four experiments. It was necessary to conduct the initial studies in unanesthetized animals to ensure that no signs or symptoms of toxicity were masked by the anesthetic. However, in subsequent experiments, animals receiving toxic doses of I were anesthetized with sodium pentobarbital unless its use was contraindicated.

[1] Didronel; Procter & Gamble Co., Cincinnati, Ohio.

0022-3549/84/0800-1097$01.00/0