A considerable quantity of the radioactivity (47% in bile duct-cannulated and 10% in intact rats) excreted in the feces of the rats given tritium-labeled I orally was identified as the parent compound. This material probably represents Compound I, which was not absorbed from the GI tract.

Radioactivity in the urine was in the form of metabolites of I. These data agree with previous reports (6, 9) of the appearance of polar metabolites in the urine. Zone scraping of the TLC plates indicates the presence of metabolites other than 11-hydroxy-Δ^9-tetrahydrocannabinol and 8,11-dihydroxy-Δ^9-tetrahydrocannabinol. Identity of this large polar fraction has not been achieved. This may be accounted for, in part, by the recently described (14) diacetyl derivative found in bile. The identity of the material remaining in the feces after exhaustive extraction is also a mystery. Unfortunately, sufficient samples of material were not available for hydrolysis studies. These are planned.

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Interfacial Adsorption of a Psychotomimetic Drug Using Liquid Scintillation

H. KIMIZUKA* and L. G. ABOOD▲

Abstract □ A study was conducted on the oil–water partitioning and interfacial adsorption of ^{3}H-2-pyrrolidylmethyl *N*-methyl cyclopentylphenylglycolate (I), an anticholinergic psychotomimetic agent. A new technique for radiotracer adsorption was developed involving the use of liquid scintillation counting. Among the physical parameters examined were partition coefficient, permeation constant, stability constant, and rate constant of I in a two-phase system of water and a lipid, didodecyl phosphate (II). II greatly accelerated the rate of oil–water partitioning of I and exhibited interfacial adsorption with I. The presence of polyanions, such as hyaluronic acid in the aqueous phase, promotes the transfer of drug from the oil to water phase. Equations found applicable to permeation of ions across membranes have been used to describe drug transfer through an oil–water interface.

Keyphrases □ Adsorption, interfacial, ^{3}H-labeled psychotomimetic agent—studied using liquid scintillation counting □ Interfacial adsorption and oil–water partitioning of radiolabeled compounds—studied using liquid scintillation counting □ 2-Pyrrolidylmethyl *N*-methyl cyclopentylphenylglycolate, radiolabeled—interfacial phospholipid adsorption and oil–water partitioning studied using liquid scintillation counting □ Anticholinergic psychotomimetic agents, radiolabeled—interfacial phospholipid adsorption and oil–water partitioning studied using liquid scintillation counting □ Liquid scintillation counting—used to measure interfacial adsorption and oil–water partitioning of radiolabeled psychotomimetic agent □ Partitioning (oil–water) of ^{3}H-2-pyrrolidylmethyl *N*-methyl cyclopentylphenylglycolate—studied using liquid scintillation counting

For a number of years, interest in this laboratory has focused on the mechanism of action of a group of anticholinergic psychotomimetic agents, particularly with regard to their ability to modify the physicochemical properties of excitatory membranes (1). A useful approach has been to examine the effect of the agents on the interfacial properties of surface films of phospholipids and membranous proteins using conventional interfacial techniques, including the measurement of surface adsorption with radioactive compounds (2).

The technique of surface adsorption involves the measurement of a radioactively labeled substance as it

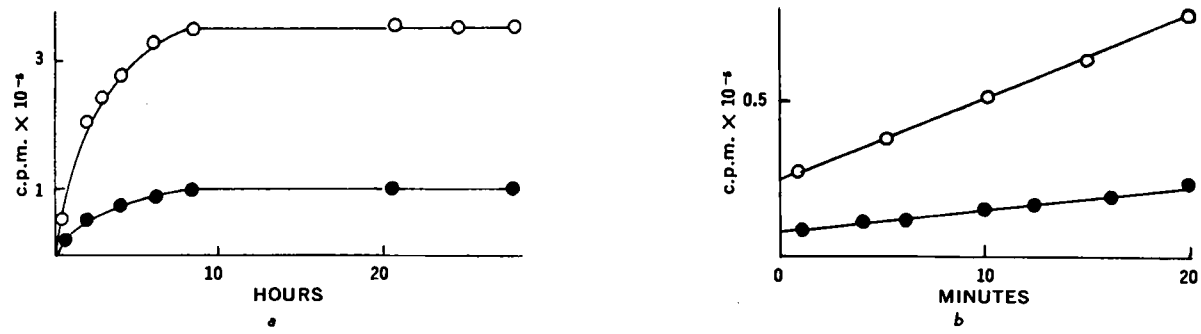

Figure 1—(a) *Time course of partitioning of I from the aqueous to the scintillator phase. Concentration of I was 0.16 mM in the presence* (○) *and in the absence* (●) *of II.* (b) *Time course of partitioning of I from the aqueous to the scintillator phase during initial period. Concentration of I was 0.16 mM in the presence* (○) *and in the absence* (●) *of II.*

interacts with a surface film (comprised of a lipid or other surfactant) by means of a geiger-mueller tube (3). One difficulty, however, has been the insensitivity of the geiger-mueller tube to soft β-radiation, particularly in the case of ^{3}H-labeled compounds.

To overcome this limitation, a new technique was developed for the measurement of interfacial interaction as well as oil–water partitioning by use of the liquid scintillation method. By means of this technique, the interaction of ^{3}H-labeled drugs with lipoidal substances can be determined with greater accuracy and simplicity. The present study is concerned with the kinetic aspects of the interfacial interaction and oil–water partitioning of the ^{3}H-labeled psychotomimetic agent, ^{3}H-2-pyrrolidylmethyl *N*-methyl cyclopentylphenylglycolate (I) employing a phospholipid, didodecyl phosphate (II).

EXPERIMENTAL

The scintillator solution was prepared by dissolving 4 g. of 2,5-diphenyloxazole and 0.25 g. of *p*-bis[2-(5-phenyloxazolyl)]benzene in 1:1 toluene (*S*). This solution (*S*) was diluted twice by volume with either toluene (*S* + *T*), or toluene solution of 1 m*M* didodecyl phosphate (*S* + *D*). After 10 ml. of an aqueous solution of ^{3}H-I was introduced in a vial, 10 ml. of the scintillator solution was carefully layered on the top solution and the radiation was counted with a liquid scintillation counter. The samples were counted immediately (within 1 min.) after preparation. By extrapolation to zero time, the time course of adsorption during the first few minutes could be determined.

The specific activity of I was determined in a mixture of 1 ml. aqueous ^{3}H-I solution of known concentration, 10 ml. ethanol, 5 ml. (*S* + *T*), and 5 ml. (*S* + *D*). The specific activity of I in this mixture was found to be (3.76 ± 0.19) × 10^{11} c.p.m./mole. By means of this standardization procedure, the specific activities (mean ± *SD*) of I were determined in the scintillator solutions (*S* + *T*) and (*S* + *D*) and were found to be (5.09 ± 0.25) × 10^{12} c.p.m./mole in (*S* + *T*) and (3.10 ± 0.16) × 10^{11} c.p.m./mole in (*S* + *D*), respectively. The aqueous I solution was adjusted to pH 2 with hydrochloric acid unless otherwise stated.

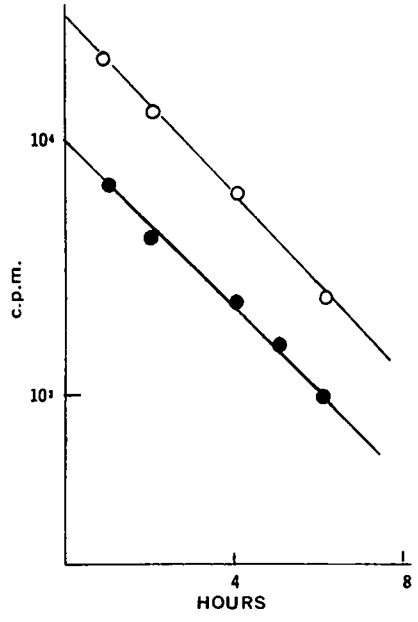

Figure 2—*Time course of partitioning, log* (I_∞ − I) versus t *in the presence* (○) *and in the absence* (●) *of II.*

RESULTS AND DISCUSSION

The increase in counting due to the partitioning of I into the scintillator phase was measured with time, and an example is shown in Figs. 1*a* and 1*b*. These figures indicate that the partition coefficient was greater in the presence of didodecyl phosphate than in its absence, although the time constants for partitioning were approximately the same under both conditions.

The semilogarithmic plots for the data are given in Fig. 2, which are expressed by the relation:

$$\ln (I_\infty - I) = \ln I_\infty - kt \qquad \text{(Eq. 1)}$$

where *I* denotes the radioactive counts at time *t*, I_∞ is the equilibrium value, and *k* is the rate constant. From Figs. 1 and 2, it is apparent that the radioactivity arises from the scintillator phase. Since the counting is proportional to the amount of ^{3}H-I in the scintillator phase, Eq. 1 may be rewritten as:

$$\ln (m_{II_\infty} - m_{II}) = \ln m_{II_\infty} - kt \qquad \text{(Eq. 2)}$$

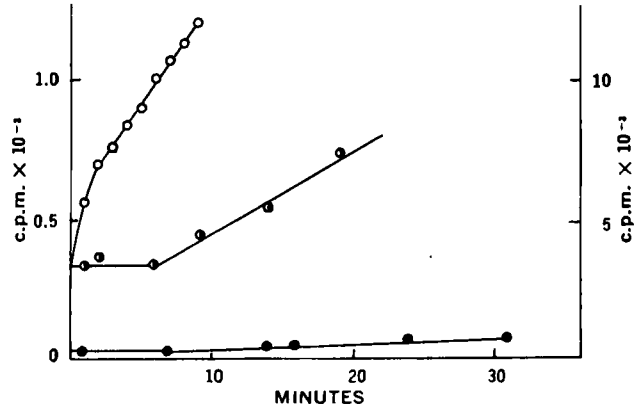

Figure 3—*Time course of partitioning of I during initial period, with II present in scintillator phase. Initial concentrations of I in the aqueous phase were: 1.6 × 10^{-5} M* (○), *1.8 × 10^{-6} M* (◑), *and 1.8 × 10^{-7} M* (●).

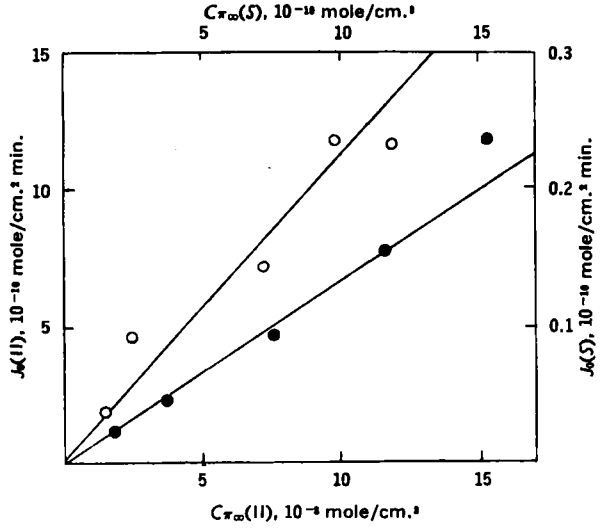

Figure 4—*Initial rate of permeation* versus *equilibrium concentration of I in the scintillator phase in the presence* (○) *and in the absence* (●) *of II.*

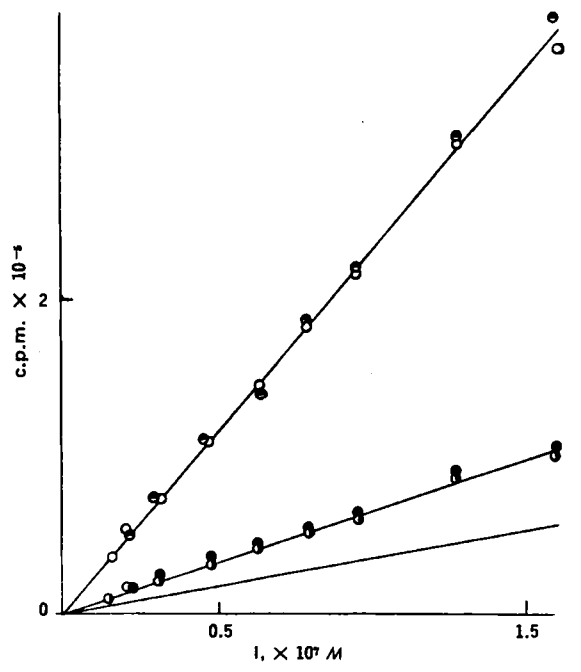

Figure 5—*Partitioning of I between scintillator and aqueous phases in the presence or the absence of II. Key:* ○ *and* ◔, *activity in presence of II in 5 ml. scintillator phase and 5 ml. scintillator + 10 ml. aqueous phase, respectively; and* ● *and* ◑, *same as above but in absence of II. Lower curve refers to the calibration by the standardization procedure described in text.*

where m_{II} denotes the amount of I present in the scintillator phase at a time t, and m_{II_∞} is the equilibrium value.

When t is small, the counting is linear with time after the first 3–7 min. (Fig. 3). At low values of t, m_{II} is also very small as compared with m_{II_∞}, so Eq. 2 may be reduced to:

$$m_{II} = m_{II_\infty} kt \qquad \text{(Eq. 3a)}$$

or:

$$C_{II} = C_{II_\infty} kt \qquad \text{(Eq. 3b)}$$

where C_{II} and C_{II_∞} are the concentrations at a time t and infinity; respectively. By differentiating Eq. 3*a* with respect to t and dividing by the interfacial area A:

$$j_0 = \frac{1}{A}\frac{dm_{II}}{dt} = \frac{m_{II_\infty}}{A} k_0 = \frac{V_{II}}{A} C_{II_\infty} k_0 \qquad \text{(Eq. 4)}$$

where j_0 and k_0 denote the rate of partitioning per unit area and the rate constant, respectively, at the small values of t; and V_{II} is the volume of scintillator phase. Values for j_0 and C_{II_∞} were determined for various concentrations of I, and the results (Fig. 4) verified the relation given by Eq. 4.

According to a previous study (4), the permeation of an ion through a membrane is expressed as:

$$j = P[C_I e^{-(ZFV+\eta)/2RT} - C_{II} e^{(ZFV+\eta)/2RT}] \qquad \text{(Eq. 5)}$$

where j denotes the steady ion flux; P is the membrane permeability; C_I and C_{II} are the concentrations of diffusible ion in phases I and II, respectively, which are separated by a membrane; Z is the charge of the ion; F is the Faraday constant; V is the transmembrane potential; η is the free energy of partition between phases I and II; R is the gas constant; and T is absolute temperature. For nonionic species, $Z = 0$ and Eq. 5 reduces to:

$$j = P[C_I e^{-(\eta/2RT)} - C_{II} e^{(\eta/2RT)}] \qquad \text{(Eq. 6)}$$

Table I—Rate Constant in Permeation

	With II	Without II	Ratio
$\tau = k^{-1}$	150 min.	120 min.	1.25
Partition constant	$K_2 = 2.89$	$K_1 = 1.12 \times 10^{-2}$	258
P	9.33×10^{-5}/cm./sec.	2.25×10^{-5}/cm./sec.	3.66
k_1	1.59×10^{-4}/cm./sec.	2.70×10^{-6}/cm./sec.	50.9
k_2	5.50×10^{-5}/cm./sec.	2.41×10^{-4}/cm./sec.	0.23

Equation 6 may also be applied to the permeation process through an oil–water interface, since the interface can be regarded as the boundary separating the aqueous (I) from the scintillator (II) phase.

Since the permeation of I from the aqueous to scintillator phase was observed in the present study, it follows that:

$$V_I C_I + V_{II} C_{II} = V_I C_0 \qquad \text{(Eq. 7)}$$

where V_I and V_{II} denote the volumes of the aqueous and scintillator phases, respectively; and C_0 is the initial concentration in the aqueous phase. When the equilibrium partition is attained, one has from Eq. 6:

$$C_{II_\infty}/C_{I_\infty} = e^{-\eta/RT} = K \qquad \text{(Eq. 8)}$$

where K denotes the partition constant, and the subscript ∞ refers to the equilibrium value. Combining Eqs. 6–8, one finds:

$$j = P(e^{-\eta/2RT} + e^{\eta/2RT})(C_{II_\infty} - C_{II}) \qquad \text{(Eq. 9)}$$

provided $V_I = V_{II} = 10$ ml. (see *Appendix*).

Since the total flux, J, is given by j multiplied by the area of the interface, A, one has from Eqs. 4 and 9:

$$J = Aj = \frac{dm_{II}}{dt} = k(m_{II_\infty} - m_{II}) \qquad \text{(Eq. 10)}$$

where:

$$k = AP(e^{\eta/2RT} + e^{-\eta/2RT})/V_{II} \qquad \text{(Eq. 11a)}$$

$$m_{II} = C_{II} V_{II} \qquad \text{(Eq. 11b)}$$

$$m_{II_\infty} = C_{II_\infty} V_{II} \qquad \text{(Eq. 11c)}$$

Integrating Eq. 10 with respect to time, Eq. 2 is obtained, which may be reduced to Eq. 4 when t is small. The values of the time constant, τ, or the reciprocal of the rate constant in the presence or absence of II in the scintillator phase are given in Table I.

After partition equilibrium was attained, 5 ml. of scintillator solution was transferred to another vial, and the radioactivity was measured in both vials (Fig. 5). It was found that the I in the scin-

tillator phase was homogeneously distributed, while the radiation from the aqueous part was within experimental error and, hence, was disregarded. The lower curve refers to the calibration by the standardization procedure described in the *Experimental* section. The standardization procedure, by permitting the determination of the concentrations of I in the aqueous and scintillator phases, makes it possible to calculate the partition constant.

Equation 6 may also be written as:

$$j = k_1 C_I - k_2 C_{II} \qquad \text{(Eq. 12)}$$

where:

$$k_1 = Pe^{-\eta/2RT} \qquad \text{(Eq. 13}a\text{)}$$

$$k_2 = Pe^{\eta/2RT} \qquad \text{(Eq. 13}b\text{)}$$

The values of P, k_1, and k_2 may be evaluated according to Eqs. 1 and 11, provided the partition constant, the volume of scintillator phase, and the area of interface are known. By assuming the interface to be spherical and measuring the meniscus height of the interface and the inner diameter of the vial, the interfacial area was found to be 5.2 cm.^{2}. The values of P, k_1, and k_2 are given in Table I, together with the time constant and the partition constant. Also given are the ratios of these values both in the presence and absence of II.

According to Eq. 4, the time constant for initial sorption, τ_0, can also be estimated from Fig. 4. The estimated values of time constant τ_0 were near to those of τ estimated from the whole curves of the time course, as indicated in Table I. The turbulence of the system involved in the initial stage of sorption would be responsible for the slight deviation of τ_0 from τ.

As seen in Table I, the partition constant K_2 is much greater than K_1, a finding suggesting the formation of a II salt of I. The formation constant, K_s, of the salt in the scintillator phase can be expressed by:

$$K_s = \frac{C_{II_\infty}^{salt}}{C_D \cdot C_{II_\infty}} \qquad \text{(Eq. 14)}$$

where $C_{II_\infty}^{salt}$ and C_{II_∞} denote the concentrations of salt and free I, respectively; and C_D is the concentration of free II.

Since the volumes of aqueous and scintillator phases are identical, the following relations hold:

$$C_{I_\infty} = C_I^0 - (C_{II_\infty} + C_{II_\infty}^{salt}) \qquad \text{(Eq. 15)}$$

$$C_{II_\infty}^{salt} = (C_{II_\infty}^{salt} + C_{II_\infty}) - C_{II_\infty} \qquad \text{(Eq. 16)}$$

and:

$$C_D = C_D^0 - C_{II_\infty}^{salt} \qquad \text{(Eq. 17)}$$

where C_I^0 and C_D^0 are the initial concentrations of I in the aqueous phase and those of II in the scintillator phase, respectively. In Eq. 15, $(C_{II_\infty} + C_{II_\infty}^{salt})$ indicates the total concentration of I in the scintillator phase that may be estimated from the radiation intensity. The concentration of free I in the scintillator phase, C_{II_∞}, may be determined from the relation:

$$K_1 = C_{II_\infty}/C_{I_\infty} \qquad \text{(Eq. 18)}$$

where K_1 is the partition constant for free I. The value of K_1 in the absence of II was found to be $(1.12 \pm 0.02) \times 10^{-2}$. Since the presence of II does not alter K_1, $C_{II_\infty}^{salt}$ can be determined according to Eqs. 16 and 18 and C_D can be determined according to Eq. 17. The values of K_s calculated according to Eq. 14 by using the data at various concentrations of I were found to be a constant within experimental error, $K_s = (5.7 \pm 0.4) \times 10^5\ M^{-1}$ (Table II). This verifies Eq. 4, which suggests the formation of a 1:1 salt between the cation of I and anion of II.

Since K_2, the partition constant in the presence of II, is given by:

$$K_2 = \frac{C_{II_\infty} + C_{II_\infty}^{salt}}{C_{I_\infty}} \qquad \text{(Eq. 19)}$$

combining this equation with Eqs. 14–19 (see *Appendix*) gives:

$$K_2 = K_1 + \frac{K_s K_1 C_D^0}{1 + K_s K_1 C_{I_\infty}} \qquad \text{(Eq. 20)}$$

When $K_s K_1 C_{I_\infty}$ is much less than unity, K_2 may be reduced to:

$$K_2 \simeq K_s K_1 C_D^0 \qquad \text{(Eq. 21)}$$

provided K_1 is much less than K_2. Since the observed value of K_2 (2.89 ± 0.25) is much greater than K_1 and $K_s K_1 C_{I_\infty}^0$ is less than unity, the approximate value of K_2 may be evaluated according to Eq. 21. A value of 3.02 is obtained, which is close to the observed value, 2.89.

The partition of I between the $(S + T)$ and the aqueous phase at pH 7.4 was also measured and the partition coefficient was found to be 9.6 ± 1.1 (Table III). The pH dependence of I may be attributed to the dissociation of I ion in aqueous phase, *i.e.*:

$$K_a = \frac{[H^+]_W [PMCG]_W}{[PMCG^+]_W} \qquad \text{(Eq. 22)}$$

where the subscript W refers to the aqueous phase, and PMCG = I.

Since the PMCG exists in the form of $PMCG^+$ and PMCG, one has from Eq. 22:

$$\frac{[PMCG]_{TW}}{[PMCG]_W} = \frac{[PMCG^+]_W + [PMCG]_W}{[PMCG]_W} = \frac{[H]_W}{K_a} + 1 \qquad \text{(Eq. 23)}$$

where $[PMCG]_{TW}$ is the total concentration of I in the aqueous phase. On the other hand, the partition coefficient of the neutral I molecule between the $(S + T)$ and the aqueous phase is defined as:

$$K_{0W} = \frac{[PMCG]_0}{[PMCG]_W} \qquad \text{(Eq. 24)}$$

Since the concentrations of I in the $(S + T)$ phase, $[PMCG]_0$, and in the aqueous phase are equivalent to C_{II_∞} and C_{I_∞} of Eq. 18, respectively:

$$K_1 = \frac{[PMCG]_0}{[PMCG]_{TW}} \qquad \text{(Eq. 25)}$$

and, hence:

$$K_{0W} = K_1\left(\frac{[H]_W}{K_a} + 1\right) \qquad \text{(Eq. 26)}$$

Table II—Constants in Partition Phenomenon[a]

Concentration					10^5
$10^{-5}\ M$					M^{-1}
C_I^0	C_{I_∞}	$C_{II_\infty}^{salt}$	C_D	C_{II_∞}	K_s
16	4.7	11.25	38.75	0.052	5.6
12.8	3.1	9.64	40.36	0.035	6.8
9.6	2.6	6.97	43.03	0.029	5.6
8.0	1.5	5.48	43.52	0.017	7.4
6.4	1.9	4.46	45.54	0.021	4.7
4.8	1.54	3.24	46.76	0.017	4.1
3.2	0.78	2.41	47.59	0.009	5.7
2.4	0.59	1.80	48.20	0.007	5.4
		Mean $K_s = (5.7 \pm 0.4) \times 10^5\ M^{-1}$			

[a] $K_1 = (1.12 \pm 0.02) \times 10^{-2}$
$K_2 = 2.89 \pm 0.24$
$K_s = (5.7 \pm 0.4) \times 10^5\ M^{-1}$
$K_2 \doteqdot K_s K_1 C_D^0 = 3.02$
Formation constant, K_s, of salt.
$(C_D^0 = 50 \times 10^{-5}\ M)$

Table III—Partition Coefficient of I

	pH	K_1
1.8 *M* I	7.4	8.0
1.8 *M* I (hyaluronic acid)[a]	7.4	8.0
9.6 I	7.4	8.0
Desorption experiment 1	7.4	13.0
Desorption experiment 2	7.4	11.0
Mean		9.6 1.1

[a] The hyaluronic acid (0.01%) was present in the aqueous phase.

Table IV—Partition Coefficients and pKa of I Ion

pH	2	7.4
K_1	1.12×10^{-2}	9.6
K_a	10^{-5}	
pKa (calc.)	5	
pKa (obs.)	5.3 ± 0.2	
K_{ow}	10	

The stability constant of I ion in aqueous solution may be estimated from the measurements of the partition coefficients of I at various pH's since K_{ow} is a constant. The value of pKa calculated from K_1's at pH 2 and 7.4 was found to be 5, in agreement with 5.3 ± 0.2 which was determined by pH titration. K_{ow} which could be determined from Eq. 26, provided K_1, K_a, and pH were known, was found to be 10 (Table IV).

In investigating the effect of II on the permeation rate of I at pH 7.4, it was found that the rate of permeation was not influenced by the presence of II in the scintillator phase (Fig. 6). At pH 7.4, virtually all of the I in the aqueous phase would be in the neutral form, so that no ionic interaction with II would occur.

Finally, the transfer of I from the scintillator to the aqueous phase was examined under various conditions (Fig. 7). At pH 7.4, the transfer of I was negligible, indicating the high partition of I in the scintillator phase at this pH. On the other hand, the presence of a strong polyanion, such as the hyaluronic acid, in the aqueous phase at pH 2 enhanced the transfer rate of I from the scintillator to the aqueous phase with or without II in the scintillator phase.

In living cells, the membrane as well as the cell interior possesses components exhibiting a high affinity to diffusible substances such as inorganic ions, metabolites, and drugs. Interfacial techniques have proven to be effective tools for examining such phenomena and, thereby, have contributed to the development of an effective experimental model of a biological membrane. The adaptation of liquid scintillation to the measurement of oil–water partitioning and interfacial adsorption should extend the usefulness of the radiotracer technique for measuring surface adsorption.

APPENDIX

Derivation of Eq. 9—Combining Eq. 6 with Eq. 7, one derives:

$$j = P[C_{I}e^{-\eta/2RT} - C_{II}e^{\eta/2RT}]$$

$$= P[(C_0 - C_{II})e^{-\eta/2RT} - C_{II}e^{\eta/2RT}]$$

$$= P[C_0e^{-\eta 2RT} - C_{II}(e^{\eta/2RT} + e^{-\eta/2RT})]$$

$$= P(e^{\eta/2RT} + e^{-\eta/2RT})\left[\frac{C_0e^{-\eta/2RT}}{e^{\eta/2RT} + e^{-\eta/2RT}} - C_{II}\right] \quad \text{(Eq. A1)}$$

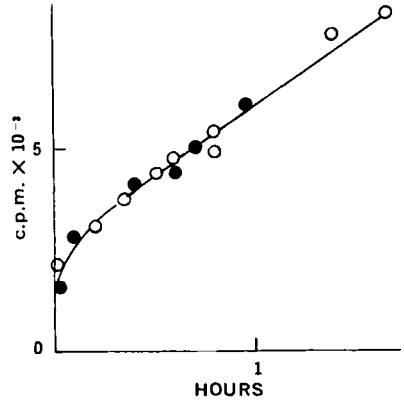

Figure 6—*Effect of II on the permeation rate of I, pH 7.4. Concentration of I was 1.8 × 10⁻⁶ M in the presence* (○) *and in the absence* (●) *of II.*

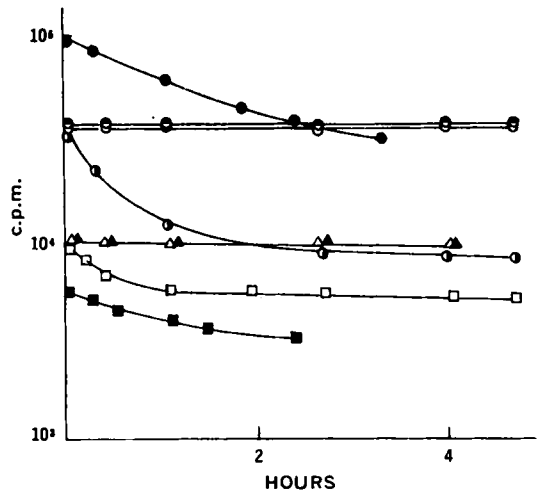

Figure 7—*Effects of adding various substances to scintillator and aqueous phases on the partition rate of I from scintillator phase to aqueous phase. Key:* ●, *in the presence of II alone, pH 2;* ○ *and* ◓, *in the presence of II at pH 7.4 with and without 0.01% hyaluronic acid (III), respectively;* ◑ *in the presence of II and 0.01% III, pH 2;* △ *and* ▲, *absence of II at pH 7.4 with and without 0.01% III, respectively; and* □ *and* ■, *absence of II at pH 2 with and without 0.01% III, respectively. III dissolved in aqueous phase and II dissolved in scintillator phase.*

From Eq. 8:

$$\frac{C_{I_\infty}}{C_{II_\infty}} + 1 = e^{\eta/RT} + 1 \quad \text{(Eq. A2)}$$

Since $V_I = V_{II}$, one has:

$$C_I + C_{II} = C_{I_\infty} + C_{II_\infty} = C_0 \quad \text{(Eq. A3)}$$

and combining Eq. A2 with Eq. A3:

$$C_{II_\infty} = \frac{C_0}{e^{\eta/RT} + 1} = \frac{C_0e^{-\eta/2RT}}{e^{\eta/2RT} + e^{-\eta/2RT}} \quad \text{(Eq. A4)}$$

Comparing Eq. A1 with Eq. A4, one obtains Eq. 9.

Derivation of Eq. 20—From Eq. 14:

$$C_{II\ salt} = K_aC_{II_\infty}C_D \quad \text{(Eq. A5)}$$

Combining Eqs. 14 and 17 and substituting in Eq. A5, one obtains:

$$C_D^0 - C_D = K_aC_{II_\infty}C_D \quad \text{(Eq. A6}a\text{)}$$

$$C_D = \frac{C_D^0}{K_aC_{II_\infty} + 1} \quad \text{(Eq. A6}b\text{)}$$

$$C_{II\ salt} = \frac{K_aC_{II_\infty}C_D^0}{K_aC_{II_\infty} + 1} \quad \text{(Eq. A6}c\text{)}$$

From Eq. 18, $C_{II_\infty} = K_1C_{I_\infty}$. Substituting Eqs. A5 and A6*c* in Eq. 19, one obtains:

$$C_{I_\infty}K_2 = K_1C_{I_\infty} + \frac{K_aK_1C_{I_\infty}C_D^0}{K_aK_1C_{I_\infty} + 1} \quad \text{(Eq. A7)}$$

Finally:

$$K_2 = K_1 + \frac{K_aK_1C_D^0}{1 + K_aK_1C_{I_\infty}} \quad \text{(Eq. A8)}$$

which is Eq. 20.

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Action of Ultrasonicated Epinephrine: Potentiation of Isometric Contraction in Isolated Papillary Muscle from Dog Quiescent Heart

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Abstract □ Epinephrine solutions were exposed to ultrasonic frequencies or to oxidation by passing oxygen through the solution until a pink color was formed. The effects of these epinephrine solutions were investigated on the physiological functions of heart papillary muscles and their contractile element, *i.e.*, actomyosin. When added to the bathing medium containing papillary muscle from beating rat hearts, the modified epinephrine produced a gradual increase in the maximum response of the muscle to electrical stimulation. If the incubating medium contained papillary muscle from quiescent dog heart, the muscle did not respond to electrical stimulation alone or in the presence of epinephrine. However, when added to the bathing medium, the sonified or the oxidized epinephrine caused initiation and potentiation of isometric contractions in response to electrical stimulation. The effects of the altered epinephrines on papillary muscles from both types of hearts were concentration and time dependent. When added with either the sonified or the oxidized epinephrine, a "serum protein" isolated from human serum intensified and stabilized the effects of both forms of epinephrine on the papillary muscle from rat or dog quiescent heart. When added to a standard actomyosin solution in imidazole–potassium chloride–ethyleneglycol tetraacetate salt buffer at pH 7.0, adenosine triphosphate caused precipitation of the contractile protein, which was assessed by the increase in absorbance and was measured spectrophotometrically at 660 nm. When the incubation mixture contained increments of the sonified or the oxidized epinephrine, the precipitating effect of adenosine triphosphate was not changed. But, if added with either modified epinephrine, the serum protein caused two- or fivefold increases in the precipitation of the contractile elements from solutions incubated in the presence of the oxidized or sonified epinephrine, respectively.

Keyphrases □ Epinephrine, ultrasonicated or oxidized—potentiation of isometric contraction in isolated heart papillary muscle □ Isometric contraction in isolated heart papillary muscle—potentiation by ultrasonicated or oxidized epinephrine □ Heart papillary muscle—potentiation of isometric contraction by ultrasonicated or oxidized epinephrine □ Ultrasonicated epinephrine—potentiation of isometric contraction in isolated heart papillary muscle

Epinephrine inotropy in cardiac muscle is characterized by two distinct alterations in the contractile process. In isolated tissue preparations (1) as well as *in situ* (2, 3), administration of epinephrine brings about an increase in systolic force or shortening, with an appreciable reduction in the duration of contraction (4).

In recent years, most studies on cardiac muscle have been performed on papillary muscle from beating cat or rat heart. When bathed or perfused with physiological buffer solution, isolated myocardial preparations spontaneously undergo contractile failure. Clark *et al.* (5) suggested that the addition of serum to the buffer solution stabilized these failing preparations. Addition of various plasma fractions or dialysates also delayed the onset of cardiac decay. This led Gabel *et al.* (6) to hypothesize that fatigue in isolated muscle preparations might also be influenced by the presence or absence of blood fractions. Other investigators (7, 8) showed that cardiac muscle maintained in plain buffer solutions lost the poststimulation potential, but it was restored by serum; they confirmed the presence of cardioactive principles in blood plasma.

The aim of the present studies is to demonstrate the initiation and potentiation of isometric contractions in electrically stimulated dog heart papillary muscle with epinephrine modified by exposure to gaseous cavitation produced by ultrasonic frequencies or by limited oxidation of the amine.

EXPERIMENTAL

Materials—Epinephrine solutions were transformed to a light-pink pigment, metaphrine, by two methods:

1. Solutions (10^{-6} *M*) of epinephrine in physiological saline were exposed to ultrasonic frequencies. A sonic oscillator[1] was employed at 20 w. at the machine maximum output of 10 kc., 0.78 amp. and 60 cycles. The solutions were sonified for 120 min. at 0–4° until the formation of metaphrines, which was assessed by the appearance of a light-pink color (9).

[1] Raytheon, model DF 101.