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Calcium–Prostaglandin Aggregation and Its Effect on Prostaglandin Uptake by Isolated Rabbit Intestine

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Abstract □ A quantitative estimate of the role of calcium ions on the lipid–water partition coefficients of prostaglandin E_1 and dinoprost suggested the possibility of prostaglandin molecules and calcium ions aggregating in a 14:1 ratio to produce a lipid-soluble aggregate. The aggregation is postulated to be a characteristic of prostaglandin molecules as compared to simple fatty acids, *e.g.*, 1-octanoic acid, which, in the presence of calcium, behave differently than the prostaglandins. The uptake of prostaglandins by the mucosal surface of the rabbit intestine increased in the presence of calcium. For example, at 25 m*M* calcium, prostaglandin E_1 was transported at approximately twice the rate as in the system containing no calcium. The uptake rate of dinoprost was estimated to be three times faster with 10 m*M* calcium than in the absence of calcium. Therefore, it is proposed that a carrier-mediated diffusion process, for both the prostaglandin molecules and calcium ions, takes place in the uptake mechanism. Diffusion coefficients ranging from 0.48×10^{-5} to 7.19×10^{-5} cm²/sec and permeability coefficients ranging from 1.04×10^{-2} to 15.6×10^{-2} cm/sec were estimated for all systems studied.

Keyphrases □ Prostaglandin E_1—aggregation with calcium ions, effect on lipid–water partition coefficients and uptake by isolated rabbit intestine □ Dinoprost—aggregation with calcium ions, effect on lipid–water partition coefficients and uptake by isolated rabbit intestine □ Calcium ions—aggregation with prostaglandin E_1 and dinoprost, effect on lipid–water partition coefficients and uptake by isolated rabbit intestine □ Partition coefficients, lipid–water—prostaglandin E_1 and dinoprost, effect of aggregation with calcium ions □ Intestinal uptake, isolated—prostaglandin E_1 and dinoprost, effect of aggregation with calcium ions

Recent studies on the physical, physiological, and biochemical relationships between prostaglandin and calcium dealt with the cellular distribution of prostaglandins (1), the prostaglandin and cellular calcium interaction (2–5), and the effect of prostaglandin on the GI tract, including its influence on glucose, water, and electrolyte absorption (6–8). Very few studies attempted to explain the physical interaction between calcium and prostaglandins (9, 10).

The present study attempts to explain the nature of this interaction and to correlate the data to prostaglandin uptake by the intestinal mucosa of the rabbit.

EXPERIMENTAL

Partition Coefficient—The *n*-octane[1]–water, 1-octanol[2]–water, and 1-octanoic acid[3]–water partition coefficients of 5,6(n)-^{3}H-prostaglandin E_1[4] (I) and 5,6,8,11,12,14,15(n)-^{3}H-dinoprost (II) were measured as follows. In 10-ml screw-capped test tubes, 2.0 ml of the oil phase and 2.0 ml of a pH 5 aqueous phase containing the desired amount of I (24.4×10^{-9} m*M*), II (2.88×10^{-9} m*M*), and calcium ions (2.5, 5.0, 10.0, 25.0, 40.0, 50.0, 75.0, 100.0, 150.0, or 200.0 m*M*) were added. Prior to equilibration, nitrogen gas was cautiously bubbled into each test tube to ensure the displacement of air from the upper void spaces. The test tubes were then tightly capped and attached with rubber bands to the shaft of a rotating-bottle disintegration apparatus[5].

The apparatus with the test tubes was put in a stainless steel tank filled with water thermostatically controlled at 25°. The motor was allowed to rotate, mixing the two phases in the test tubes for 30 min. After equilibration, each test tube was detached from the apparatus and allowed to stand for 15 min at 25° for complete phase separation. Then 1.0 ml of each oil and aqueous phase was individually pipetted and assayed for I and II in a three-channel liquid scintillation spectrometer[6]. The calculated partition coefficients were the averages of four samples for each oil–water system.

The same procedure was followed for the estimation of the partition coefficient while varying the concentration of I and II and keeping the concentration of calcium ions constant (5 or 50 m*M*).

Partition Coefficient of 1-Octanoic Acid—Since prostaglandins are considered to be fatty acids, it was essential to compare their partition behavior with that of a fatty acid such as 1-octanoic acid in the presence of calcium ions. A potentiometric method was used for the quantitative analysis of 1-octanoic acid in the aqueous phase. A standard aqueous 1-octanoic acid solution (32.0 m*M*) was prepared and potentiometrically[7] titrated with 0.01 *N* sodium hydroxide. The standard solution was employed as the aqueous phase in the partition experiments after the addition of the desired amount of calcium ions (0, 100, or 200 m*M*). The oil phase chosen was 1-octanol.

Thus, for a single calcium-ion concentration system, two sets of 250-ml separators were prepared. For the 0 m*M* calcium system (A and A′),

[1] Eastman Kodak Co., Rochester, N.Y.
[2] Riedel-De Haen AG, Seelze-Hannover, West Germany.
[3] Hopkin and Williams Ltd., Chadwell Heath, Essex, England.
[4] Radiochemical Center, Amersham, Buckinghamshire, England.
[5] Ernest D. Menold, Lester, Pa.
[6] Packard Tri-Carb model 3320, Packard Instrument Co., Downers Grove, Ill.
[7] Radiometer, pH meter model 22, Copenhagen, Denmark.

Table I—Effect of Varying Calcium Concentration on the Apparent Partition Coefficient[a] (k') of I in *n*-Octane–Water, 1-Octanol–Water, and 1-Octanoic Acid–Water Systems

Calcium-Ion Concentration, mM[b]	$k' \times 10^{-2}$, *n*-Octane–Water	k', 1-Octanol–Water	k', 1-Octanoic Acid–Water
0	3.20[c]	59.68[c]	17.67[c]
2.5	3.04	66.12	17.50
10.0	3.07	69.34	19.70
25.0	3.20	74.25	19.29
40.0	3.39	80.14	21.38
50.0	3.48	86.53	20.75
75.0	3.75	85.92	22.39
100.0	3.96	88.97	23.92
150.0	—	91.85	24.70
200.0	4.05	98.61	26.40

[a] The k' values are averages of at least four samples. [b] At pH 5. [c] The k' values at 0 mM calcium are values for k, the true partition coefficient.

separator A contained 200 ml of 32 mM 1-octanoic acid as the aqueous phase, while separator A′ contained 200 ml of double-distilled water as the aqueous phase. To sets A and A′, 0.5 ml of 1-octanol as the oil phase was added. For the 100 mM calcium system (B and B′), separator B contained 200 ml of 32 mM 1-octanoic acid with 100 mM calcium ions in the aqueous phase, while B′ contained only 200 ml of 100 mM calcium ions as the aqueous phase. A 0.5-ml aliquot of 1-octanol was added to sets B and B′ as the oil phase. For the 200 mM calcium system (C and C′), separator C contained 200 ml of 32 mM 1-octanoic acid with 200 mM calcium ions in the aqueous phase, while C′ contained only 200 ml of 200 mM calcium as the aqueous phase. A 0.5-ml aliquot of 1-octanol was added to sets C and C′ as the oil phase.

The aqueous phases in all sets were adjusted to pH 5 before equilibration. The separators were shaken after attachment to the rotating-bottle disintegration apparatus for 30 min at 25°. After equilibration was completed, 100 ml of the aqueous phase was filtered through analytical filter paper. The filtrate was potentiometrically assayed for 1-octanoic acid left in the aqueous phase. The volume of 0.01 N sodium hydroxide consumed by sets A′, B′, and C′ was subtracted as controls from the volume consumed by sets A, B, and C, respectively. If the difference in volume of base consumed was not altered, calcium ions had no effect on the partition behavior of 1-octanoic acid between the 1-octanol and the water phase.

Uptake of I and II by Isolated Rabbit Intestine—The treatment of animals, apparatus, and sampling and assay of samples were described previously (11). Ringer's solution was not employed in the present study. Instead, four calcium chloride solutions (0, 10, 25, and 50 mM) were prepared and made isotonic with sodium chloride. The pH of all solutions was adjusted to 5. Into these isotonic solutions, the desired amount of I (24.4×10^{-9} mM) or II (2.88×10^{-9} mM) was added.

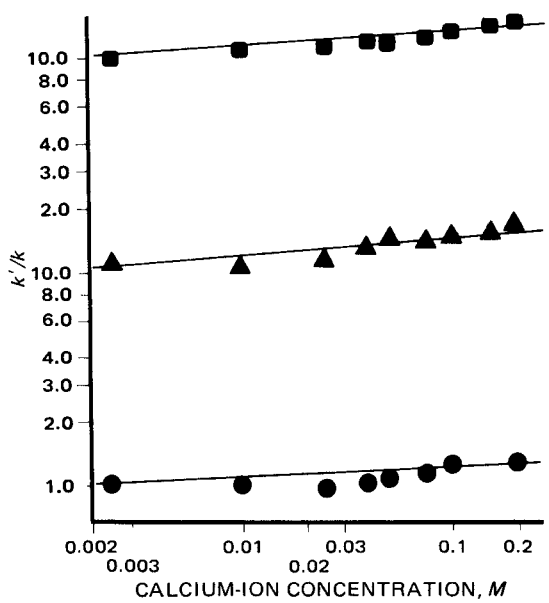

Figure 1—*Least-squares line plot of log* k′/k versus *log calcium concentration as applied to Eq. 8 for I. Key:* ●, n-*octane–water;* ▲, *1-octanol–water; and* ■, *1-octanoic acid–water.*

Table II—Effect of Varying Calcium Concentration on the Apparent Partition Coefficient[a] (k') of II in *n*-Octane–Water, 1-Octanol–Water, and 1-Octanoic Acid–Water Systems

Calcium-Ion Concentration, mM[b]	$k' \times 10^{-2}$, *n*-Octane–Water	k', 1-Octanol–Water	k', 1-Octanoic Acid–Water
0	1.86[c]	125.25[c]	37.40[c]
2.5	2.02	130.00	36.70
10.0	2.08	134.50	39.40
25.0	2.05	138.50	45.90
40.0	2.22	136.00	45.00
50.0	2.37	146.75	47.60
75.0	2.52	161.75	51.30
100.0	3.12	169.00	53.40
150.0	3.43	178.50	57.00
200.0	3.63	189.25	60.20

[a] The k' values are averages of at least four samples. [b] At pH 5. [c] The k' values at 0 mM calcium are values of k, the true partition coefficient.

Calculations—*Partition Coefficient Studies*—The true partition coefficient, k, is defined as:

$$k = \frac{[PG]_{TO}}{[PG]_{TW}} \qquad \text{(Eq. 1)}$$

where $[PG]_{TO}$ represents the total concentration of prostaglandin in the oil phase, and $[PG]_{TW}$ is the total concentration of prostaglandin in the water phase at equilibrium. At pH 5 [the pKa of prostaglandins is ~6.3 (12)] and at 0 mM calcium-ion concentration, Eq. 1 completely applies. But in the presence of calcium ions in the aqueous phase and at pH 5, it is assumed that the total prostaglandin concentration in the aqueous phase is:

$$[PG]_{TW} = [PG]_{FW} + [PG(Ca^{2+})_n]_W \qquad \text{(Eq. 2)}$$

where $[PG]_{FW}$ is the concentration of the free prostaglandin in equilibrium with the prostaglandin–calcium complex, $[PG(Ca^{2+})_n]_W$, in the aqueous phase. Therefore, the apparent partition coefficient, k', can be defined as:

$$k' = \frac{[PG]_{TO}}{[PG]_{FW} + [PG(Ca^{2+})_n]_W} \qquad \text{(Eq. 3)}$$

If it is assumed that the reaction mechanism of the formation of the complex, $[PG(Ca^{2+})_n]_W$, is:

$$PG + nCa^{2+} \rightleftharpoons PG(Ca^{2+})_n$$

then:

$$K_d = \frac{[PG]_{FW}[Ca^{2+}]_W{}^n}{[PG(Ca^{2+})_n]_W} \qquad \text{(Eq. 4)}$$

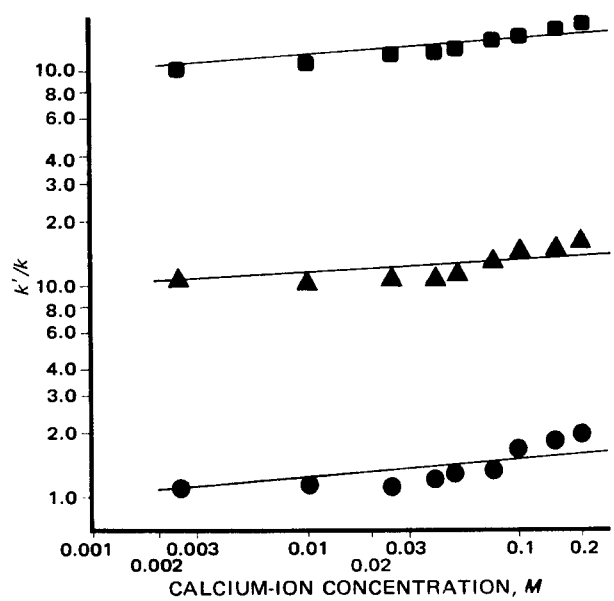

Figure 2—*Least-squares line plot of log* k′/k versus *log calcium concentration as applied to Eq. 8 for II. Key:* ●, n-*octane–water;* ▲, *1-octanol–water; and* ■, *1-octanoic acid–water.*

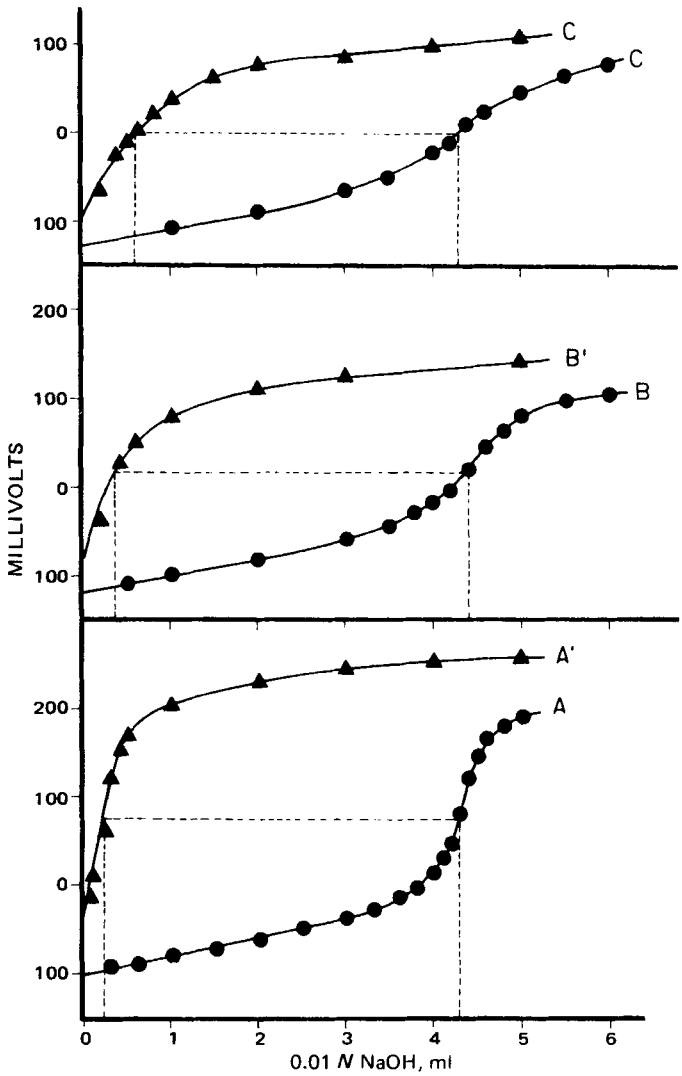

Figure 3—*Potentiometric titration plot of millivolts* versus *milliliters of 0.01* N *sodium hydroxide for 1-octanoic acid in the aqueous phase left after partitioning. Key: A and A' represent the 0 m*M *calcium system, and the volume of base consumed was 4.10 ml; B and B' represent the 100 m*M *calcium system, and the volume of base consumed was 4.05 ml; and C and C' represent the 200 m*M *calcium system, and the volume of base consumed was 3.70 ml.*

where K_d is the dissociation constant of the complex. Rearranging Eq. 4 and substituting for $[PG]_{FW}$ into Eq. 3 would result in:

$$k' = \frac{[PG]_{TO}[Ca^{2+}]_W{}^n}{([PG(Ca^{2+})_n]_W)(K_d + [Ca^{2+}]_W{}^n)} \quad \text{(Eq. 5)}$$

Eagling *et al.* (10) reported K_d values in the range of 24 ± 8; since $[Ca^{2+}]$ is in millimoles per liter, one can assume that $K_d > [Ca^{2+}]_W{}^n$. Therefore, Eq. 5 is reduced to:

$$k' = \frac{[PG]_{TO}[Ca^{2+}]_W{}^n}{([PG(Ca^{2+})_n]_W)(K_d)} \quad \text{(Eq. 6)}$$

Dividing the numerator and the denominator of Eq. 6 by $[PG]_{TW}$, substituting with Eq. 1, and rearranging yield:

$$\frac{k'}{k} = \frac{[Ca^{2+}]_W{}^n}{\frac{[PG(Ca^{2+})_n]_W}{[PG]_{TW}} K_d} \quad \text{(Eq. 7)}$$

Taking the logarithm of both sides of Eq. 7, one gets:

$$\log \frac{k'}{k} = n \log[Ca^{2+}]_W - \log \frac{[PG(Ca^{2+})_n]_W}{[PG]_{TW}} K_d \quad \text{(Eq. 8)}$$

From Eq. 8, the plot of $\log k'/k$ *versus* $\log [Ca^{2+}]_W$ should be linear with a slope equal to n.

Intestinal Uptake Studies—The apparatus and the mathematical model utilized were as reported previously (11). The following relationships describe the situation for the uptake rate:

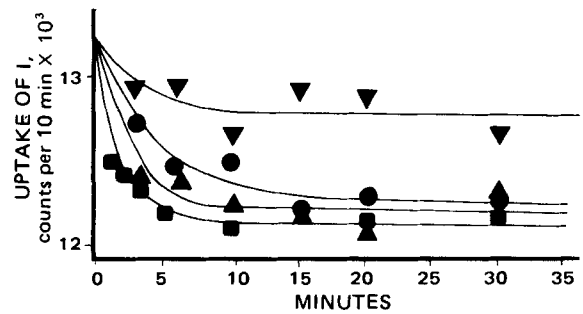

Figure 4—*Plot of* ^{3}*H-labeled I in counts per 10 min left in solution* versus *time in minutes. Key:* ●, *0 m*M *calcium;* ■, *10 m*M *calcium;* ▲, *25 m*M *calcium; and* ▼, *50 m*M *calcium.*

$$\frac{d[PG]_{TW}}{dt} = -\frac{D_W A}{h V_W}[PG]_{TW} \quad \text{(Eq. 9)}$$

Separating variables and integrating yield:

$$\ln [PG]_{TW} = -\frac{D_W A}{h V_W} t + \text{constant} \quad \text{(Eq. 10)}$$

where D_W represents the aqueous solute diffusion coefficient, A is the total surface area of exposed mucosal surface, h is the diffusion layer thickness, V_W is the aqueous bulk volume, and t is time. Therefore, when the partition coefficient, k', of the solute is relatively large, the data are expected to deviate from linearity at large t. Consequently, a semilogarithmic plot of $[PG]_{TW}$ *versus* t yields a straight line at the initial data points whose slope $[-(D_W A/hV_W)]$ is the initial uptake rate constant. Values of h, V_W, and A were experimentally measured and reported (11). The value of D_W can be calculated from measurement of the initial rate. The permeability coefficient, $P = D_W/h$, is also calculated.

RESULTS

Partition Coefficient of I in Oil–Water Systems—Table I shows the true (k at 0 mM calcium ions) and the apparent (k') partition coefficients calculated for I in n-octane–water, 1-octanol–water, and 1-octanoic acid–water systems. When Eq. 8 was applied to the data of Table I, Fig. 1 was obtained. The best straight lines through all points were drawn utilizing the method of least squares. The best least-squares line fits shown in Fig. 1 gave average slopes of approximately 0.07. Thus, the value of n in Eq. 8 is 0.07. Therefore, for every 14 molecules of I, one ion of calcium is needed to produce the complex.

Partition Coefficient of II in Oil–Water Systems—The true (k at 0 mM calcium ions) and apparent (k') partition coefficients of II are reported in Table II. Figure 2 represents a plot of $\log k'/k$ *versus* $\log [Ca^{2+}]$ of the data in Table II. The average slope of the least-squares lines in Fig. 2 was calculated to be around 0.07 and, therefore, is equal to n in Eq. 8. Thus, 14 molecules of II interact with one ion of calcium to produce the complex.

Effect of Calcium Ions on Partitioning of 1-Octanoic Acid in 1-Octanol–Water System—Figure 3 represents the data of the potentiometric titration of 1-octanoic acid with 0.01 N sodium hydroxide. For the 0 mM calcium system, 4.10 ml of 0.01 N sodium hydroxide was consumed for titrating the 1-octanoic acid remaining in the aqueous phase

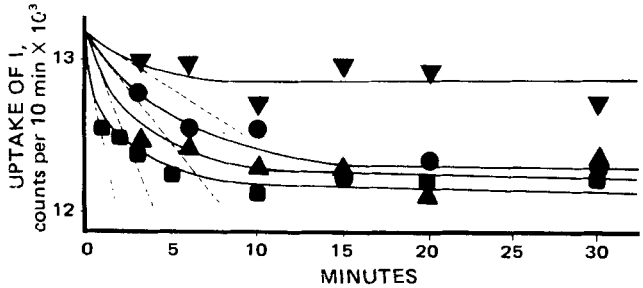

Figure 5—*Semilogarithmic plot of* ^{3}*H-labeled I in counts per 10 min left in solution* versus *time in minutes. Key:* ●, *0 m*M *calcium;* ■, *10 m*M *calcium;* ▲, *25 m*M *calcium; and* ▼, *50 m*M *calcium. The dotted lines join the early experimental data points. Their slopes were employed in the treatment of the data.*

Table III—Estimated Initial Rates and Relative Initial Rates of I and II into Rabbit Intestine as a Function of Calcium-Ion Concentration

Calcium-Ion Concentration, mM[a]	Initial Rate Constant[b], min^{-1}	Relative Initial Rate Constant[c]
	Compound I	
0	0.0163	1.00
10.0	0.0492	3.00
25.0	0.0242	1.48
50.0	0.0064	0.40
	Compound II	
0	0.0395	1.00
10.0	0.0552	1.40
25.0	0.0948	2.40
50.0	0.0208	0.525

[a] At pH 5. [b] Calculated from the first few experimental points of the semilogarithmic plot. [c] Calculated by dividing the initial rate in the presence of calcium ions by the initial rate at 0 mM calcium.

after equilibration. The volumes of base consumed for the 100 and 200 mM calcium systems were 4.05 and 3.70 ml of 0.01 N sodium hydroxide, respectively. It was concluded, therefore, that the distribution of 1-octanoic acid between 1-octanol and water was not drastically influenced by calcium ions. For example, 4.10 and 4.05 ml of 0.01 N sodium hydroxide were needed to titrate the remaining 1-octanoic acid in the aqueous phase, after equilibration, for the 0 and 100 mM calcium-ion systems, respectively.

Effect on Partition Coefficient of Varying Prostaglandin Concentration while Keeping Calcium Concentration Constant—There was no observable effect on the apparent partition coefficient when varying the I and II concentrations while keeping the calcium concentration constant.

Uptake of I by Isolated Rabbit Intestine—Figure 4 is a rectangular coordinate plot of I (in counts per 10 min) remaining in solution *versus* time in minutes. Each point on the graph represents at least four experimental runs. Figure 5 represents the corresponding semilogarithmic plot of I concentration in counts per 10 min *versus* time. The dotted lines are extensions of the slopes to the curves through the initial data points.

The calculated initial rate constants from the slopes are reported in Table III. The data indicated an increase in the initial rates at 10 and 25 mM calcium ions as compared to the 0 mM calcium concentration. At 50 mM calcium, the rate decreased to a value smaller than the rate at the 0 mM calcium-ion concentration.

Uptake of II by Isolated Rabbit Intestine—Figures 6 and 7 represent the rectangular and semilogarithmic plots, respectively, of II in counts per 10 min left *versus* time in minutes. The data for II showed an increase in the initial rate constant and relative initial rates at 10 and 25 mM calcium as compared to the 0 mM calcium-ion concentration system (Table III). At 50 mM calcium, the rate decreased to a lower value than in the absence of calcium ions.

DISCUSSION

The results of the partition coefficient studies revealed that I and II interacted with calcium to produce a lipid-soluble complex. As compared with ordinary fatty acids, I and II showed an increase in the oil–water partition coefficient in the presence of calcium ions. The partition coefficient of 1-octanoic acid between oil and water was not altered in the presence of calcium ions. Therefore, the acid (pKa ~6.3) at pH 5 apparently did not favor coordination with the ionic species, thus resulting in

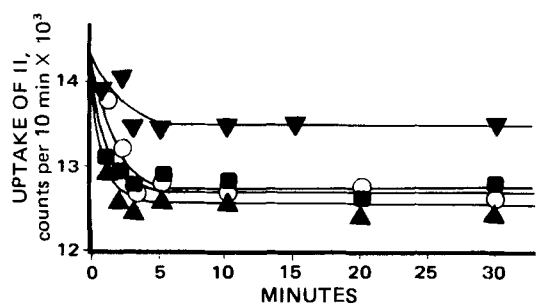

Figure 6—*Plot of ^3H-labeled II in counts per 10 min left in solution* versus *time in minutes. Key:* ○, *0 m*M *calcium;* ■, *10 m*M *calcium;* ▲, *25 m*M *calcium; and* ▼, *50 m*M *calcium.*

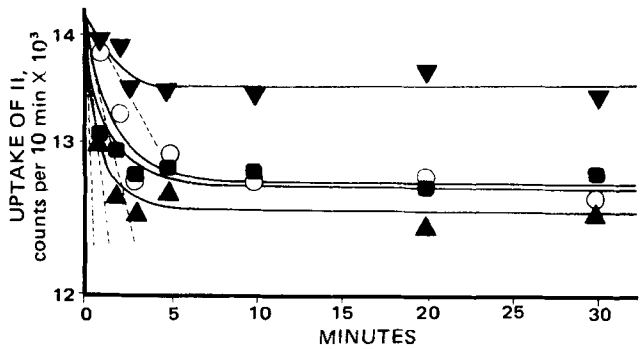

Figure 7—*Semilogarithmic plot of ^3H-labeled II in counts per 10 min left in solution* versus *time in minutes. Key:* ○, *0 m*M *calcium;* ■, *10 m*M *calcium;* ▲, *25 m*M *calcium; and* ▼, *50 m*M *calcium. The dotted lines join the early experimental data points. Their slopes were employed in the treatment of the data.*

an unsubstantial increase in the oil–water partition coefficient. If conditions were identical with I and II, their oil–water partition coefficients would remain unchanged in the presence of calcium. However, there was a substantial increase in the partition coefficients. Therefore, although I and II may not have favored coordination with calcium at pH 5, they were intermolecularly aggregating (9) to produce aggregates in a ratio of 14 prostaglandin molecules to one calcium ion. The formation of the lipid-soluble aggregate could be explained on the basis of intermolecular hydrophilic and/or hydrophobic bonding, probably similar to surfactant aggregation in micelle formation.

Tables I and II show that the partition coefficients of I (which is less polar than II) were higher in the less polar oil–water systems, namely, the *n*-octane–water and 1-octanoic acid–water systems. Even though the presence of calcium ions increased the lipid solubility of I and II, the inherent polarity of the prostaglandin molecules in the aggregated state showed the same effect on the k' values.

The transport studies of I and II through the mucosal surface of the isolated rabbit intestine revealed a direct influence of calcium ions on prostaglandin uptake. The apparatus and techniques employed (11) assured constant hydrodynamics at the mucosal surface and, therefore, allowed the quantitative estimation of initial rates, diffusion coefficients, and permeability coefficients.

Table III reports the estimated initial uptake rates for I and II by the rabbit intestine in the absence and presence of calcium. At 10 and 25 mM calcium, the relative initial uptake rates increased but were followed by a decrease at 50 mM calcium. The decrease at 50 mM calcium may be attributed to the toxic effect of calcium on the mucosal membrane at such a relatively high concentration. The increase in the relative initial uptake rates at 10 and 25 mM calcium may result from an increase in the mucosal–water partition tendency of prostaglandins in the prostaglandin–calcium aggregated form; *i.e.*, the calcium ions and/or the prostaglandin molecules could be acting as carrier species (9) in the uptake process.

Table IV includes the estimated diffusion and permeability coefficients of I and II utilizing the data in Table III. The estimated diffusion coefficient values of I and II in the absence of calcium should theoretically be equal when calculated by the Stokes–Einstein approximation (D_W = 2–4 × 10^{-5} cm^2/sec). Table IV revealed the D_W value of I to be almost twice as large as that of II at 0 mM calcium, which is in the order of magnitude of the ratio of their partition coefficients. The estimated

Table IV—Estimated Diffusion and Permeability Coefficients of I and II Uptake into Rabbit Intestine as a Function of Calcium-Ion Concentration

Calcium-Ion Concentration, mM[a]	Diffusion Coefficient, $cm^2/sec \times 10^{-5}$	Permeability Coefficient, $cm/sec \times 10^{-2}$
	Compound I	
0	2.92	6.34
10.0	4.26	9.26
25.0	7.19	15.60
50.0	1.57	3.41
	Compound II	
0	1.23	2.70
10.0	3.73	8.10
25.0	1.83	4.00
50.0	0.48	1.04

[a] At pH 5.

diffusion coefficients for I and II increased as the calcium concentration increased and then decreased at the 50 m*M* level. The corresponding permeability coefficients agreed fairly well with the sequence of the results. At 0 m*M* calcium, the estimated permeability coefficient of I is almost double that of II, which is also in the order of magnitude of the ratios of their partition coefficients. The permeability coefficients increased with an increase in the calcium-ion concentration, followed by a decrease at the 50 m*M* calcium level.

It is, therefore, proposed that both the prostaglandin molecules and the calcium ions constituting the aggregates crossed intact the diffusion barrier into the mucosal surface. Both species were self-carried in a fashion similar to a carrier-mediated process through the intestinal membrane.

These results may be important in understanding prostaglandin movement through biological fluids and membranes.

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Thermal Decomposition of Amorphous β-Lactam Antibacterials

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Abstract □ Thermal decomposition rates for amorphous samples of penicillin G potassium, cephalothin sodium, cefamandole sodium, and cefamandole nafate were determined as a function of water content and temperature. Even when rigorously dry, amorphous cephalosporins were at least one order of magnitude less stable than the corresponding unsolvated crystalline form. Absorbed water generally increased both the number of decomposition products and the net decomposition rate. Reaction kinetics were usually apparent first order, but an anomalously high effective reaction order was observed in several systems. Nonlinear Arrhenius plots were observed, and a qualitative model based on molecular relaxation in glasses is proposed. Although decomposition rates at 25° were small for dry samples, even slight decomposition produced visually detectable changes. Thus, the unsolvated crystalline form was noticeably more stable, even at 25°.

Keyphrases □ Decomposition, thermal—amorphous samples of various antibacterials, effect of water content and temperature □ Cephalosporins, various—thermal decomposition of amorphous samples, effect of water content and temperature □ Penicillin G potassium—thermal decomposition of amorphous samples, effect of water content and temperature □ Antibacterials, various—thermal decomposition of amorphous samples, effect of water content and temperature

Cephalosporins are often difficult to obtain in crystalline form and, even when crystallized, the products may be partially amorphous (1). Thus, information on the stability of amorphous forms is necessary. Several organic reactions were studied in crystalline solids (2–4), but amorphous solids have received little attention. The intuitive notion that an amorphous solid is more reactive than the corresponding crystalline form has some experimental support (5–11) and is probably a useful generalization. However, crystalline reactions are often only slightly slower than the corresponding reactions in the liquid state (2) and some crystalline reactions are considerably faster (12, 13). While the decomposition of cephalosporins and penicillins in aqueous solution has been studied (14–22), data regarding thermal decomposition in the amorphous solid state are limited to several brief reports where the water content of the sample was either high or unspecified (8–11).

This study investigated the thermal decomposition rates of several β-lactam antibacterials in their amorphous and unsolvated crystalline forms as a function of temperature and water content. The amorphous samples studied were amorphous to X-rays and were nonbirefringent when examined microscopically under polarized light. The compounds chosen were penicillin G potassium and three cephalosporins: cephalothin sodium, cefamandole sodium, and cefamandole nafate.

EXPERIMENTAL

Materials—Crystalline cephalothin sodium and crystalline penicillin G potassium were commercial samples[1]. The corresponding amorphous samples were prepared by freeze drying from a 20% aqueous solution. To avoid partial crystallization, the solutions were frozen and partially dried at low temperature (−20°) before allowing the temperature to increase

[1] Eli Lilly and Co., Indianapolis, Ind.