Ionized Calcium Buffering
in the Transfused Anhepatic Patient:
Ab Initio Calculations of Calcium
Ion Concentrations*†

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ABSTRACT

Homeostatic control of ionized calcium can be volatile during liver transplantation, particularly during the anhepatic stage. Recently, an opportunity arose to evaluate an 11-year-old girl who developed persistent ionic hypocalcemia during a prolonged anhepatic period subsequent to the failure and removal of the graft. The patient was remarkable for having survived a 34-hour anhepatic interval before a second and successful orthotopic liver transplant. Ionic hypocalcemia (ionized calcium <1 mmol per L) coexisted with significant hypercalcemia (total calcium >5 mmol per L) during this anhepatic interval. The discrepancy was due to high concentrations of citrate, which accumulated from the multiple transfusions of citrated blood, and the inability to metabolize citrate in the anhepatic state. Using a mathematical model to solve for free calcium ion concentration in the presence of multiple ligands, it is demonstrated that prolonged hypercitricemia markedly alters the calcium ion buffering properties of blood, and these changes must be recognized in order to prevent adverse clinical consequences of ionic hypocalcemia.

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Introduction

Hepatic disease serious enough to warrant liver transplantation is inevitably accompanied by myriad biochemical abnormalities, but sometimes overlooked are the metabolic consequences of mas-
sive transfusion in the anhepatic patient. It is well known that the citrate content of transfused blood can induce ionic hypocalcemia, but the depression of ionized calcium (Ca++) is usually transient owing to the rapid conversion of citrate to acetyl CoA and oxaloacetate by hepatic adenosine triphosphate (ATP)-citrate lyase enzyme.

During orthotopic liver transplantation, however, there is necessarily a period of time in which the patient is anhepatic, and during this time calcium homeostasis can be substantially disrupted by persistently high concentrations of citrate from transfused blood. Precipitous changes in serum Ca++ concentration are of immediate concern owing to their potential for adversely affecting cardiac rhythm and contractility. Ickx and co-workers3 reviewed Ca++ measurements on 26 patients undergoing liver transplantation and found that these levels typically decreased throughout the dissection phase from a mean of 1.08 mmol per L before surgery to 0.89 mmol per L during the anhepatic phase. They increased to a mean of 1.23 mmol per L following revascularization and closure. The rates of transfusion and therapeutic calcium administration were both greatest during the anhepatic phase.

Barat and colleagues1 examined calcium measurements in 20 pediatric patients undergoing liver transplantation and found that these levels typically decreased throughout the dissection phase from a mean of 1.08 mmol per L before surgery to 0.89 mmol per L during the anhepatic phase. They increased to a mean of 1.23 mmol per L following revascularization and closure. The rates of transfusion and therapeutic calcium administration were both greatest during the anhepatic phase.

Case History

N.S. is an 11-year-old white female with a long standing history of hepatic cirrhosis of unknown etiology. At six months of age, she developed hematemesis that resolved with ice and saline lavage. Hepatomegaly was discovered at age one year, following a viral syndrome. No other gastrointestinal symptoms were present, but she had persistently elevated liver enzymes and was referred to the University of Florida for a liver biopsy. The biopsy revealed micronodular cirrhosis without evidence of active inflammation. Laboratory studies for infectious, autoimmune, or metabolic causes of hepatic disease were negative except for a mildly elevated antibody titer to cytomegalovirus, indicating prior infection. Throughout her childhood she was closely monitored and repeat biopsies were obtained at ages 3 and 8 years, with no change in the diagnosis. She exhibited normal growth and development despite slowly progressive liver and spleen enlargement, fluctuating elevations of liver enzymes, and borderline thrombocytopenia. No ascites, edema, jaundice, or rashes were evident.

In August of 1990, during an annual visit to the clinic, a surveillance alpha-fetoprotein was markedly elevated at 3863 ng per mL (normal 0 to 9 ng per mL). Abdominal ultrasound and computerized tomography (CT) both confirmed the presence of two hepatic lesions suspicious for hepatocellular carcinoma. She was admitted to Shands Hospital in November, 1990 for a complete metastatic disease workup and liver transplant evaluation. Bone marrow biopsy, magnetic resonance imaging, chest CT, and a bone scan failed to reveal evidence of metastatic spread. She was placed on the national donor list and began therapy with Adriamycin at a dose of 10 mg per m² per week and cyclosporine (100 mg per d).
A suitable donor was identified on January 5, 1991, and she underwent an orthotopic liver transplant the following morning. Her post-operative course was notable for persistent hypovolemia. A routine ultrasound 18 hours post-operatively demonstrated the absence of portal venous blood flow, with patency of the hepatic artery. She was returned to the OR emergently for thrombectomy and revision of the anastomosis. Despite this intervention, intra-abdominal hemorrhage increased, and no blood flow was discernible in either the portal vein or hepatic artery. Massive blood support and intraoperative plasma exchange failed to control bleeding, and the grossly necrotic graft was removed at 02:15 A.M. on January 8, 1991.

In the post-operative period, a discrepancy was noted between the whole blood Ca++ (1.0 mmol per L) and total serum calcium (5.6 mmol per L). Over the ensuing 12 hours, the patient was maintained by plasmapheresis every four hours, involving an average of 2,100 cc of fresh frozen plasma and 280 cc of ACD-A anticoagulant. Boluses of calcium chloride were administered when the Ca++ fell below 1.0 mmol per L. On the evening of January 8, she was placed on continuous arterio-venous hemofiltration and dialysis to manage fluid overload and markedly elevated sodium, potassium, and calcium. After 30 hours with total absence of liver function, a second donor liver was located, and she returned to the OR at 08:45 A.M. on January 9, 1991 for retransplant. Revascularization of the second graft was complete at 11:45 A.M. By 6:00 P.M., her Ca++ had stabilized in the normal range and her total calcium returned to 2.48 mmol per L. Her clinical course and calcium concentrations are summarized in figure 1.

**Methods**

Total calcium was measured on a Paramax autoanalyzer* using an o-cresol-
phthalein complexone method. Serum calcium concentrations greater than 5.0 mmol per L were confirmed by atomic absorption spectrometry.† Ionized calcium was measured by ion selective electrode on a STAT Profile 6 analyzer.‡ Citrate was quantitated using an enzymatic method.§

Equilibrium concentrations of the various calcium species were calculated using software developed in the Center for the Study of Lithiasis and Pathological Calcification at the University of Florida. Details of the computer program appear elsewhere.2

Discussion

Calcium Ion Buffering in Serum

The speciation of calcium in blood was extensively studied by K. O. Pedersen, who described his results in a series of papers appearing in Scandinavian Journal of Clinical Laboratory Investigation between 1969 and 1972.9,10,11,12,13,14,15,16,17,18 Ionized calcium concentration in blood is regulated primarily by the buffering capacity of a large population of relatively weak (log $K_s = 2.0$) calcium binding sites on albumin.18 Analysis of binding data for the calcium-albumin complex indicates that there are 12 equivalent calcium binding sites on an albumin molecule. Approximately half of the calcium in blood is bound to these sites, although this amount is sufficient to saturate only 20 percent of the total calcium binding capacity of albumin. Higher affinity binding sites occur on other serum proteins, but the low concentration of these sites limits their contribution to five percent or less of total protein-bound calcium, except in the relatively rare instances of poly- or monoclonal gammopathies.5

About 20 percent of the diffusible fraction of calcium in blood is complexed. Stability constants ($K_s$) for many of the calcium complexes in blood were calculated by Toffaletti et al20,21 using data obtained from equilibrium gel filtration experiments. Among normal blood constituents, oxalate forms the most stable calcium complex,5 although calcium oxalate is only sparingly soluble and normal serum oxalate concentration is very low (0.02 mmol per L). The majority of complexed calcium in blood is in the form of calcium carbonates (log $K_s = 0.73$).20 Citrate binds calcium more tightly (log $K_s = 3.1$) than either bicarbonate or albumin, but accounts for a third or less of total complexed calcium. The concentration of citrate in normal blood is 0.10 mmol per L, which is less than 1/20th the total calcium concentration and an order of magnitude less than $Ca^{++}$ concentration. Thus, the citrate concentration is insufficient to contribute measurably to $Ca^{++}$ buffering capacity, and albumin emerges as the preeminent calcium ion buffer in normal blood.

Although use of the McLean-Hastings nomogram7 for estimating calcium ion is widespread, it has been demonstrated that total calcium concentration, taken alone or in conjunction with albumin concentration, does not correlate well with ionized calcium concentration.4 The poor correlation is the expected result of a weakly buffered system.

Citrate as a Calcium Ion Buffer

There is a substantial difference between the stabilities of the calcium-albumin and calcium-citrate complexes, and therefore it can be predicted that excess citrate will significantly alter the calcium ion buffering properties of
blood. Recently Martin\(^6\) reviewed equilibriums calculations for metal ion buffers in physiological systems. He pointed out that for highly stable metal-ligand complexes, the concentration of free metal ion in the presence of ligand is determined by the ratio of the total concentration of ligand to metal, by the relationship:

\[
\text{[free ion]} = \frac{R}{(1 - R)K_s} \quad \text{Eq. 1}
\]

where \(R = \frac{[\text{metal}]}{[\text{ligand}]}\). In the derivation of this equilibrium expression, it is assumed that free ion concentration is insignificant when compared to the total concentration of metal. Thus, the expression:

\[
K_s = \frac{[\text{bound ligand}]}{[\text{free ion}][\text{free ligand}]} \quad \text{Eq. 2}
\]

is replaced with:

\[
K_s = \frac{[\text{metal}]/[\text{free ion}]}{([\text{ligand}]/[\text{metal}])}
\]

or:

\[
[\text{free ion}] = \frac{[\text{metal}]}{([\text{ligand}]/[\text{metal}])K_s} \quad \text{Eq. 3}
\]

since \([\text{bound ligand}] = [\text{metal}] - [\text{free ion}] \equiv [\text{metal}],\) and \([\text{free ligand}] = [\text{ligand}] - [\text{bound ligand}] \equiv [\text{ligand}] - [\text{metal}].\) Multiplying the numerator and denominator in the right side of equation 3 by \(1/[\text{ligand}]\) gives equation 1. Total ion and ligand concentrations are usually known or can be easily measured, which simplifies the calculation of free metal ion concentration. For calcium in serum, however, neither \([\text{CaP}]\) nor \([\text{CaCit}^-]\) can be replaced with total calcium concentration to simplify the expression. Moreover, calcium forms three complexes with citrate (\(\text{CaCit}^-, \text{CaHCit, and CaH}_2\text{Cit}^+\)), the relative amounts of which depend on pH. Finally, as \(R\) in equation 1 approaches unity (i.e., the citrate concentration approaches the total calcium concentration), buffering capacity diminishes.

The result is a system in which \(\text{Ca}^{++}\) concentration is not well buffered, and can change precipitously in response to small variations in citrate or calcium concentration. Martin\(^6\) compared this behavior to the endpoint in a titration curve. When citrate concentration exceeds the available (non-albumin bound) calcium, however, the system becomes highly buffered and the \(\text{Ca}^{++}\) concentration is held quite low.

A rigorous treatment of this equilibrium expression would also require that activity coefficients be taken into account. In dilute solutions, these coefficients approach unity, but they will be significantly depressed by the relatively high ionic strength of blood. The stability constants given previously are \textit{apparent} stability constants measured in serum or plasma and, therefore, compensate for the ionic strength of these matrices. Stability constants are customarily given under conditions of \(25^\circ\)C and zero ionic strength; these standard values would be substantially higher than the stability constants mentioned previously.

It is interesting to note the rapidly fluctuating \(\text{Ca}^{++}\) concentration during the period that calcium was being infused in this patient (figure 1). This behavior may be indicative of the poor buffering capacity as total calcium and citrate concentrations approached equivalence. Equation 1 provides only a rough approximation for the moderately stable \(\text{CaCit}^-\) complex, however, and frequent infusions of calcium chloride are likely to account for much of the fluctuation in \(\text{Ca}^{++}\) concentration. Total calcium began to decline, and \(\text{Ca}^{++}\) rose to normal late in the anhepatic period, presumably owing to the cessation of plasma exchanges and removal of citrate by hemodialysis. Following the second
transplant, Ca\(^{++}\) levels stabilized at slightly below normal but followed closely the total calcium levels, which were also marginally depressed. Citrate concentration returned to normal after recovery of hepatic function by the second graft.

**AB INITIO CALCULATIONS OF CALCIUM ION CONCENTRATION**

Conservation of mass requires that the total calcium concentration is the sum of all species containing calcium. Free calcium ion concentration can therefore be expressed by a set of nonlinear simultaneous equations in \([Ca^{++}]\), \([CaL]\), and \([L]\), where \(L\) (ligand) can be albumin, citrate, bicarbonate, etc. A mathematical solution to these equations is best approached by using an algorithm that approximates successive solutions until self-consistency is reached. In the course of research into the mechanisms of urolithiasis, Finlayson developed a computer program for doing such calculations in order to predict the rate of intrarenal crystal growth.\(^2\) These ab initio models were used by the present authors for predicting the calcium ion concentration with respect to total calcium in the presence of various concentrations of calcium-binding constituents in serum. A partial list of parameters available for equilibrium \([Ca^{++}]\) calculations by the EQUIL software is given in table I.

In figure 2 is illustrated the effect of various citrate concentrations on Ca\(^{++}\) assuming normal albumin, total protein, bicarbonate, phosphate, and pH. It is evident that the relationship of Ca\(^{++}\) concentration to total calcium is relatively stable even when citrate is increased 10-fold above normal to 1.0 mmol per L. This observation is consistent with the presence of a large number (7.5 mmol per L) of relatively weak calcium binding sites on albumin and the normally low citrate concentration in blood. The \([Ca^{++}]\)/[total calcium] curve is, however, progressively more depressed as citrate concentration increases 30- to 900-fold above normal. Significantly, at high citrate concentrations, Ca\(^{++}\) remains well below physiologically appropriate concentrations except at very high total calcium, owing to the greater buffering capacity of the more stable calcium citrate complex. The comparative efficiency of the citrate-buffered system with respect to the albumin-buffered system is apparent in figure 3. At 0.1 to 1.0 mmol per L citrate concentrations, Ca\(^{++}\) is very sensitive to albumin concentration, whereas at 5.0 mmol per L citrate, changes in albumin concentration have a significant effect on Ca\(^{++}\) only when total calcium exceeds the citrate concentration.

**TABLE I**

<table>
<thead>
<tr>
<th>Parameters Used by EQUIL Software for Calculating Free Calcium Ion Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
</tr>
<tr>
<td>Carbonate</td>
</tr>
<tr>
<td>Lactic acid</td>
</tr>
<tr>
<td>Albumin</td>
</tr>
<tr>
<td>Phosphate</td>
</tr>
<tr>
<td>Potassium</td>
</tr>
<tr>
<td>Oxalate</td>
</tr>
<tr>
<td>Urate</td>
</tr>
<tr>
<td>Malate</td>
</tr>
<tr>
<td>Nitrate</td>
</tr>
</tbody>
</table>

\(\log K_s = 1.7\) vs. \(2.0\), but the inorganic phosphate concentration in adults is low compared to the concentration of calcium binding sites on albumin (1.0 vs. 7.5 mmol per L) or bicarbonate (25 mmol per L). Osteogenic activity in children and adolescents, however, results in higher serum phosphate levels.
Figure 2. Predicted ionized calcium (Ca\(^{++}\)) concentration based on total calcium at citrate concentrations of 0.1 (normal) to 9.0 mmol per L. Calculated values assume pH = 7.4 and normal physiological concentrations of albumin, bicarbonate, and phosphate.

Serum phosphate in this patient during the anhepatic period reached 2.7 mmol per L (reference range for children = 1.45 to 1.78 mmol per L). This elevation was most likely due to metabolic abnormalities associated with absent hepatic function and depressed renal function. However, normal pediatric serum phosphate can be as much as twice the adult levels, and the contribution of the calcium phosphate complex to the non-ionic diffusible fraction of plasma calcium would be predicted to increase correspondingly. Calculated concentrations of calcium citrate and phosphate complexes in normal adult, pediatric, and this patient's serum are compared in table II. These results for normal adult serum are similar to the data reported by Toffaletti et al.\(^{20}\) In a normal pediatric patient, however, the concentration of calcium phosphates is increased by 63 percent, and in this patient, calcium phosphates apparently exceeded total calcium carbonates, which normally predominate among complexed calcium species.

Other Effects on Calcium Ion Concentration

Since profound hepatic failure is characterized by multiple biochemical and metabolic disturbances, the current authors were curious as to whether or not variations in other calcium binding constituents, such as carbonate, lactate, and sulfate, or potential competition from sodium or magnesium for cation binding sites, might have an observable effect on the buffering capacity of blood. To examine this, the concentration of each constituent listed in table I was independently varied by 20 percent from normal in each direction, and the Ca\(^{++}\) concentration was recalculated. The largest effect on Ca\(^{++}\) was observed when pH
Figure 3. Predicted ionized calcium (Ca\textsuperscript{2+}) concentration based on total calcium at citrate concentrations of 0.1 (normal), 1.0, and 5.0 mmol per L and albumin concentrations from 0.5 to 10 g per dL (normal = 3.5 to 5.5 g per dL). Calculated values assume pH = 7.4 and normal physiological concentrations of bicarbonate and phosphate.
TABLE II

Ab Initio Calculations of Calcium Species

<table>
<thead>
<tr>
<th>mmol/L</th>
<th>Normal Adult</th>
<th>Normal Pediatric</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calcium</td>
<td>2.4</td>
<td>2.4</td>
<td>5.7</td>
</tr>
<tr>
<td>Total citrate</td>
<td>0.10</td>
<td>0.10</td>
<td>5.9</td>
</tr>
<tr>
<td>Total phosphate</td>
<td>1.0</td>
<td>1.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Ca(citrate)⁻</td>
<td>0.038</td>
<td>0.039</td>
<td>3.33</td>
</tr>
<tr>
<td>Ca(HCO₃)⁺ + Ca(CO₃)</td>
<td>0.080</td>
<td>0.080</td>
<td>0.10</td>
</tr>
<tr>
<td>Ca(PO₄)⁻ + Ca(HPO₄)</td>
<td>0.030</td>
<td>0.049</td>
<td>0.11</td>
</tr>
</tbody>
</table>

was adjusted; Ca⁺⁺ varied from 1.96 to 1.22 mmol per L. Physiological variations of 20 percent in pH are, however, incompatible with life. Variations in total calcium concentration from 20 percent below to 20 percent above normal changed Ca⁺⁺ from 1.38 to 2.14 mmol per L. Albumin had a lesser effect on Ca⁺⁺ (see figure 3), and the effect of changes in bicarbonate concentration were minimal (1.78 to 1.73 mmol per L). No other constituent in table I altered Ca⁺⁺ concentration by more than one percent. It is evident from these calculations that the Ca⁺⁺ in normal blood is primarily sensitive to the albumin concentration, and at high citrate concentration (>5 mmol per L), Ca⁺⁺ is regulated almost exclusively by the buffering ability of citrate.

**CLINICAL CONSEQUENCES OF IONIC HYPERCALCEMIA**

Cardiac dysrhythmia and generalized tetany are the most widely recognized clinical manifestations of low ionized calcium. Munoz et al⁸ suggested an additional complication in liver transplant patients that may be related to disrupted calcium homeostasis. They described ectopic soft tissue calcification in seven patients following liver transplantation and noted that these patients had been transfused with significantly greater amounts of blood products and had significantly higher parathyroid hormone (mid-molecule assay) levels than a matched group of 12 liver transplant patients who had no evidence of calcium deposition. Although there were no significant differences in total or ionized calcium levels between the two groups, these investigators suggested that transient hypercalcemia and transiently elevated Ca⁺⁺, which may have occurred during therapeutic infusion of calcium, resulted in calcification of soft tissue. It was not possible for us to detect a pattern of elevated intact parathyroid hormone levels related to either calcium infusion or decreased Ca⁺⁺ concentration in this patient, although CT and echocardiographic studies revealed calcifications in brain and heart tissue, respectively. The solubility of crystalline calcium citrate, Ca₃(C₆H₅O₇)₂ • 4H₂O, at 25°C is 1.48 mmol per L, and this raises a question of whether or not calcium citrate concentrations 50 times the normal may result in precipitation and calcium deposition. This compound contains three equivalents of calcium and two of citrate, so concentrations of these constituents exceeding 4.44 mmol per L and 2.96 mmol per L, respectively, have the potential for precipitating calcium citrate. Both of these limits were exceeded in this patient. Physiological temperature, however, and the decreased activity product of calcium and citrate at the relatively high ionic strength of blood would increase the solubility of calcium citrate. Whether or not precipitation of calcium citrate resulted in the soft tissue calcium deposition described by Munoz and co-workers is, therefore, highly speculative. Calculations of relative supersaturations for various calcium complexes in this patient's serum indicate that, among the predicted calcium complexes, apatite (Ca₅(PO₄)₃OH) was nearest its limit of solubility.
Conclusion

The calcium measurements in this patient illustrate an extreme case of hypercitricemia resulting from massive transfusion and a prolonged absence of hepatic function. Persistent ionic hypocalcemia was accompanied by markedly elevated total calcium as the excess citrate buffered free calcium at well below normal concentrations. Hemodialysis during the anhepatic period helped to remove citrate and release calcium ion. Following a successful transplant and recovery of hepatic function, citrate concentration fell to normal and calcium status returned to normal. Maintenance of ionized calcium within clinically acceptable limits was difficult in the presence of high citrate concentrations; aggressive infusion of supplemental calcium achieved only limited success. Although the concentration of calcium ion ordinarily depends most heavily on albumin and total calcium, citrate at high concentrations eclipses the calcium ion buffering capacity of albumin and dramatically alters the total calcium:ionized calcium ratio in blood. Extreme hypercitricemia results in apparently discordant laboratory values for total and ionized calcium, and the physician must be aware of the altered calcium ion buffering properties of blood in the transfused anhepatic patient. Therapeutic measures aimed at citrate removal are required before satisfactory calcium status can be restored.

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Thanks are extended to Dr. Daniel L. Purich, of the Department of Biochemistry and Molecular Biology, for his helpful comments. We would also like to acknowledge the following members of the University of Florida Liver Transplant Team, for their contributions to the care of this patient: Max R. Langham, Jr., M.D., Samuel M. Mahaffey, M.D., Matthew E. Brunson, M.D., Richard J. Howard, M.D., Joel A. Andres, M.D., Donald A. Novak, M.D., Gary L. Davis, M.D., C. J. Paris, P.A., and Sandra Haiman, R.N.

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