which was consistent for the morphine chromophore (2).

The remaining 6.6 mg of the compound was converted to the sulfate salt by dissolving the sample in ether and adding ether saturated with sulfuric acid. A white crystalline material (2.0 mg) was obtained and dried. Its IR spectrum (potassium bromide) was superimposable with that of an authentic morphine sulfate sample.

These data served to confirm the presence of morphine in poppy plants grown from seeds obtained at a local supermarket.

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Thermodynamics of Gaseous Anesthesia of Mice

Keyphrases □ Anesthesia—gaseous, thermodynamics of equilibrium process, evaluation of Ferguson principle, mice □ Thermodynamics—gaseous anesthesia, evaluation of Ferguson principle, mice □ Ferguson principle—applicability to thermodynamics of gaseous anesthetics

To the Editor:

Evidence was presented recently indicating that the gaseous anesthesia of mice is largely a gas–biophase equilibrium process (1). This evidence consists principally of the observations that gaseous anesthetic potencies can be correlated against gas–oil distribution coefficients (1) but not against oil–water or 1-octanol–water partition coefficients (2). Since gaseous anesthesia has the characteristics of an equilibrium process, it is appropriate to establish the apparent thermodynamic variables pertaining to the anesthetic process in mice. This report presents such an attempt. The results indicate that the Ferguson principle (3), as presently formulated, is in error. A revised expression of the Ferguson principle is a direct outcome of this study.

The Clausius–Clapeyron equation for the distribution of a gas with a condensed phase is given by:

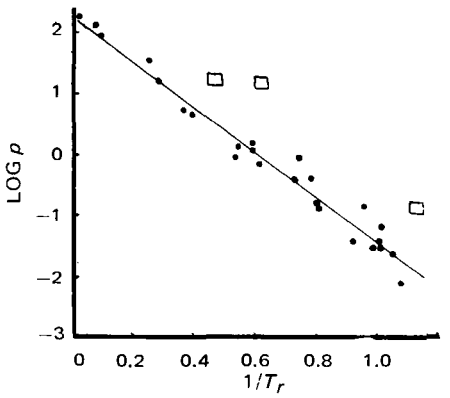

Figure 1—*Graph of anesthetic pressure against the reciprocal of the physiological reduced temperature. Compounds not following the relationship (fluoromethane, perfluoromethane, and perfluoroethane) are identified by the symbol* □.

$$\left(\frac{\partial \ln a_2}{\partial T}\right)_p = \left(\frac{\partial \ln p}{\partial T}\right)_p = \frac{\Delta H_v}{RT^2} = \frac{\Delta S_v}{RT} \qquad \text{(Eq. 1)}$$

where p is the partial pressure of the gas (presumed ideal) at an absolute temperature, T, and pressure, P, and a_2 is the thermodynamic activity of the gas solubilized in the condensed phase.

Equation 1 is appropriate for the determination of the apparent enthalpy and entropy of vaporization, ΔH_v and ΔS_v, at each temperature T from the slopes of graphs of log p against $1/T$ or log T, respectively. However, if the distribution of a number of gases with a condensed phase is of interest, one must either determine the partial pressure variation with temperature for each gas or, following the Principle of Corresponding States, approach the problem in terms of reduced thermodynamic variables so that the same equation of state will apply to each gas.

The reduced thermodynamic variables, P_r, V_r, and T_r, for a substance are obtained by dividing the critical values, P_c, V_c, and T_c, for the substance into the corresponding thermodynamic variables, P, V, and T. Critical values define the point where a gas can no longer be condensed into a liquid phase. When con-

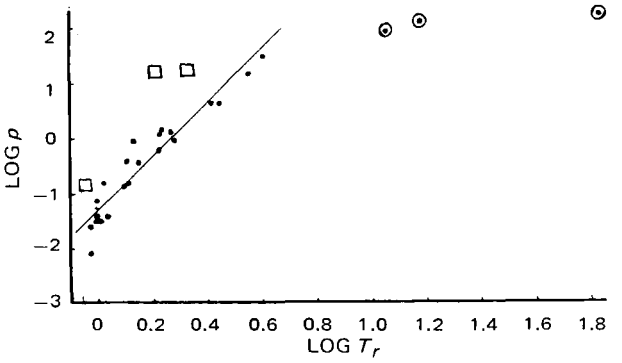

Figure 2—*Graph of anesthetic pressure against the logarithm of the physiological reduced temperature. Compounds not following the relationship (fluoromethane, perfluoromethane, and perfluoroethane) are identified by the symbol* □. *Anesthetics whose boiling points are near 0°K (helium, hydrogen, and neon) and which may be expected to depart from general trends are identified using the symbol* ⊙.

Table I—Minimum Anesthetic Pressures to Inhibit the Righting Reflex of Mice and the Physiological Reduced Temperatures for the Gases

Gas	Log p[a]	T_{BP}, °K[b]	$T_r = \frac{T}{T_{BP}} = \frac{310}{T_{BP}}$
Helium	2.28	4.5	68
Hydrogen	2.14	20.6	15.0
Neon	1.94	27.1	11.4
Nitrogen	1.52	77.3	4.01
Hexafluoroethane	1.19	194.1	1.60
Tetrafluoromethane	1.24	145.4	2.13
Argon	1.18	87.5	3.54
Methane	0.66	111.6	2.77
Krypton	0.65	121.1	2.56
Nitrous oxide	0.18	184.6	1.68
Ethylene	0.15	169.4	1.83
Ethane	0.11	184.5	1.68
Xenon	−0.02	165.1	1.88
Propane	−0.05	231.0	1.34
Acetylene	−0.15	189.5	1.63
Dichlorodifluoromethane	−0.40	243.3	1.27
Propylene	−0.40	225.4	1.37
Cyclopropane	−0.80	239.6	1.29
Trichloromonofluoromethane	−0.82	296.8	1.04
Fluoromethane	−0.85	351.3	0.88
Chloromethane	−0.85	249.1	1.24
Iodomethane	−1.15	315.5	0.98
Ethyl chloride	−1.40	285.4	1.08
Ethyl bromide	−1.40	311.5	0.99
Ether	−1.52	307.7	1.01
Dichloromethane	−1.52	313.8	0.99
1,1-Dichloroethane	−1.59	330.5	0.94
Chloroform	−2.08	334.4	0.93

[a] From Refs. 1 and 5. [b] Boiling points at 1 atm. (from "Handbook of Chemistry and Physics," R. C. Weast and S. M. Selby, Eds., Chemical Rubber Co., Cleveland, Ohio, 1967, pp. D113–D137).

sidering gases at 1 atm, the critical temperature, T_c, can be replaced by the boiling point, T_{BP}, in determining reduced temperature, since the temperatures of substances at their boiling points are within 0.66 of their critical temperatures, provided hydrogen bonding or other association effects do not occur in the gaseous or condensed phases (4).

Gases at the physiological temperature of mice, 37° (310°K) (5), differ in relation to their physiological reduced temperature, as obtained from the relation $T_r = 310/T_{BP}$. Table I shows the physiological reduced temperatures for the gaseous anesthetics studied by Miller *et al.* (1, 6). The Clausius–Clapeyron relation (Eq. 1), expressed in terms of physiological reduced temperature, may thus be used to compare the partial pressures of gases for the anesthesia of mice. The criterion of anesthesia is the loss of the righting reflex.

Figures 1 and 2 show the partial anesthetic pressures of gases toward mice plotted according to the two alternative integrated forms of the Clausius–Clapeyron equation. In each graph the fluorinated hydrocarbon, fluoromethane, and the perfluoro compounds, perfluoromethane and perfluoroethane, depart from the general linear trend, perhaps because of the unique solubility properties of fluorinated hydrocarbons. The complimentariness expected on the basis of Eq. 1 between Figs. 1 and 2 is generally real-

Table II—Estimation of the Thermodynamic Activities for Some Gaseous Anesthetics

Gas	Boiling Point, T_{BP}[a]	Enthalpy of Vaporization, ΔH_v[a]	Partial Pressure Pure Liquid, log p^0	Anesthetic Partial Pressure, log p	Thermodynamic Activity, log (p/p^0)
Helium	4.2	20	1.025	2.28	1.255
Hydrogen	20.4	216	2.158	2.14	−0.018
Neon	27	450	3.317	1.94	−1.377
Nitrogen	77	1330	2.826	1.52	−1.306
Tetrafluoromethane	145	2650	2.358	1.24	−1.118
Hexafluoroethane	195	3860	1.598	1.19	−0.408
Argon	87	1560	2.808	1.18	−1.628
Methane	112	1960	2.438	0.66	−1.778
Krypton	121	2160	2.369	0.65	−1.719
Nitrous oxide	184	3960	1.905	0.18	−1.725
Ethylene	169	3240	1.894	0.15	−1.744
Ethane	184	3510	1.688	0.11	−1.578
Xenon	165	3020	1.863	−0.02	−1.883
Propane	231	4490	1.076	−0.05	−1.026
Propylene	225	4400	1.167	−0.40	−1.567
Cyclopropane	114	4536	5.470	−0.80	−6.270
Chloromethane	249	5150	0.884	−0.85	−1.734

[a] From J. H. Hildebrand and R. L. Scott, "The Solubility of Nonelectrolytes," Dover, New York, N.Y., 1964, pp. 435–439.

ized, except in Fig. 2 where the points for helium, hydrogen, and neon are far from the line. Since these compounds have boiling points close to 0°K, aberrant behavior with respect to other compounds is to be expected in a graph involving log T.

Least-squares regression of the points contained in Figs. 1 (omitting those for the deviant fluorocarbons) and 2 (omitting those for the deviant fluorocarbons, helium, hydrogen, and neon) provides the slopes from which, respectively, apparent enthalpies and entropies of vaporization can be estimated. The derived relationships are expressed by:

$$\Delta H_v = 16.81(\pm 0.32)T_r^2 \quad \text{(Eq. 2)}$$

$$\Delta S_v = 22.31(\pm 1.56)T_r \quad \text{(Eq. 3)}$$

In principle, at a reduced temperature of 1, the constants appearing in Eqs. 2 and 3 should be identical. The fact that they are not, but are nearly so, may be attributed to the use of a biological end-point, the loss of the righting reflex, in making partial pressure assignments for the anesthetic gases. Nevertheless, given the uncertainties in the partial pressure values, the agreement found is remarkable.

Inspection of Table I shows that the reduced temperatures for many important gaseous anesthetics are generally not far from 1, *e.g.*, chloroform, $T_r = 0.93$; ether, $T_r = 1.01$; and cyclopropane, $T_r = 1.29$. The apparent entropy of vaporization of these substances from the biophase responsible for anesthesia in the mouse is thus on the order of 20 cal/mole-°K. This finding may be contrasted with the Trouton's rule value of 21 cal/mole-°K for the entropy of vaporization of nonassociating substances. These anesthetics thus appear to be dissolving in a nonpolar phase; and since the process is universal to the gaseous anesthetics under consideration, the same nonpolar biophase must be involved for them all. No special character to anesthesia is imparted by having electronegative atoms on carbon-bearing hydrogen atoms, as was conjectured recently (2).

A particularly important conclusion can be drawn from the finding of a nonzero slope to the lines shown in Figs. 1 and 2, since this finding designates that the Ferguson principle must be restated. The Ferguson principle as presently accepted states that, under equilibrium or near equilibrium conditions, compounds must accumulate to a certain level in an organism or tissue to elicit an equivalent biological response and that this level of accumulation is directly measured by the thermodynamic activities of the compounds in the phase external to the organism or tissue.

This statement signifies that all gaseous anesthetics would have the same thermodynamic activities at the biological end-point measured as the loss of the righting reflex of mice. If this was indeed true, then the slope of the data presented in Figs. 1 and 2 would have to be zero according to Eq. 1. That is, ΔH_v and ΔS_v would be zero, signifying that gaseous anesthetics behave as if they form an ideal solution with the biophase of mice. The nonzero slope to the line in Figs. 1 and 2 shows that the "solution" formed by gaseous anesthetics in a biophase is not ideal. Hence, *the presently accepted statement of the Ferguson principle applies only if the properties of drugs in a biophase are similar to those of an ideal solution.*

The nonideal behavior of the biosolubilization of gaseous anesthetics provides a basis for understanding the origins of linear free energy relationships in relation to anesthesia. Were the properties of drugs in a biophase to be in accord with the Ferguson principle, no linear free energy relationships could exist, since there would be no variation in enthalpy or entropy. However, the Ferguson principle, as revised to apply to the ideal case, is invalid for gaseous anesthesia. Hence, there are variations in enthalpy and entropy that can be related to corresponding variations in model systems.

In further support of the conclusion that the Ferguson principle is invalid for gaseous anesthesia, the thermodynamic activities for some gaseous anesthetics were determined. The relationship given by Eq. 4 was used, where the vapor pressure of the pure liquid, p^0, was determined using Eq. 5, as suggested by Hildebrand and Scott (7):

$$\log (p/p^0) = \log a_2 \quad \text{(Eq. 4)}$$

$$\log p^0 = \frac{\Delta H_v}{2.3R}\left(\frac{T - T_{BP}}{TT_{BP}}\right) \quad \text{(Eq. 5)}$$

Table II shows the results of these determinations. Log p^0 at the physiological temperature of 37° (310 °K) was relatively constant and of much greater magnitude than the partial pressures of anesthesia given by log p. The calculated thermodynamic activities were found to be roughly constant, due principally to the relatively constant value of log p^0, the anesthetic potencies being small in comparison. There is thus gained the illusion that anesthetics in a biophase behave ideally, since for ideal solutions $a_2 = 1/p_2^0$ when the vapor pressure is measured at 1 atm. Therefore, the Ferguson principle has been founded on an observation that by its very nature excludes the variation in anesthetic partial pressure.

The thermodynamic description of the gaseous anesthesia of mice resulting from this study has led to a restatement of the Ferguson principle. All of the observations made by directly applying thermodynamic principles to the anesthetic potencies are in full accord with the gas–oil distribution studies conducted by Miller *et al.* (1). The distinction here is that thermodynamics have been applied solely to the biological observations rather than in relating the biological observations to the behavior of a model system.

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Relationship between Quinidine Plasma and Saliva Levels in Humans

Keyphrases □ Quinidine—plasma and saliva concentrations compared, humans □ Saliva—determination of quinidine levels, correlated with plasma levels

To the Editor:

Increasing applications of the principles of pharmacokinetics to clinical situations (1) have emphasized the need for rapid, noninvasive techniques for monitoring drug concentrations in biological fluids, especially with pediatric patients or whenever a large number of serial samples is indicated. Since several drugs such as salicylate (2), sulfonamides (3), barbiturates (4), tetracyclines (5), phenytoin (6), theophylline (7), and digoxin (8) are secreted in saliva, this measurement appears to have potential use, especially when these levels can be correlated with plasma levels in patients.

There are no previous reports concerning salivary concentrations of quinidine. However, blood level monitoring of quinidine is occasionally useful clinically, since the drug exhibits an extremely narrow range between a usually effective serum level (2 μg/ml) and a usually toxic serum level (8 μg/ml) (9).

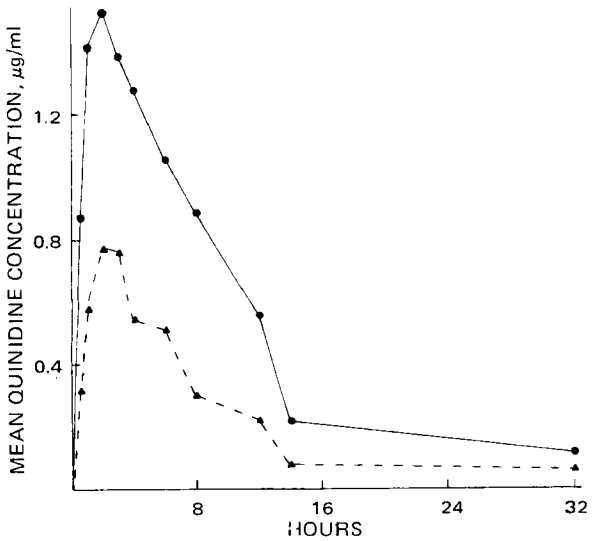

Figure 1—*Mean plasma and saliva concentrations as a function of time for Subject 1. Each concentration is the mean of two determinations. Key: ●, plasma concentrations; and ▲, saliva concentrations.*

Table I—Relationship between Plasma and Saliva Concentrations of Quinidine in Three Subjects

Subject	Mean Plasma–Saliva Ratio (±*SD*)	Correlation Coefficient	Statistical Significance
1	2.29 (±0.89)[a]	0.885	$p < 0.001$
2	1.55 (±0.70)[a]	0.681	$p < 0.01$
3	2.35 (±0.70)[b]	0.825	$p < 0.001$

[a] Eighteen determinations. [b] Nineteen determinations.

Thus, a technique that would obviate the need for collecting serial blood samples would be of value.

The data reported here on saliva levels of quinidine were obtained in conjunction with a larger study on the bioequivalency of various commercially available quinidine sulfate tablets in humans. The specific value of this study relates best to the possible use of saliva in bioavailability studies rather than for clinical monitoring.

Three adult, normal, male subjects involved in the bioequivalence study were selected randomly and, in addition to providing blood samples (by venipuncture), were requested to expectorate into 10-ml test tubes from approximately 5 min before until 5 min after each blood collection or until 3–5 ml of mixed saliva had been obtained. Blood and saliva quinidine levels were determined at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, and 32 hr after administration of two 200-mg tablets of any one of four brands of quinidine sulfate. These same subjects repeated this procedure a second time with at least a 1-week interval between administrations. The values reported in Table I for each subject were pooled from the two different runs and involve 18 or 19 determinations/subject since saliva levels were not always sufficiently high to be determined at the 24- and 32-hr time periods.

Aliquots (0.5 ml) of plasma or saliva (centrifuged to remove sputum) at each time period were assayed by the fluorometric method of Cramer and Isaksson (10) with only slight modifications. Blank specimens of serum and saliva were assayed in the same manner as the samples, and the appropriate blank corrections were applied. Figure 1 shows a plot of plasma and saliva quinidine concentrations as a function of time for one of the three subjects (Subject 1 of Table I).

Although a significant correlation exists between plasma and saliva levels of quinidine, differences in the extent of correlation are evident among the three subjects (Table I). While a number of studies (2, 6) showed an even better correlation between plasma and saliva levels of other drugs, the present study nonetheless suggests that quinidine can be detected in human saliva in measurable concentrations and that these concentrations are related to their corresponding plasma levels. The pH of the saliva samples was not measured, although fluctuations in saliva pH could possibly account for the observed intrasubject variability in plasma saliva ratios for the weak base quinidine (11).

Plasma samples in this study consisted of both bound and unbound drug, and better correlations might have resulted if unbound quinidine in plasma