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

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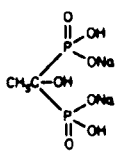
Abstract □ The acute intravenous toxicity of disodium dihydrogen (1-hydroxyethylidene)diphosphonate (etidronate disodium; I) and the mechanism of this toxic response have been investigated in 40 beagle dogs. The intravenous toxicity of I is dependent on the total dose administered and the length of the 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.

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Electrocardiography—Electrocardiograms were obtained according to standard procedures (19) on an electrocardiograph unit[2]. Serial six-lead electrocardiograms were obtained immediately prior to dosing and at 5, 10, 15, and 60 min after the start of the infusion. In all experiments, a continuous lead II electrocardiogram was obtained during and for 1–3 min after the start of the infusion.

Infusions—Indwelling catheters[3] were inserted percutaneously into the cephalic vein to facilitate infusions. Dose solutions were delivered manually from a syringe. In the "rescue" study, I was infused into one cephalic vein, and the calcium solution was infused simultaneously into the other cephalic vein.

Dose Solutions—All solutions of I were prepared in sterile distilled water and adjusted to pH 7.4. Solutions were filtered[4] (0.22 μm) into sterile serum bottles. The concentrations of the dose solutions varied with the intended dose level. The diphosphonate concentrations employed were (x to y mg of I/mL, z mg/kg): 1.9 to 4.0, 2; 5.7 to 5.8, 4; 14.5 to 14.7, 8; 27.0 to 32.0, 16; 38.3 to 49.4, 28; 41.1 to 64.0, 32; and 52.8 to 58.4, 37 and 44, and 55.3, 60.

Ten percent calcium gluconate for injection[5] was used as supplied; the concentration of calcium was 9.2 mg/mL. Physiological saline[6] was used as the control infusion solution.

Experiment I—Part 1 of experiment I was designed to determine the acute toxicity of I when doses of 2, 4, 8, 16, and 32 mg of I/kg (two dogs per dose level) were administered intravenously over a 15-s interval. Six males and four females were used. Part 2 of experiment I was a determination of the acute toxicity of I using doses of 2, 4, 8, 16, 28, 37, and 44 mg of I/kg (two dogs per dose level) administered intravenously over a 2-min interval. Nine males and five females were used. These two studies were designed to examine the toxic effects and influence of the duration of the infusion on the toxicity of intravenously administered I.

Experiment II—This experiment was designed to examine the relationship between diphosphonate blood levels and the physiological response to three nonlethal dose levels (2, 16, and 32 mg of I/kg) administered intravenously over a 2-min interval. Six dogs (one male and one female per dose level) were used. In this experiment silicone rubber cannulas were surgically implanted into the jugular vein on the day prior to the infusion to facilitate serial blood sampling. The cannulas were kept patent with heparinized saline (125–250 U/mL).

The diphosphonate dose solution was labeled with carbon-14 in the 1-position, [^{14}C]I, so that the diphosphonate blood level could be monitored by standard radioassay procedures using a scintillation fluid[7]. Total calcium and total orthophosphate were analyzed on an autoanalyzer. Calcium was determined using calcein (20), phosphorus was determined colorimetrically (21), and ionized calcium was determined with a calcium electrode. Serial blood samples were collected immediately prior to the start of the infusion and then at the following intervals: 0.5, 1, 1.5, 2, 3, 5, 10, 15, and 30 min, 1, 2, 4, 24, 48, and 72 h, and 1 week. All analyses were performed on the serum.

Experiment III—A calcium "rescue" experiment was designed to determine if the effects of a lethal intravenous dose of I could be prevented by the simultaneous intravenous infusion of an ionized calcium salt. Preliminary infusions using two dogs (one male and one female) confirmed the lethality of 60 mg of I/kg administered over a 2-min interval. Two other dogs (one male and one female) received only calcium gluconate at 20 mg of Ca^{2+}/kg in order to determine generalized and electrocardiographic responses to 2-min infusions of an ionized calcium salt. Subsequent to the above, two female dogs received infusions of 60 mg of I/kg simultaneously with calcium gluconate (20 mg of Ca^{2+}/kg) over a 2-min interval.

Experiment IV—This experiment served as a control for the preceding series of experiments. Four dogs (two males and two females) received 2-min intravenous infusions of physiological saline.

General Reactions and Anesthesia—The reactions of the dogs to infusions of I were monitored by continuous observation during and immediately after the infusion, and daily for up to 2 weeks. These data were considered important in defining the potential hazards of intravenous administration of I in humans.

Sodium pentobarbital decreases respiration and may affect the cardiovascular system such that the electrocardiogram and distribution of I would be altered (22–24); therefore, anesthesia was not employed in experiments I and II. Having established the relationship between calcium and I in the blood, and the coincident electrocardiographic changes in the conscious dog, dogs receiving lethal doses of I were anesthetized for experiment III.

[2] Burdich Model EKS; Kauffman-Goodwin, Columbus, Ohio.
[3] Sherwood Medical Industries, St. Louis, Mo.
[4] Millipore Corp., Bedford, Mass.
[5] W. A. Butler, Cincinnati, Ohio.
[6] Abbott Laboratories, North Chicago, Ill.
[7] Triton X-100; Rohm & Haas.

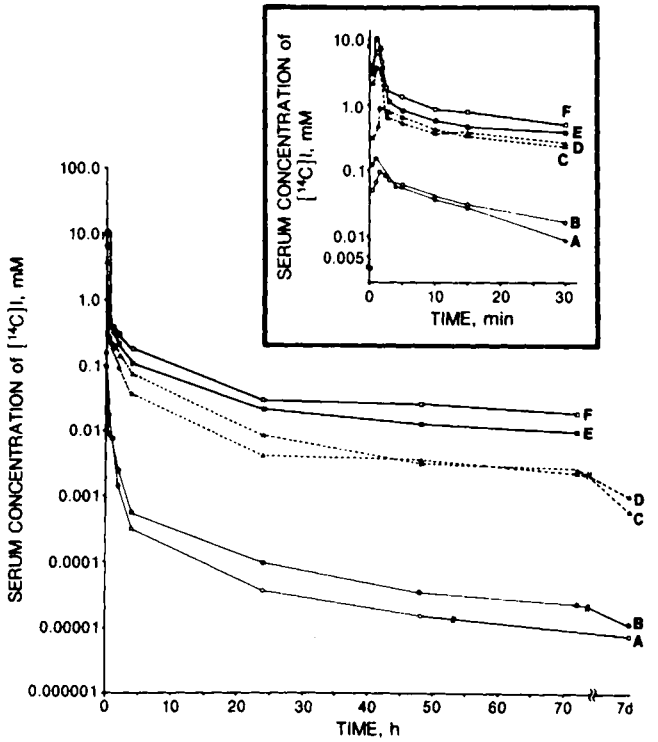

Figure 1—*Concentration of [^{14}C]I in the serum of dogs during and after 2-min intravenous infusions of 2 (A,B), 16 (C,D), and 32 (E,F) mg of [^{14}C]I/kg. Inset shows the first 30 min during and after the infusion.*

RESULTS

Acute Toxicity Studies (Experiment I)—*15-s Infusions*—No drug-related electrocardiographic effects were observed during or after infusions of 2–8 mg of I/kg. At a dose level of 16 mg/kg, an increased respiratory rate and a transient increase in heart rate were observed in both dogs during the infusion. The heart rate returned to normal within 5 min after the start of the infusion. Daily observations, lasting up to 2 weeks, did not reveal adverse effects in dogs receiving up to 16 mg/kg. At a dose level of 32 mg/kg, clonic contractions preceded death, which was observed in both dogs as a cessation of respiration. Electrocardiographically, both animals receiving 32 mg of I/kg developed lethal ventricular arrhythmias within 2–3 min after starting the infusion; tachycardia, QT prolongation, heart block, premature ventricular contractions, and finally, ventricular fibrillation were noted sequentially in both dogs.

2-min Infusions—No significant electrocardiographic effects were observed during or after infusions of 2–8 mg of I/kg. At doses of 16 or 28 mg of I/kg, transient tachycardia was the only consistent electrocardiographic effect and, in all dogs, the heart rate had returned to normal within 5 min after the start of the infusion. As the dose level increased to 37, and finally to 44, mg of I/kg, the dogs resisted restraint and electrocardiographic changes became evident. Electrocardiographically, slight, persistent sinus tachycardia occurred at a dose level of 37 mg of I/kg. Dogs that received 44 mg of I/kg exhibited severe, persistent tachycardia, T waves of increased magnitude, prolonged QRS intervals, ST segment elevation, and premature ventricular contractions. At a dose level of 44 mg of I/kg, one of the two dogs died.

Physiological Response to Nonlethal Doses of I (Experiment II)—*2-min Infusion*—The electrocardiograms of dogs receiving 2 mg of [^{14}C]I/kg appeared essentially normal. At a dose of 16 mg of [^{14}C]I/kg there was a transient increase in heart rate and a prolongation of the QT interval in both dogs. Within 5 min after start of the infusion, these had returned to normal (predose) values. At 32 mg of [^{14}C]I/kg a predominant sinus tachycardia and a prolongation of the QT interval occurred in both dogs. In one dog, these had returned to normal within 5 min, but in the other dog the tachycardia persisted for 30 min. In the latter dog, premature ventricular contractions were observed between 2 and 3 min after the start of the infusion. In dogs receiving 32 mg of I/kg, respiratory rates increased and the dogs resisted restraint. These reactions were absent within 1 min after cessation of the infusion. No toxic symptoms were observed the day after infusion in any dog, and all dogs survived.

Diphosphonate, Total and Ionic Calcium, and Total Phosphorus Levels in Serum—The peak [^{14}C]I serum levels occurred for all dose levels within the 2-min infusion interval and then decreased exponentially (Fig. 1). The

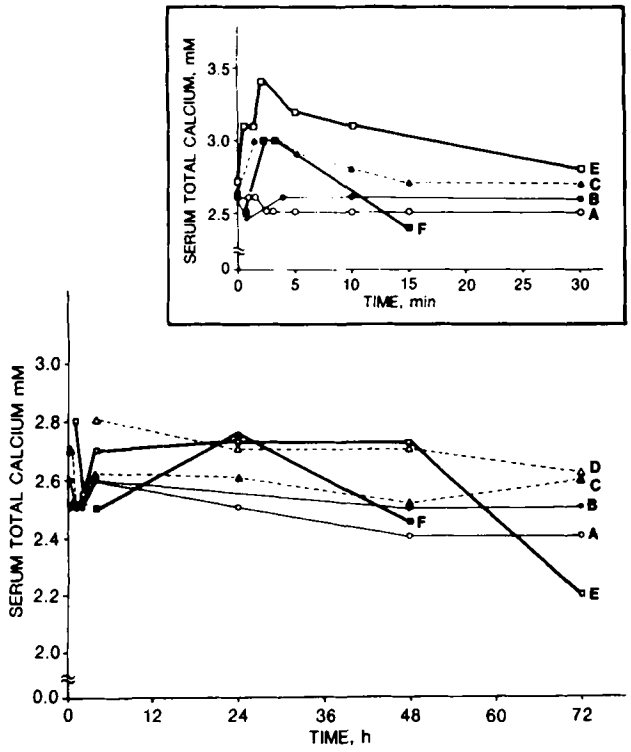

Figure 2—*Concentration of total calcium in serum of dogs during and after 2-min intravenous infusions of 2 (A,B), 16 (C,D), and 32 (E,F) mg of [^{14}C]-I/kg. Inset shows the first 30 min during and after the infusion.*

highest peak serum concentration achieved at each dose level was: 0.15 mM (2 mg of [^{14}C]I/kg), 3.8 mM (16 mg of [^{14}C]I/kg), and 10 mM (32 mg of [^{14}C]I/kg).

At the two highest dose levels (16 and 32 mg of [^{14}C]I/kg) total serum calcium increased 0.4–0.7 mM within 5 min after the start of infusion (Fig. 2), while serum phosphorus for these same animals decreased 0.2–0.5 mM within 15 min (Fig. 3). There was a decrease in ionic calcium within the 2-min infusion interval that was proportional to the serum [^{14}C]I level (Fig. 4). For example, the highest [^{14}C]I serum level (10 mM) corresponded to the lowest

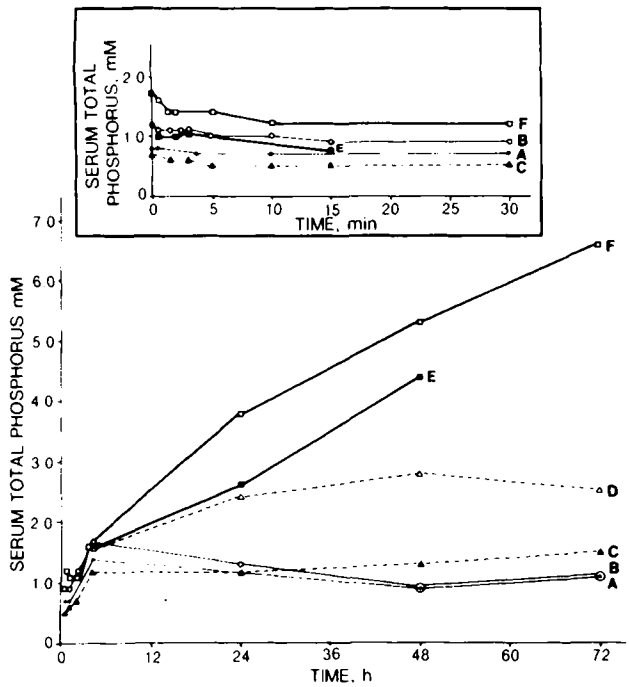

Figure 3—*Concentration of serum orthophosphate in dogs during and after 2-min intravenous infusions of 2 (A,B), 16 (C,D) and 32 (E,F) mg of [^{14}C]I/kg. Inset shows the first 30 min during and after the infusion.*

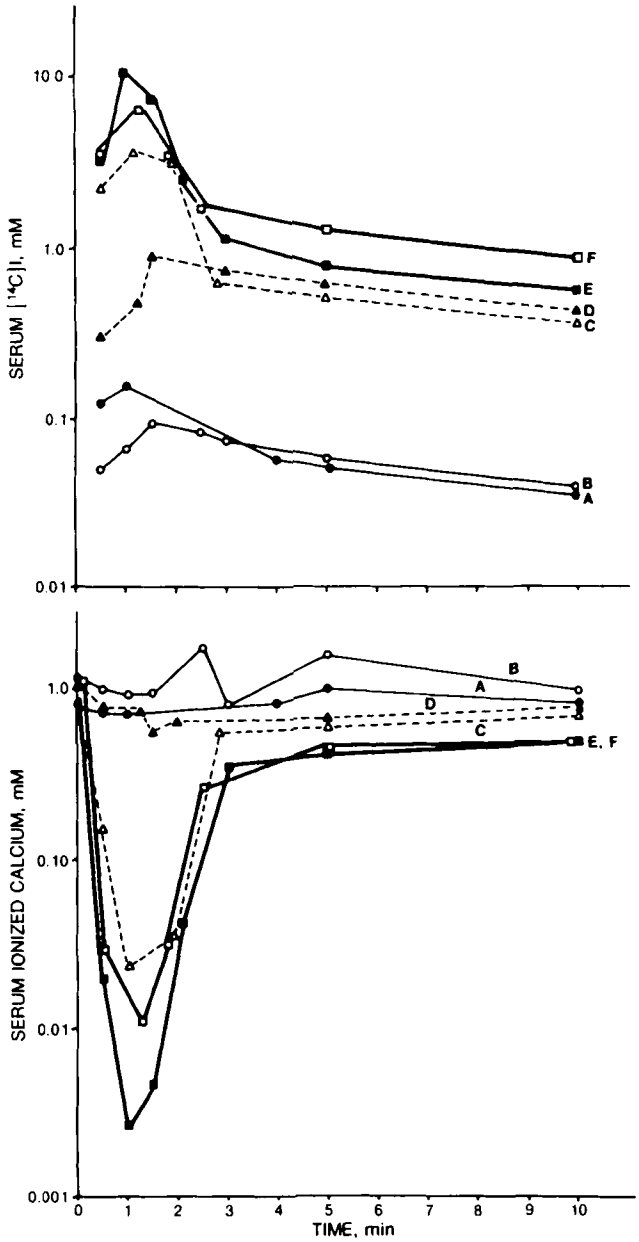

Figure 4—*Concentration of [^{14}C]I and of ionized calcium in the serum of dogs during and after 2-min intravenous infusions of 2 (A,B), 16 (C,D), and 32 (E,F) mg of [^{14}C]I/kg.*

ionized calcium level attained (0.001 mM). The lowest [^{14}C]I blood levels were achieved with a dose of 2 mg of [^{14}C]I/kg and did not alter the baseline ionized calcium (~1 mM). In dogs receiving the two highest levels of bisphosphonate, ionized calcium fell precipitously and then rose rapidly to normal levels within 5 min after the start of the infusion (Fig. 4), while total calcium, after increasing, did not return to normal levels until ~30 min after the start of the infusion (Fig. 2). In these same dogs, serum phosphorus (as orthophosphate) showed a progressive and steady increase (Fig. 3) up to 72 h after the start of infusion (up 5.4 mM). In the dog which exhibited the greatest increase in serum phosphorus, there was a concomitant decrease in both total and ionized calcium at the 72-h evaluation.

Calcium "Rescue" (Experiment III)—Both dogs infused with 60 mg of I/kg died. Within 1 min after initiation of the infusion, tachycardia was evident in both dogs. Electrocardiographic changes were similar to those which occurred with lethal doses in experiment I. Infusions of calcium gluconate, on the other hand, were associated with emesis and transient electrocardiographic changes consistent with hypercalcemia.

Animals that received a combination of I and calcium gluconate did not display any significant electrocardiographic effects during the first 5 min of the experiment. Sinus tachycardia was observed in one dog 1 h postdose. Negative P waves and second degree atrioventricular block were observed in the other dog through the 15-min tracing, but this arrhythmia was absent in

the 1-h tracing. The dogs that received I plus calcium gluconate appeared normal during the 2-week postdose observation period.

Control Infusions (Experiment IV)—No general reactions were observed in response to 2-min intravenous infusions of saline. No significant electrocardiographic changes were observed in these dogs.

DISCUSSION

The intravenous administration of high doses of I can produce rapid death; however, the toxic effect is both time and dose dependent. The lethal toxicity of I relates directly to the concentration of I achieved in the blood. Identical doses administered over a shorter period of time produce a higher blood concentration and result in higher toxicity.

The toxicity of I is directly related to its ability to bind or complex with the circulating calcium of the blood. For a given infusion interval, when the intravenous dose of [^{14}C]I was increased, the concentration in the blood rose proportionally (Fig. 1). At the two highest dose levels (16 and 32 mg of [^{14}C]I/kg), the concentration of ionized calcium in the blood fell, with the maximum depressions of ionized calcium coinciding in time with the peak blood levels of [^{14}C]I (Fig. 4). The ionized calcium returned rapidly to near-normal concentrations within 5 min, despite the significant concentration of I beyond this time. The use of electrocardiography during infusions of increasing diphosphonate doses was found to be a useful diagnostic tool for detecting progressive signs of increasing cardiotoxic effects. The electrocardiographic effects observed at the high dose levels of I are consistent with depressions in ionized calcium and indicate the sensitivity of heart muscle to low ionized calcium levels, in spite of elevations in total calcium (Fig. 2). No effect on ionized calcium levels was seen at the 2-mg/kg dose of I administered over a 2-min interval, and no significant electrocardiographic changes were observed in these dogs.

The rise in total calcium observed with high parenteral doses of I (Fig. 2) has been observed previously (25). For the animals showing the greatest rise in total calcium, serum phosphorus fell (Fig. 3). The rise in total calcium reflects mobilization of calcium to satisfy ionized calcium homeostasis in the blood. The fall in serum phosphorus levels probably does not relate to the rise in total calcium since ionized calcium fell. The fall in phosphorus is probably effected by stimulation of parathyroid hormone release (26) with consequent clearance of phosphorus by the kidneys. After returning rapidly to normal (as did the total calcium and ionized calcium levels), serum phosphorus increased slowly and steadily above normal for up to 72 h after the infusion (Fig. 3), reaching a maximum level that was 3–4 times normal in dogs receiving the highest dose of I. This delayed and elevated serum phosphorus level has been observed on numerous occasions in humans given high daily oral doses of I (20 mg of I/kg), and is attributed to an increased tubular reabsorption of orthophosphate by the kidney (27).

The lethal effects (depression in ionized calcium) of the very high doses of I can be overcome by simultaneous infusion of an ionized calcium salt, such as calcium gluconate. Similar studies with another diphosphonate (disodium dichloromethylenediphosphonate) have confirmed this "rescue" effect[8]. In these studies, calcium gluconate maintained normal serum ionized calcium, while total calcium rose above normal until shortly after the infusion period. In the present study, in the absence of calcium gluconate, maximum depression in ionized calcium would be expected during the first 5 min after the start of infusion (see Fig. 4). During this period, electrocardiographic changes were observed but, with simultaneous infusion of calcium gluconate, normal electrocardiographic patterns were obtained. Calcium chloride can achieve the same effects as calcium gluconate[8], but the gluconate salt is preferred (28), since toxic effects of calcium administration have been reported and the chloride salt seems more toxic than the gluconate salt.

This series of studies describes the lethal and sublethal toxic responses of I administered over two infusion intervals. The studies define the dose levels of I which can be administered safely by intravenous injection. In addition, the studies provide insight into the mechanism of toxicity, and describe a means to prevent death associated with lethal doses of intravenously administered disodium dihydrogen (1-hydroxyethylidene)diphosphonate.

[8] M. D. Francis and C. L. Slough; unpublished results.

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