Technetium-99m Calcium Phytate—Optimization of Calcium Content for Liver and Spleen Scintigraphy: Concise Communication

Julie Campbell, Johan C. Bellen, Richmond J. Baker, and David J. Cook

Institute of Medical and Veterinary Science, Adelaide, South Australia

The addition of ionic calcium to technetium-99m stannous phytate produced an agent with improved splenic uptake. In a series of patients, it was found that molar ratios of 2:1 and 3:1 produced scans of optimal diagnostic quality. Studies in mice showed dose-related changes in the biodistribution. Electron microscopy demonstrated that the addition of calcium produced progressive aggregation of the colloid as the molar ratio was increased. Chemical analysis of sodium phytate was essential to obtain accurate ratios.

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Technetium-99m stannous phytate is an excellent liver scanning agent by virtue of its reaction with plasma calcium ions to produce an insoluble radiocolloid (*1*). However, splenic uptake is frequently insufficient to provide images of diagnostic quality (*2*). It has been observed that the addition of calcium before injection improves the spleen image (*2,3*), but the optimal molar ratio of calcium to phytate has not been reported.

With the aim of providing an easily prepared, high-quality radiopharmaceutical, we have investigated the effect of varying the proportion of calcium to phytate on the biological behavior of this preparation.

MATERIALS AND METHODS

Kit preparation. Kits were prepared containing the equivalent of 20 mg anhydrous sodium phytate* and 1.0 mg $SnCl_2 \cdot 2H_2O$ in 0.5 ml water for injection at pH 5.0. They were stored at −20°C in rubber-closed vials containing positive nitrogen pressure. Labeling was carried out by the addition of 9.5 ml pertechnetate (Tc-99m) in water for injection.

A buffered calcium solution was prepared using $CaCl_2 \cdot 2H_2O$ (1.37 g) and KH phthalate (0.5 g) in 100 ml water for injection, pH 3.2, so that 0.25 ml of calcium solution was equimolar to the phytate content of one vial. Various ratios of calcium to phytate were prepared by replacing pertechnetate with stock calcium solution.

Analysis of sodium phytate. A discrepancy in the formulation of sodium phytate as either $C_6H_6Na_{12}O_{24}P_6$* (containing 37–42% H_2O) or $C_6H_9Na_9O_{24}P_6$ (*4*) led to an investigation of the chemical composition of the product. The phytic acid content was determined by a modification of the method of Samotus and Schwimmer (*5*) (R. K. Barnes, personal communication). The water content was determined by heating at 120°C to constant weight, and sodium was analyzed by atomic absorption spectroscopy.

Chromatography. The pertechnetate content of the Tc-99m stannous phytate and calcium colloids was determined by ascending chromatography using ITLC-SG medium,† with ethanol as solvent.

Electron microscopy. Particle sizing of stannous phytate and the various calcium phytate colloids was investigated using an electron microscope and formvar-coated grids. Solutions were prepared with membrane-filtered (0.22 μ) doubly distilled water, spotted onto grids within 3 min of preparation, then allowed to dry in air.

Animal studies. Organ distribution studies were performed in female Balb/c mice weighing 20–22 g. Groups of four mice were studied at each of two phytate levels, 0.25 ml (25 mg/kg) or 10 μl (1.0 mg/kg). The mice were

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For reprints contact: Richmond J. Baker, PhD, Div. of Nuclear Medicine, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia 5000.

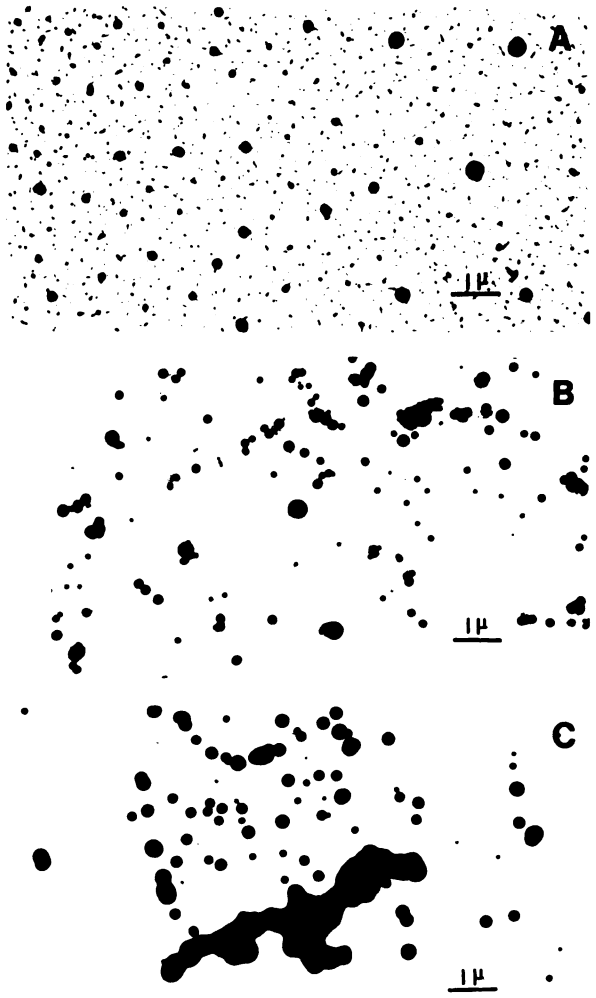

FIG. 1. Electron micrographs of Tc-99m phytate preparations containing (A) no calcium, and calcium:phytate molar ratios of (B) 2:1, and (C) 6:1.

killed, and samples excised 30 min after injection. Using suitable standards, the radioactivity content of blood, lungs, liver, spleen, stomach, intestine, kidneys, femora, muscle, urine, and carcass was determined. The sum of the radioactivity in all organs was within 5% of the injected dose.

For the preparation containing no calcium (25 mg phytate/kg) the carcasses were dissected further and uptake in femur and bone marrow determined. The effect of injection rate was also studied using the 1:1 ratio.

Clinical trial. In order to assess the effect of calcium on the quality of liver and spleen scans, the ratio of calcium to phytate used in clinical studies was varied from 0 to 4:1. Patients with a moderate or high probability of hepatocellular disease, known splenic disease, or major scan abnormalities were excluded from the study. Eleven to 15 patients were investigated at each ratio. The scans were graded by an impartial observer according to the relative intensity of liver and spleen.

RESULTS

Analysis of sodium phytate. The material examined was found to have the following composition: water 14.4%, phytic acid 66.3%, sodium 20.9%. This analysis agrees with a formulation as the nona-sodium salt (*4*) (calculated for $C_6H_9O_{24}P_6Na_9$ + 14.4%H_2O: phytic acid, 65.9%; sodium, 20.7%).

Chromatography. All preparations were found to have less than 2% free pertechnetate. Additional vials stored at −20°C for 12 mo labeled equally well.

Electron microscopy. Electron micrographs of the stannous phytate preparation revealed the presence of small particles 0.1-0.2 μ in diameter, and a general background of smaller, needle-like particles (Fig. 1A) which were visible only in the preparation without calcium. At the 2:1 calcium:phytate ratio (Fig. 1B) there was a discrete population 0.1-0.2 μ diameter, together with some aggregates about 1 μ in length. The size of aggregates increased as the ratio increased, until at the 6:1 level (Fig. 1C) aggregates in the order of 6 μ were seen, together with smaller particles.

Organ distribution studies. Preliminary work with the 1:1 Ca:phytate preparation showed that changes in the distribution of radioactivity could be produced by varying the rate of injection. The most marked difference was found in the carcass uptake, which decreased from 9.12% (8.08-10.00) for a fast bolus injection to 2.45% (2.34-2.62) for an injection carried out over 50 sec. To standardize the procedure, all subsequent experiments were carried out using rapid injection.

The change in organ distribution on the addition of calcium is shown in Fig. 2. The high carcass uptake observed in the absence of calcium at the 25 mg/kg dose was associated with a generalized distribution of activity in muscle, fat, skin, and bone. However, uptake in the two femora was low (0.18%), with only 29% of this radioactivity contained in the marrow.

Clinical results. The results of clinical studies are presented in Fig. 3. With calcium:phytate ratios of 0:1 and 1:1, splenic intensity was less than that of the liver in ~60% of scans. When this occurred, the studies were clinically unsatisfactory. However, at the 2:1 and 3:1 level, all scans were found to be satisfactory, even though 25% of studies showed spleen with greater intensity than liver. At the 4:1 level, the proportion of scans with greater splenic intensity rose to 50%. Typical scan qualities can be seen in Fig. 4.

DISCUSSION

It has been reported (*1*) that the distribution of Tc-99m phytate in mice can be altered by changing the molar ratio of stannous ion to phytate and by the amount of phytate injected. The results presented here show that

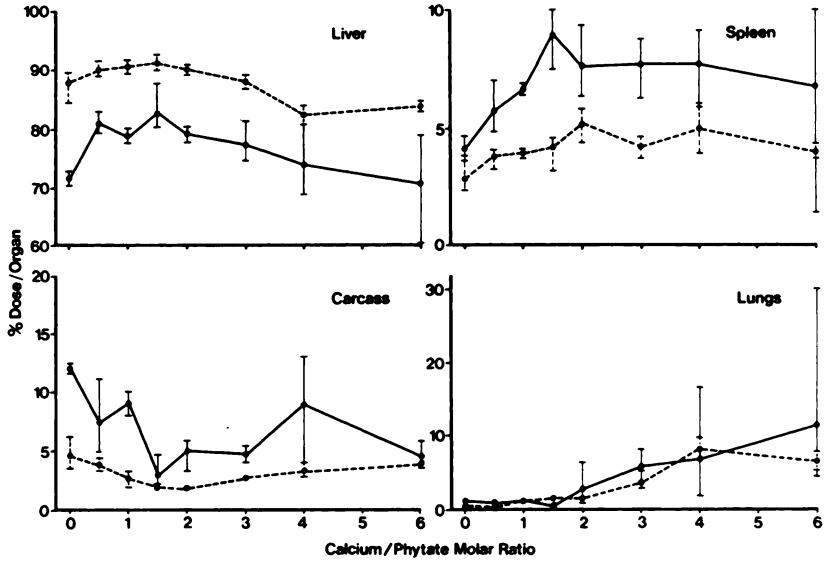

FIG. 2. Organ uptake of Tc-99m in mice as the calcium:phytate molar ratio was increased from 0 to 6:1, at two dose levels of sodium phytate: 25 mg/kg (solid line), and 1.0 mg/kg (broken line). Mean values and ranges are shown.

the rate of injection is also important if high doses (25 mg/kg) are used. At this level, generalized distribution of radioactivity in the tissues of the carcass may be related to reduced availability of plasma calcium ions. As determined in our laboratory, the serum calcium level of Balb/c mice is 2.53 millimol/l. Thus, for a blood volume of 1.1 ml (*6*), approximately 40% of the available calcium would be removed for each molar equivalent incorporated into the colloid formed in vivo.

The high dose level produced liver uptake of up to 83% at a calcium:phytate ratio of 1.5:1. With the 1.0 mg/kg dose, uptake in the liver was consistently higher, peaking at 91%. Both levels showed variable lung uptake as the proportion of calcium increased, due to the formation of larger aggregates, as demonstrated by electron microscopy.

Stannous phytate has been examined by Ege and Warbick (*7*) using electron microscopy and found to contain particles with a mean size of 9 nm when prepared with 20:1 phytate:tin ratio. Davis et al. (*2*) reported that

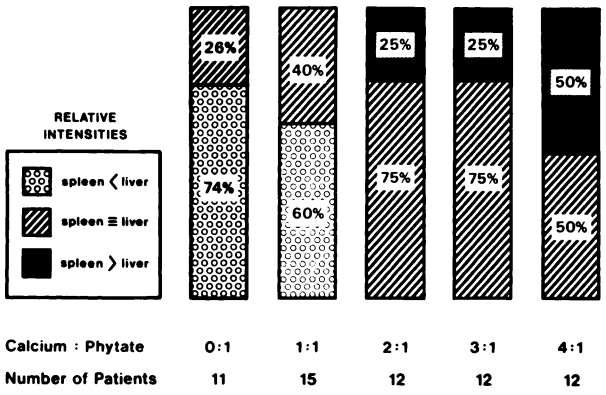

FIG. 3. Comparison of liver and spleen intensities in small series of patients where calcium:phytate molar ratio was varied from 0 to 4:1.

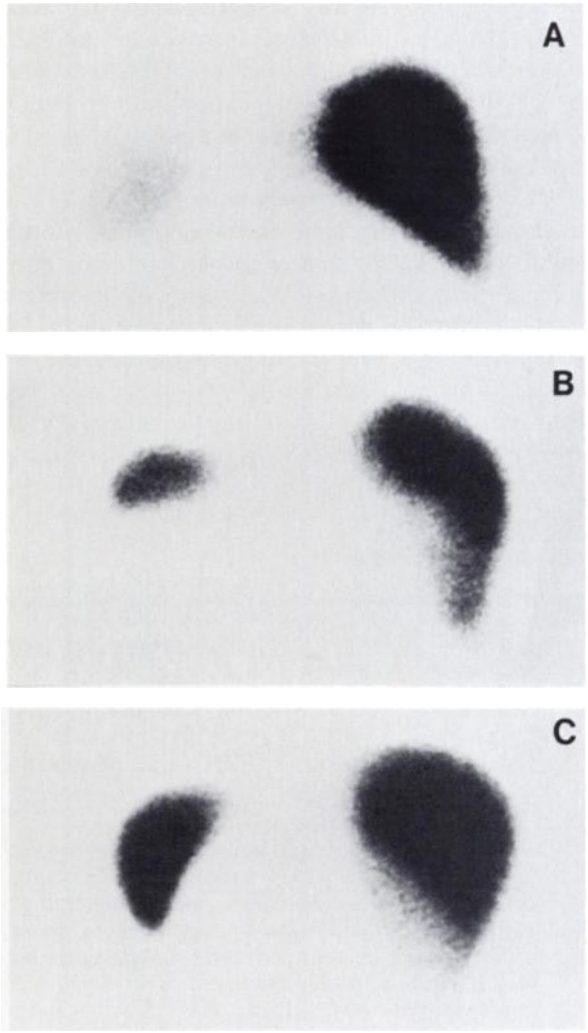

FIG. 4. Liver and spleen scintigrams obtained with large-field camera, showing increasing splenic uptake as calcium:phytate molar ratio was increased from (A) 0:1 to (B) 2:1, and (C) 4:1.

a calcium phytate colloid suitable for liver and spleen scanning has a mean particle size of 0.3 μ, as determined by Nuclepore filtration, a method giving activity against size distribution. The particle-sizing results reported here appear to correlate well with the work of Davis, but they conflict somewhat with the results of Ege and Warbick. This may be due to the lower ratio of phytate:stannous ion (5:1) used in the present study. Ege and Warbick removed soluble salts by washing the grids, which may account for the absence of needle-like particles in their preparations; these are likely to be crystallized salts. Washing was not performed in the present study, since it might have resulted in the removal of some colloidal material.

The marked improvement of splenic uptake shown in patient studies was reflected in results obtained from the distribution study in mice. Uptake by the spleen effectively doubled as calcium was added. This increase was also shown by the spleen:liver concentration ratio (% dose/g), which increased from 0.36 at the 0:1 ratio to 0.66 at the 4:1 ratio. Although these results parallel the human situation, it is clear that primates, as proposed by Davis et al. (*2*), would provide more acceptable models. However, this may not be a practical approach for many laboratories. The use of mice should then be satisfactory, provided a low dose level is used.

The clinical results indicate that calcium:phytate ratios of 2:1 and 3:1 are suitable as routine liver and spleen imaging agents. Improved splenic uptake is probably associated with the presence of larger particles. We observed that the 2:1 colloid, on standing, remained suspended for several hours, whereas the 4:1 and 6:1 colloids sedimented in under 1 hr with the formation of visible aggregates. Because of the large particles, rapid sedimentation, and high lung uptake noted in mice, the 6:1 ratio was not administered to patients.

In our laboratory, the 2:1 ratio is now used as the preferred agent, producing scans of comparable diagnostic quality with those performed using Tc-99m sulfur colloid, with the advantage of faster preparation.

FOOTNOTES

* British Drug Houses Ltd., Poole, England.
† Gelman Instrument Co., Ann Arbor, MI.

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