Citrate Toxicity During Massive Blood Transfusion

Walter H. Dzik and Scott A. Kirkley

THE USE OF sodium citrate as the blood anticoagulant for transfusion science dates from 1914 to 1915 and the almost simultaneous publication of the work of four independent investigators. Articles from Hustin,[1] Agote,[2] Weil,[3] and Lewisohn,[4] published between May 1914 and January 1915, each reported the successful use of citrated blood for human transfusion. Because citrate was known to be toxic to animals, Lewisohn carefully titrated the minimum concentration of citrate required to prevent clotting. Despite the demonstration by Weil that citrated blood could be stored for several days and still be effective,[3] and the discovery by Rous and Turner[5] that citrated blood supplemented by dextrose was capable of more prolonged storage, citrated blood was not quickly accepted by the general medical community. Though used with success in limited trials on the battlefield in World War I,[6] transfusions with citrated blood were often associated with chills and fever which were incorrectly attributed to the citrate. Febrile reactions were so common with citrated blood that during the 1920s most transfusions consisted of the rapid transfers of nonanticoagulated whole blood, and the use of stored citrated blood did not become commonplace until the mid-1930s.[7]

While improvements in transfusion technology and the establishment of blood banks made the administration of blood a standard procedure in the operating room, blood usage was generally limited to a few units for any given patient. Advances in knowledge of the biochemistry of citrate and calcium led to an improved understanding of their interaction as well as the relationship of serum ionized $Ca++$ to total serum calcium. The development of citrate toxicity due to acute hypocalcemia was demonstrated in dogs as early as 1944.[8] After World War II surgical techniques of increas-

ing complexity required rapid transfusion of larger volumes of blood. In 1955 several cases of 'citric acid intoxication' following transfusion were reported by Bunker.[9] In the years that followed, numerous investigations were published and considerable controversy developed regarding the management of citrate toxicity during massive transfusion. Recognition and therapy of citrate toxicity was enhanced by the widespread application in the 1970s of ion selective electrodes capable of accurate measurement of the level of ionized $Ca++$. With the development of advanced trauma care, liver transplantation, and prolonged extensive surgical procedures in pediatrics, there has been a renewed interest in the role of citrate toxicity in the setting of ultramassive transfusion.

This review focuses on citrate toxicity during massive blood replacement in adults. The chemistry of the citrate calcium interaction; the dose, distribution, metabolism, and excretion of citrate; the toxic effects of citrate; and the treatment of citrate induced hypocalcemia are discussed. Occasionally we have offered a personal view based on our own experience with massive transfusion during hepatic transplantation. Citrate toxicity is also discussed in most general reviews of massive transfusion.[10,11]

CHEMISTRY OF CITRATE

Citric acid (molecular weight 192 daltons) is a ubiquitous organic compound with three ionizable carboxyl groups. With three pKs (3.14, 4.77, and 6.39) all <7.4, the majority of citrate present in the body has all three carboxyl groups ionized (Fig 1). These ionized carboxyl groups are responsible for the major pharmacologic action of citrate, the binding of divalent cations. This binding is accomplished by having two of the valences occupied by the divalent calcium ion. Because of the third ionized carboxy group, citrate is still highly soluble in aqueous media even when bound to a divalent cation. Citrate may bind to any of the metallic divalent cations and subsequently lower the concentration of the ionized form of that cation. While most reports dealing with the effects of citrate deal with its effects on ionized calcium, well documented depressions of magnesium have

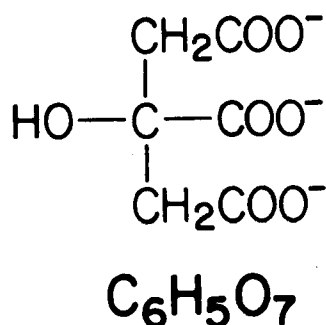$$CH_2COO^-$$
$$|$$
$$HO-C-COO^-$$
$$|$$
$$CH_2COO^-$$

$$C_6H_5O_7$$

Fig 1. Chemical structure of citrate.

also been reported.[12,13] Citrate binds slightly stronger to $Mg++$ (formation constant 2.9×10^3) than it does to $Ca++$ (formation constant 1.88×10^3).[12,14,15] Citrate is found in all human cells and is an intermediary in the Kreb's citric acid cycle. Because the citric acid cycle takes place within the mitochondria of a cell, tissues with a high number of mitochondria per cell (such as liver, skeletal muscle, and kidney) contain larger amounts of those enzymes responsible for the production and metabolism of citrate. While neither a common nor routine laboratory test, plasma citrate levels can be measured by several methods. One common method involves incubation of plasma with bacterial citrate lyase in the presence of zinc ions. The reaction produces oxaloacetate which is then acted upon by malate dehydrogenase resulting in the production of NADH from NAD. Production of NADH is measured spectrophotometrically after the serum proteins are precipitated. The normal adult plasma concentration of citrate is from 0.9 mg/dL to 2.5 mg/dL when measured with the citrate lyase method (Table 1). Slightly higher levels are found in children and in patients with hepatic or renal disease.[16,17]

MEASUREMENT OF CALCIUM

Early in this century it was realized that calcium exists in biologic fluids in at least two forms, one diffusable across a dialysis membrane, the other

Table 1. Normal Adult Concentrations of Citrate and Ionized Calcium

	Citrate	Ionized Calcium
mg/dL	0.9–2.5	4.5–5.4
mEq/L	0.14–0.39	2.3–2.7
mmol/L	0.047–0.130	1.1–1.4

nondiffusable. The nondiffusable form was found to be bound to serum proteins, especially albumin. By the 1930s most researchers in the field agreed that the diffusable portion of calcium existed in two states, bound to diffusable small ligands such as lactate or citrate, and in the free or ionized state.[18] These three states exist in equilibrium in the plasma and it is generally agreed that ionized $Ca++$ is the physiologically active form. In the healthy human, approximately 47% of calcium is in the ionized form, 40% bound to serum proteins (mostly albumin), and approximately 13% bound to smaller ligands.[19] These proportions differ with changes in pH, protein concentration, ligand concentration, and total calcium concentration.

While measurement of total serum calcium remains useful for gross or chronic disturbances of serum calcium, acute changes in ionized $Ca++$ are often missed with these measurements. Most laboratories use dye binding methods such as orthocresolphthalein or arsenazo III dye where the change in absorption by the dye is proportional to the total calcium concentration.[20] Atomic absorption spectrophotometry is also used and is an accurate and reproducible method, but is rarely automated and requires careful maintenance and standardization. While these methods have good accuracy and precision, the difficulty is that total calcium measurements may not accurately reflect the concentration of ionized calcium which is the physiologically active form.

Early attempts to determine the ionized $Ca++$ relied on nomograms for estimation of ionized $Ca++$ from measured total calcium. In 1935, McLean and Hastings, in their extensive monograph on calcium in body fluids, developed a nomogram for estimating the concentration of ionized $Ca++$. The nomogram was based on the total calcium and total serum protein concentration at a set pH and temperature.[18] With the advent of the more rapid ion selective electrode measurement of ionized $Ca++$, this nomogram and derivations of it, have been shown to give poor estimates of the ionized $Ca++$ levels.[21,22,23] The inaccuracies of early nomograms likely result from the assumption that pH and small ligand concentrations had little effect on ionized $Ca++$ concentration. Another outdated method for determining ionized $Ca++$ is the method of Soulier which estimated the ionized $Ca++$ by its effect on the thromboplastin time of decalcified

plasma. This method is poor for ionized Ca+ + greater than 1.5 mmol/L (3 mEq/L) and can not measure values <0.5 mmol/L (1 mEq/L).[19]

The modern era of measurement of ionized Ca+ + began with the introduction of ion selective electrodes: Initially, ion selective electrodes were limited by inference from other blood and serum components. Present day ion selective electrodes operate by comparing the binding of ionized Ca+ + with one side of a non-permeable membrane which is specific for binding calcium ions and comparing it to a reference solution bathing the other side of the membrane.[20] If fewer Ca+ + ions adhere to one side of the membrane than the other, an electric potential is set up which can be measured via a second reference electrode, in contact with the serum sample, acting as a salt bridge. The membranes bind the calcium by either an ion exchange mechanism, where the binding moiety of the membrane forms a calcium salt, or by forming a neutral but sterically and electrostatically favorable binding pocket for the calcium ion.[20] Both these methods give measurements which are rapid, highly reproducible, and can be adjusted to sample whole blood, plasma, or serum. Measurement devices which employ ultrafiltration or dialysis are not as useful because ligand bound calcium, including citrate bound calcium is also measured.[20] One drawback to the ion selective electrode is the lack of a standard reference method, making results between different devices and different laboratories difficult to compare. Work is presently being done to come to an agreement for such a standard. Thus,

normal ranges depend on the individual laboratory. Estimations of the normal range of ionized Ca+ + are given in Table 1.

CITRATE DOSAGE, METABOLISM, AND CLEARANCE

Dosage and Distribution

The level of citrate in the bloodstream during massive transfusion results from the net balance of citrate dose versus citrate removal. Due to the complex interrelation of several factors affecting citrate delivery and removal, predicting the level of citrate and consequently its effect on the level of ionized Ca+ + is extremely difficult.[24] The dose of citrate is determined by several factors including the particular blood component, the anticoagulant preservative formulation, the rate of administration, the recipient blood volume, and the duration of administration. The citrate burden of various anticoagulant preservative formulations is shown in Table 2. Citrate (MW = 189) is present as both trisodium citrate dihydrate (MW = 291) and citric acid monohydrate (MW = 210) in most formulations. Given the molecular weights, citrate represents 65% and 90% of trisodium citrate and citric acid respectively. The concentration of citrate can be estimated for whole blood, red blood cells, and fresh frozen plasma (FFP) or platelets (Table 2). The highest concentration of citrate is naturally found in FFP. Although for many years acid-citrate-dextrose (ACD) solution presented the greatest citrate doseto the blood recipient, the newest additive formulations (AS-3) have the

Table 2. Citrate Content of Various Anticoagulant–Perservative Formulations

	ACD	CPD/CPDA-1	AS-1	AS-3
Grams trisodium citrate (2H$_2$O)	1.485	1.656	1.656	2.244
Grams citric acid (H$_2$O)	.540	.206	.206	.248
Grams citrate per unit	1.451	1.261	1.261	1.681
Concentration of citrate per liter (mmol/L)	7.6	6.7	6.7	8.9
Concentration of citrate (mg/dL)* in:				
Whole Blood	280	246	206	274
Packed RBC	87	76	54	181
FFP	436	384	384	384
Quantity of citrate (mg)* in:				
Whole Blood	1451	1261	1261	1681
Packed RBC	200	176	176	596
FFP	976	843	843	843

* Calculated assuming a 450 mL donation; HCT, 41, and no movement of citrate into cells. For ACD, CPD and CPDA-1 assumes the production of packed RBC with Hct, 80; FFP, 230 mL; and platelet concentrate, 55 mL. For AS-1 and AS-3 assumes production of red blood cells with final Hct, 56; FFP, 230 mL; and platelet concentrate, 55 mL.

highest quantity of citrate. AS-3 red blood cells deliver three times the quantity of citrate as ACD or CPD packed cells.

For any given blood product, the rate of administration and the size of the recipient are the key determinants of citrate administration. Thus, studies of citrate toxicity generally refer to mg citrate/kg recipient/minute. For example, one unit of CPDA-1 whole blood administered to a 70 kg man over five minutes corresponds to 1261 mg citrate/70 kg/5 min or 3.6 mg citrate/kg/min. Citrate is rapidly removed by the liver and kidney. However, the distribution of citrate also plays an important role in determining the citrate level following transfusion. Although the relative importance of metabolism v redistribution of citrate is not fully investigated, citrate can be considered as a first order approximation to be distributed throughout the extracellular fluid space. This distribution occurs within five minutes of infusions of mild-to-moderate quantities of citrate. For example, in one study 500 mL of citrated blood was infused over five minutes to adults (4 mg citrate/kg/min × 5). Citrate levels were measured every minute. At the end of the infusion, the measured citrate level was 66% that which would have been predicted had citrate remained in the intravascular space. Within three minutes after stopping the infusion, the blood level was equal to that which would have been predicted had citrate distributed itself over the extracellular volume.[25] After infusion of 2.35 mg citrate/kg/min over ten minutes, the concentration of citrate at the end of the infusion was equal to that which would have been expected from redistribution over the extracellular volume.[9] However, rapid challenges of large quantities of citrate can exceed redistribution, metabolism, and excretion.[26] Five patients receiving a mean of 5.5 mg citrate/kg/min over 15.6 minutes developed average peak citrate levels of 62 mg/dL which was 1.5 times greater than the expected citrate level assuming complete extracellular redistribution.[27] As a result of metabolism and redistribution outside the vascular space, there is an initial rapid exponential decline in the concentration of citrate after cessation of rapid blood infusion. Further removal of citrate results from continued metabolism and renal excretion. Due to the permeability of cells to citrate and the apparent large volume of distribution, complete metabolism and excretion following prolonged

rapid transfusion would be expected to take several hours. Evidence for such delayed clearance has been found in studies of metabolism and renal excretion of citrate following massive transfusion.[28,29]

The duration of citrate administration is also a key determinant of the blood citrate level. Platelet-pheresis studies with relatively constant low dose citrate infusion over approximately 100 minutes have shown that the citrate level attained is only 25% of that which would be predicted from redistribution throughout the extracellular space. Without metabolism and excretion of citrate during the period of infusion, toxic levels of citrate would have developed.[30,31] In two independent studies very similar blood citrate levels developed in individuals receiving 4 mg citrate/kg/min over five minutes as were found in individuals receiving only 1.6 mg citrate/kg/min over 113 minutes.[30] Thus, prolonged rapid blood transfusion would be expected to result in higher citrate levels than equally rapid infusions of short duration. This is of clinical importance in settings such as hepatic transplantation where rapid transfusion may continue for hours. With prolonged duration of blood administration, citrate metabolism and excretion (rather than distribution) become the most important defense against the development of toxic concentrations of citrate.

Another means for the body to deal with a low ionized calcium due to a citrate load is to mobilize stores of calcium. Parathyroid hormone (PTH) levels are measurably elevated within 2 to 4 minutes after administration of citrate[32] and reach peak levels between 5 and 15 minutes after infusion.[13] Elevations in PTH have been found both experimentally and during surgical procedures requiring blood support in adults and during exchange transfusions in infants.[13] During plasmapheresis, one study found that the ionized calcium remained low with continuous citrate infusion but the total calcium dropped at first, then returned to near normal levels. Ultrafilterable calcium, which equals the total ionized Ca + + plus ligand bound Ca + + (but not the protein bound calcium) continued to rise during the apheresis procedure.[13] This is probably a result of calcium mobilization with most of the newly mobilized calcium being bound to the infused citrate. Protein bound citrate decreased by 35% during the procedures, representing a significant buffer of

calcium when ionized Ca + + levels drop. These changes may be affected by temperature, pH and other alterations in addition to the variability of various PTH assays among different methods and laboratories.[33]

Citrate Metabolism

The metabolism of citrate involves multiple biochemical pathways (Fig 2). Citrate can directly enter the Kreb's tricarboxylic acid cycle to be completely metabolized to CO_2 and H_2O, can participate in fatty acid and amino acid synthesis, and can be converted to glucose via gluconeogenesis. Each of these pathways will be reviewed in turn. Overall, the complete oxidation of citrate results in the production of CO_2 and H_2O. Complete metabolism of ionic citrate^{-3} to nonionic endproducts consumes three H^+ ions according to:

$$C_6H_5O_7^{-3} + 4.5\ O_2 + 3\ H+ \rightarrow 4\ H_2O + 6\ CO_2$$
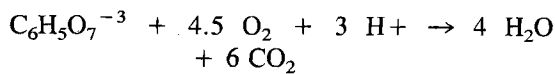
Exogenous citrate can be actively transported through the mitochondrial membrane to participate in Kreb's cycle reactions. Tricarboxylic acids (citrate, isocitrate, aconitate) are actively transported across the mitochondrial membrane by carrier molecules. Movement of citrate into the mitochondria is linked to movement of malate out of the

mitochondria. Once inside the mitochondria, two carbons of citrate are removed and two molecules of CO_2 formed. With additional turns of the Kreb's cycle, the original carbons of citrate are converted to CO_2. Since turning of the Kreb's cycle regenerates cycle intermediates from the carbons introduced by acetylCoA, metabolism of exogenous citrate exclusively by Kreb's cycling would not reduce the concentration of citrate nor have a net effect on acid/base balance. Instead, the Kreb's cycle intermediates are able to be metabolized by additional pathways. Within the mitochondria, oxaloacetate can be converted to pyruvate by the action of pyruvate carboxylase, which under normal conditions serves to convert pyruvate to oxaloacetate in an anapleurotic reaction. Conversion to pyruvate requires biotin as a cofactor, consumes a second $H+$ ion, and yields CO_2. The pyruvate formed can be then converted to acetylCoA in a series of reactions that consume the third $H+$ ion and yield an additional CO_2.

However, other Kreb's cycle intermediates are also able to be transported through the mitochondrial membrane. Alpha-ketoglutarate and malate are two such intermediates. As the concentration of cycle intermediates increases inside mitochondria following citrate administration, alpha-ketoglutarate and malate would be expected to leave
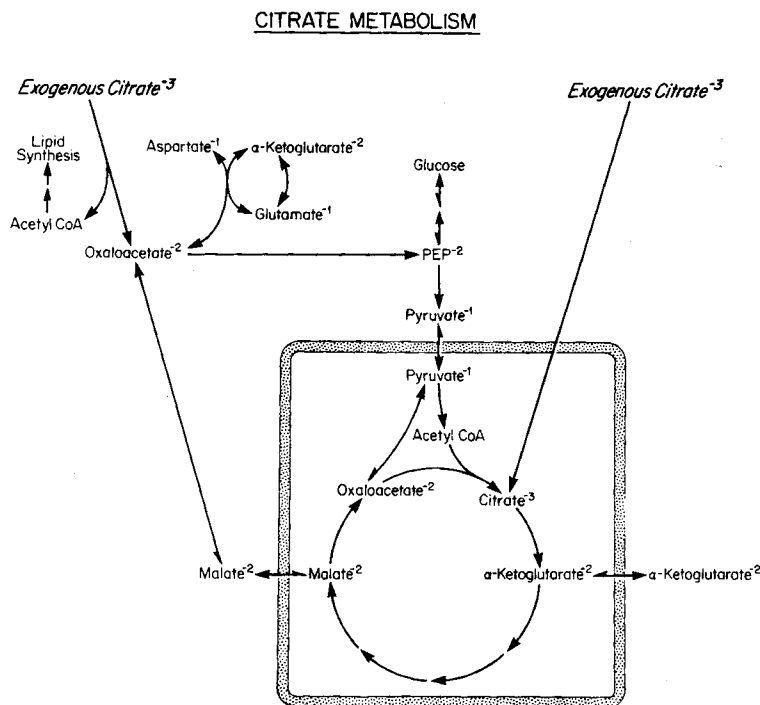
CITRATE METABOLISM

Fig 2. Metabolism of citrate.

the mitochondria. Evidence suggests that metabolism of exogenous citrate results in net transport of malate outside the mitochondria. Because the metabolism of citrate to malate involves incomplete turning of the Kreb's cycle, the normal balance of H+ ion production and consumption is not maintained and a net single H+ ion is consumed. Once in the cytoplasm, malate is able to be converted to oxaloacetate which can participate in two biochemical pathways described below.

Oxaloacetate is a ketoacid and as such can undergo a transaminase reaction with glutamate to form aspartate and alpha-ketoglutarate. The reaction is reversible with no net loss of oxaloacetate carbons and no net production or consumption of H+ ion. A second pathway of cytoplasmic oxaloacetate metabolism involves conversion to phosphoenolpyruvate (PEP) by PEP carboxykinase. This enzyme is a key enzyme in gluconeogenesis and is found in cells such as those of the liver, kidney, and skeletal muscle. The reaction requires energy in the form of GTP and results in the release of CO_2. Thus, exogenously administered citrate can be converted to PEP through oxaloacetate. Once formed, PEP can be converted to glucose via the enzymes of gluconeogenesis. However, during conditions of ATP depletion the metabolism of PEP to pyruvate is favored. The formation of pyruvate from PEP consumes an additional H+ ion. Pyruvate is freely permeable to the mitochondria. Once inside the mitochondria, it can be converted to acetylCoA in a series of reactions that release CO_2 and consume one H+ ion. The acetylCoA is then free to combine with mitochondrial oxaloacetate and be metabolized to CO_2 and H_2O. Thus, the complete metabolic breakdown of the six carbons of citrate may also occur via pathways that include extramitochondrial enzymes, produce CO_2, and consume three H+ ions. Citrate conversion to glucose via gluconeogenesis yields fewer CO_2 molecules but also consumes three H+ ions.

An additional biochemical pathway of citrate metabolism is that provided by citrate cleaving enzyme (Fig 2). This enzyme, ATP-citrate lyase is found in liver and adipose cells and splits cytoplasmic citrate in the presence of CoASH to form acetylCoA and oxaloacetate. One H+ ion is consumed in the reaction. The acetylCoA formed is not permeable to the mitochondria, but is able to participate in the biosynthesis of fatty acids. Thus,

increased cytoplasmic citrate as a result of massive transfusion might be expected to temporarily stimulate fatty acid synthesis. The cytoplasmic oxaloacetate formed as a result of citrate cleavage may then be further metabolized according to the reactions outlined above. The metabolism of cytoplasmic citrate via citrate cleaving enzyme to oxaloacetate and then to PEP, pyruvate, and mitochondrial acetylCoA also consumes three H+ ions.

The rate limiting step of citrate metabolism following massive transfusion is unknown. Evidence from liver transplantation and from studies of renal excretion of citrate suggest that alkalemia slows the metabolism of citrate presumably by retarding movement of citrate and malate across the mitochondrial membrane. Whether factors which might influence the activity of citrate cleaving enzyme exert an overall effect on the metabolism of administered citrate is less well studied.

Citrate Clearance

Clearance of citrate is highest in those organs which receive a high proportion of the cardiac output and which are composed of cells with numerous mitochondria.' Cells which are dependent on glycolysis for their energy needs, such as red blood cells, have low levels of citrate and do not remove citrate from the circulation. Liver, kidney, and skeletal muscle are responsible for most of the metabolism and excretion of citrate.

Since the 1930s, investigators have recognized that basal serum citrate levels are slightly higher in patients with chronic liver disease than in those without liver disease.[16,34] In normal fasting humans, about 20% of the endogenous citrate in the serum is removed with each pass through the liver.[16,34] In one study when a perfusate containing 54 mg/dL citrate was presented to isolated calf livers, there was a rapid initial clearance of 50% of the citrate over the first thirty minutes followed by a more gradual clearance of the remaining 50% over the next two and a half hours.[35] Convincing evidence of the importance of the liver in citrate clearance in humans is seen during liver transplantation when there is a marked rise in serum citrate with concomitant drop in ionized Ca++ during the anhepatic phase of the surgery with a rapid reversal of these changes when the new liver is perfused.[22,36,37] Citrate clearance by the liver appears to be greatly reduced during hy-

pothermia, increasing as the patient is warmed. Hypotension with decreased hepatic perfusion also leads to a decrease in clearance of citrate by the liver.[28]

The kidney is also essential for citrate clearance. Some investigators feel that the kidney is the most important organ for citrate clearance since it can metabolize large amounts of citrate and also can excrete nonmetabolized citrate in the urine.[38,39,40] Estimates of the amount of exogenous citrate handled by the kidney range from 20%[28,36] to 68%[40] of a given load in humans. Most of the uptake of citrate by the kidney is from reabsorption of filtered citrate in the proximal tubule but up to 30% of the total renal uptake is peritubular uptake from postglomerular blood.[41] The proximal tubular cells, which are rich in mitochondria, are responsible for the metabolism of citrate. Normally <1% of the citrate filtered by the kidney is excreted in the urine, but in the presence of high plasma concentration of citrate or alkalemia, there is a dramatic increase in the fractional excretion in

the urine.[42] Up to 60% of filtered citrate may appear in the urine of humans during metabolic alkalosis.[41] This increase in excretion results from inhibition of citrate transport into mitochondria by the tricarboxylic acid carrier. Other conditions leading to increased urinary excretion of citrate include high levels of other organic acids of the Krebs' cycle such as fumarate or malate and specific inhibitors of the Krebs' cycle pathways such as fluorocitrate and malonate. Calcitonin, vitamin D, calcium, and magnesium have also been shown to increase citrate excretion.[41] Conversely, the excretion of citrate is reduced during acidosis.[42] The effects of $K+$ and bicarbonate levels on citrate excretion appear to be due to their effects on acid base balance rather than a direct effect of the ion.[42] In the clinical setting, clearance of citrate by the kidney may also be limited by the decreased glomerular filtration rate and renal ischemia that frequently develops during massive transfusion. More work is needed to assess the relative contributions of the liver and kidney in citrate me-
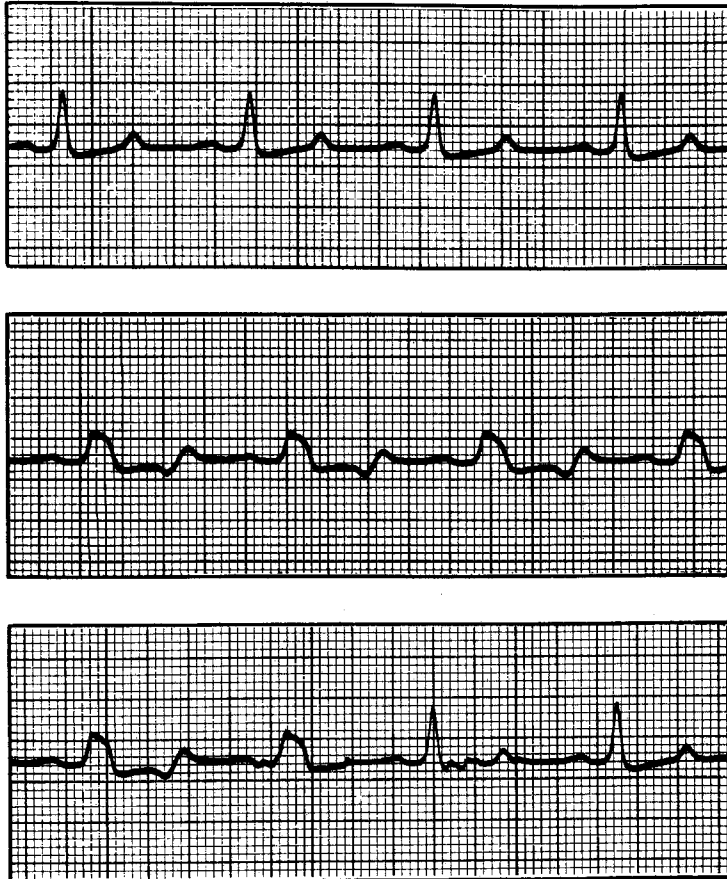

Fig 3. Effect of rapid citrate infusion on the EKG. The top panel shows a prolonged QoTc interval. Following a period of rapid transfusion, the ionized calcium fell to 0.6 mEq/L and a widened QRS complex developed (middle panel). The effect was reversed with intravenous administration of calcium (bottom panel).

tabolism and excretion during massive citrate loads.

TOXICITY OF CITRATE

Effects on the EKG

Depression of the ionized Ca + + has characteristic effects on the EKG. The most commonly recognized effect is prolongation of the QT interval (Fig 3). The QT interval corresponds to the time from contraction to repolarization and varies with the heart rate. The corrected QT interval (QTc), which takes into account the effect of heart rate, is the time from the origin of the QRS complex to the end of the T wave divided by the square root of the RR interval. The corrected QT interval may also be defined by substituting the time from the origin of the QRS complex to the origin of the T wave rather than the end of the T wave, and is then referred to as the QoTc.[43] The QTc in normal adults ranges from 350 msec to 440 msec[44] and the QoTc from 180 msec to 240 msec.[45] The measured QT interval for a particular individual is influenced by numerous factors including age, sex, autonomic innervation, myocardial ischemia, antiarrhythmics, hypothyroidism, and severe hypothermia in addition to hypocalcemia.[46]

The relationship between hypocalcemia and a prolonged QT interval has been recognized for many years. In early studies, sodium citrate was infused into normal conscious volunteers or patients under anesthesia and the prolongation of the QT interval observed.[26,27,47] Similar studies in dogs documented similar effects on the EKG and demonstrated that the effects were abolished with administration of calcium.[48,49,50] Despite the reproducible effect of citrate on the QT interval, studies in patients undergoing transfusion showed that the QT interval was an unreliable guide to administration of supplemental calcium.[24] The relationship between the level of ionized Ca + + and the length of the QT interval during rapid transfusion was found to be nonlinear and has been described by both logarithmic[51] and hyperbolic[45] curves. At mild depressions of the ionized Ca + + (>1.75 mEq/L), little or no effect on the QoTc is seen. At more moderate depressions of ionized Ca + + (1.0 mEq/L to 1.75 mEq/L), the average QoTc interval for a group of individuals will become prolonged.[45,51] This prolongation

increases more sharply at severely depressed levels of ionized Ca + + (0.25 mEq/L to 1.0 mEq/ L).[45,51] A study of twenty adults undergoing intraoperative rapid transfusion correlated corrected QT intervals and ionized Ca + + and found the correlation coefficient was only r = .61.[45] The correlation was slightly higher using QoTc compared with QTc. The 95 percent confidence intervals indicated that a given QoTc was often associated with a twofold or greater range in predicted ionized Ca + + levels.[45] Because the range of ionized Ca + + associated with any given QTc interval is broad, measurement of the QTc interval cannot be substituted for accurate measurement of the ionized Ca + + in patients likely to develop citrate toxicity during massive transfusion.

The effect of citrate infusion on the QT interval has also been studied in the setting of apheresis procedures uncomplicated by many of the confounding variables found during intraoperative massive transfusion. One study of 15 apheresis procedures measured a 32% average decrease in the level of ionized Ca + + with a corresponding prolongation of the QT interval by an average of 80 msec (range 50 msec to 120 msec).[30] These effects occurred during an average citrate infusion of 1.58 mg/kg/min for 113 minutes resulting in a mean postprocedure citrate level of 26.7 mg/dL. Although the QT interval correlated with the rate and dose of citrate administered, the level of ionized Ca + + could not be predicted from the QT interval. Following cessation of the procedure, the QT interval returned to normal in 2 to 15 minutes which was significantly less than the time required for levels of ionized Ca + + and citrate to return to normal. In another study of 12 plateletpheresis procedures, prolongations in the QT, QTc, QoT, and QoTc were correlated with the level of ionized Ca + + during procedures that infused citrate at an average rate of 1.38 mg/ kg/min.[52] Although the best correlation was found with the QoTc (r = .59), the QoTc could not predict the level of ionized Ca + + . Among individuals with depressed levels of ionized Ca + + , only 9 of 30 QoTc measurements were longer than the longest baseline reading. In addition, the authors found that the level of ionized Ca + + could not be predicted by the degree of prolongation of the QT interval over baseline for a given individual. In a third study of 79 platelet-

pheresis procedures levels of ionized $Ca++$ ranging from 2.25 to 1.35 mEq/L were found to be in linear relation to levels of serum citrate ranging from 2 to 40 mg/dL.[31] However, the average QTc prolonged by only 13 msec following the procedure.

When ionized $Ca++$ levels become severely depressed, other effects on the EKG are observed. Early reports of ventricular fibrillation and cardiac arrest during massive transfusion lack complete documentation of electrolyte and acid/base disturbances.[53,54,55] Progressive severe hypocalcemia would be expected to result in prolongation of the QRS complex. Fig 3 shows an intraoperative EKG from an adult patient in our hepatic transplantation program. Before the onset of rapid transfusion, the tracing showed a normal QRS morphology but a prolonged QoTc interval. In the middle panel is shown the EKG during a period of rapid blood infusion with a corresponding pH = 7.26, $K+$ = 5.2, Temperature = 92°F and ionized $Ca++$ = 0.6 mEq/L. Following two grams of intravenous (IV) calcium chloride and no change in pH, $K+$, or temperature, the EKG returned to normal (bottom panel). Such dramatic effects on the EKG are rare outside the setting of liver transplantation or rapid blood exchange in neonates.

The mechanism of prolongation of the QT interval during citrate toxicity and hypocalcemia is likely related to the plateau phase of the myocardial action potential.[56] The action potential of ventricular depolarization/repolarization is divided into discrete phases which are regulated by selective movement of ions across the myocardial cell membrane. The initial rapid depolarization results from a closure of $K+$ channels and an opening of fast moving $Na+$ channels. This corresponds temporally to the QRS tracing on the EKG. Depolarization is then sustained by a balanced slow inward movement of $Ca++$ and $Na+$ and a slow outward movement of $K+$. Gradually the potential across the membrane becomes more negative as $K+$ ions leave the cell. Once a threshold is reached, fast $K+$ channels open and a sudden movement of $K+$ outside the cell repolarizes the membrane. Myocardial repolarization is less well coordinated for the aggregate of myocardial cells than depolarization and, therefore, results in a broad wave of repolarization (T wave). The duration of the QT interval is, therefore, dependent on the duration of the action potential plateau. A reduction in extracellular ionized $Ca++$ results in a decrease in the slow outward $K+$ current and prolongation of the plateau. Because the action potential plateau without the T wave is measured by the QoTc, the QoTc correlates better with the degree of hypocalcemia than the QTc. Whether citrate anions have any independent effect on myocardial permeability to $Ca++$ or $K+$ and exert any effect on the action potential plateau independent of direct lowering of the extracellular concentration of ionized $Ca++$ is not well understood.

Effects on Ventricular Performance

In 1883, Sydney Ringer demonstrated that the isolated frog heart was capable of sustained contractions when suspended in saline.[57] One year later, Dr Ringer published a second paper in the same journal noting that the water used to prepare the saline of his previous study was not distilled water, but rather pipe water obtained from the New Water Company, London.[58] Chemical analysis showed it to be contaminated with appreciable quantities of calcium. Ringer repeated his experiments and discovered that sustained beating of the heart was dependent on the presence of extracellular calcium. In the century that has followed, an enormous body of information has developed which documents the importance of calcium in myocardial performance. An excellent review has been recently published.[59]

For over thirty years it has been recognized that massive transfusion with citrate toxicity and hypocalcemia would be expected to decrease cardiac performance and early studies in both humans[47,60,61] and animals[49,62,63,64] supported the concept. Nevertheless, acceptance of citrate toxicity was not without some degree of controversy.[26] The development of cardiac surgery and radical cancer surgery in the 1950s prompted several investigations into the cardiovascular effects of citrate. In an early study, Bunker examined the effect of transfusion on blood pressure and on serum levels of citrate and total calcium.[9] Twenty-four patients receiving a median of 3,500 mL of blood developed serum citrate levels of 10 mg/dL. The median rate of citrate infusion was 0.92 mg/kg/min (range 0.3 to 6). In 10 patients, systolic blood pressure fell to <100 mmHg. In a subsequent more detailed study in 1962, Bunker examined the cardiovascular effects of direct citrate

infusions into six lightly anaesthetized adults undergoing stripping of leg varicosities.[27] The patients were challenged with citrate at rates ranging from 3.7 to 7.4 mg/kg/min for 9 to 19 minutes. Recipients developed citrate levels of 30 to 77 mg/dL. In 5 of 6 individuals, there was a decline in stroke volume and left ventricular work, with a corresponding rise in pulse. Mean arterial pressure in these patients decreased 26%. The cardiovascular abnormalities corrected promptly with stopping the citrate or with infusion of calcium chloride. The one patient who developed no measurable cardiovascular changes was the patient who received the lowest citrate challenge (3.7 mg/kg/min) and who developed the lowest blood citrate level (30 mg/dL). In the same report, a similar effect was observed in dogs at citrate levels of 50 to 75 mg/dL. Serious cardiovascular depression or death occurred in the animals at citrate levels of 50 to 190 mg/dL and rates of citrate infusion of 10 to 15 mg/kg/minute. Subsequent studies in dogs by other investigators showed similar overall results.[48,50,65]

During the 1970s knowledge of the effects of citrate on left ventricular performance was refined. Rather than infusing citrate, these studies involved infusions of citrated blood versus recalcified citrated blood to which heparin was added. In one study of six patients undergoing open heart surgery,[66] infusion of warm citrated blood at 150 mL/min for three minutes resulted in a 28% decrease in cardiac output with no consistent change in mean arterial blood pressure, but with a rise in left atrial pressure. In contrast, the infusion of heparinized, recalcified, citrated blood at the same rate resulted in an 18% mean increase in cardiac output. Although administration of 200 to 300 mgm of (IV) calcium chloride had little effect on cardiac output prior to the infusion of the citrated blood, the same dose of calcium administered after the transfusion caused a mean increase of 24% in cardiac output. In the same study, the authors bled dogs to a mean systolic arterial pressure of 50 mmHg which was maintained for 15 minutes. Infusion of citrated blood at rates ranging from 2 to 3 mL/kg/min to 6 to 9 mL/kg/min was compared with the infusion of recalcified, heparinized blood at 6 to 9 mL/kg/min. Although the infusion of recalcified blood consistently resulted in an increase in aortic pressure and cardiac output with little rise in left

atrial pressure, animals receiving an equal volume of citrated blood incompletely restored aortic blood pressure and cardiac output and developed considerable elevation in left atrial pressures. The more rapid the rate of citrated blood infusion, the greater the depression in left ventricular performance. Four of nine dogs receiving citrated blood at maximal rates of 6 to 9 mL/kg/min suffered cardiac arrest. Thus, the overall effect of transfusion with citrated blood was to blunt the normal left ventricular response to volume loading.

The blunting of the left ventricular response to volume loading resulting from the rapid administration of citrated blood was conclusively demonstrated in man in a well designed 1976 study.[67] Nine patients undergoing coronary revascularization were studied. Each had normal ventricular function with ejection fractions >70 percent. Before cardiopulmonary bypass, the patients were transfused with two units of citrate-phosphate-dextrose (CPD) blood. Each unit was 37 C, pH 7.4 and <48 hours old. One unit was recalcified and heparinized and the other was heparinized but not recalcified. Each patient served as his own control. The order of transfusions was randomized and blinded to those recording cardiovascular measurements. Each unit of blood was transfused over three minutes (2 mL/kg/min for 3 min). After the first transfusion was administered, the equivalent volume of blood was removed, the patient allowed to return to a stable state, and the second unit infused. Cardiovascular measurements were taken every 45 seconds during the transfusions. The results from this study are shown in Figs 4 and 5. Although patients receiving one unit of citrated blood in three minutes increased left ventricular performance in response to volume, the magnitude of increase was blunted compared to those individuals receiving recalcified heparinized blood. This blunted response to volume loading was accompanied by a 27% decrease in the level of ionized Ca + + at the end of the three minute transfusion.

Several factors appear capable of rendering the myocardium more sensitive to the depressant effects of rapid infusions of citrated blood. Acidemia, hyperkalemia, and hypothermia have all been recognized to increase susceptibility to the cardiodepressant effects of citrated transfusions. Following early studies of cardiac autotransplanta-
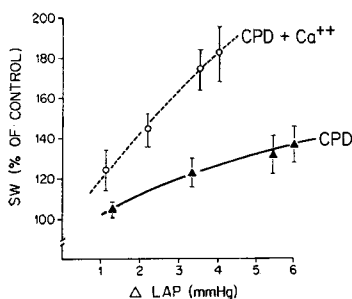
Fig 4. Effect of citrate on left ventricular performance. There is a blunted response to volume challenge with citrated blood compared with recalcified blood. Reprinted with permission.[67]

tion in dogs which suggested that denervation rendered such animals more susceptible to the effects of citrate,[68] a study was done which documented that the decline in left ventricular performance with citrated transfusions was even worse in animals that had been pretreated with a beta blocker (propranolol).[69] Albumin infusions which can further chelate ionize Ca + + were shown to have a minor deleterious effect on the level of ionized Ca + + and on cardiac performance in a group of patients resuscitated after trauma.[70] Regional or global myocardial ischemia results in a decrease in left ventricular performance during hypocalcemia.[71] A variety of drugs including anesthetic agents and calcium channel blockers are likely to also worsen the myocardial depression of hypocalcemia.

Recently there has been renewed interest in the effects of massive transfusion on cardiac per-
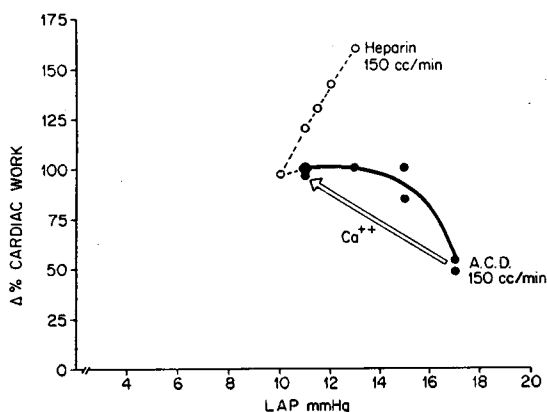
Fig 5. Effect of citrate on left ventricular performance. The blunted response to volume loading with citrated blood is reversed following calcium administration. Reprinted with permission.[66]

formance in the setting of hepatic transplantation.[72,37,36,22,73] Patients undergoing hepatic transplantation are uniquely susceptible to the development of citrate toxicity. Liver transplantation frequently involves rapid, massive transfusion of prolonged duration. A large proportion of the blood support is usually in the form of fresh frozen plasma with its high citrate concentration. The patients have decreased ability to metabolize citrate during the phase of surgery when the liver is absent (anhepatic phase). Finally, liver transplant patients frequently develop other metabolic abnormalities known to exacerbate citrate toxicity; hypothermia from extensive exposure of viscera, hyperkalemia from cellular release by the grafted liver, acidemia from decreased tissue perfusion and inability to metabolize lactic acid, and in some patients a decreased ability to mobilize calcium from bony stores as a result of preoperative hepatic osteodystrophy. One study noted hypotension in association with elevated central cardiac pressures and response to calcium treatment in two of eleven transplant recipients.[37] A recent detailed study of nine adults undergoing liver transplantation monitored cardiac performance with Swan-Ganz catheterization.[36] Median citrate levels rose to 113 mg/dL during the anhepatic phase of surgery. Calcium supplementation (mean 4.3 gm) was administered to prevent a fall in ionized calcium below approximately 50% of baseline thus maintaining ionized Ca + + > .56 mmol/L. During the period of peak citrate effect, there was a significant decline in left ventricular performance (cardiac index, stroke index, and left ventricular stroke work index) without any significant change in left or right cardiac filling pressures or systemic vascular resistance. The period of left ventricular depression was also characterized by hypothermia (33.6C). However, myocardial contractility was corrected by calcium supplementation without any change in body temperature. Following revascularization of the new hepatic graft, the levels of citrate and calcium gradually returned to their preoperative baseline.

The mechanism of decreased ventricular performance during citrate toxicity is presumably due to interference with the calcium coupled signal for myocardial cell contraction. Calcium moves into the myocardial cytosol from at least two sources during cell contraction. With the arrival of each action potential, external calcium rushes into the cell via the system of transverse T tubules of the

sarcolemma. This inward calcium movement then triggers the release of a second, quantitatively larger wave of calcium release from the sarcoplasmic reticulum. The increased cytosolic calcium then binds to troponin C thus changing the configuration of the thin filament so that actin is able to bind to myosin. The contractile response is a graded one, with maximal contractility requiring the presence of maximal levels of intracellular calcium. Citrate likely interferes with myocardial contractility by decreasing the availability of extracellular ionized Ca + +, thus reducing the stimulus for calcium release by the sarcoplasmic reticulum. In addition, intracellular citrate and/or its metabolites, eg, oxaloacetate, may directly bind intracellular calcium and thus decrease actin-myosin interaction. At low levels of myocardial work, citrate would be expected to have minimal effect since there is an excess of calcium available to the myofibrils. Because maximal myocardial response is dependent on maximal calcium availability, citrate would be expected to exert its greatest depressant effect on the maximally stressed heart. This blunting of left ventricular response to increasing stimulus as a result of excess citrate was demonstrated experimentally in clinical studies described above and in Figs 4 and 5.

Effects on Coagulation

Although patients undergoing massive transfusion frequently demonstrate multiple abnormalities of hemostasis, citrate toxicity is not felt to have any impact on coagulation. The depression of ionized Ca + + required to produce abnormalities of coagulation has been generally considered to be so low that severe cardiac dysfunction would develop before any abnormalities of coagulation could occur. Early studies which helped define the role of calcium in blood coagulation found that calcium levels of 0.2 mmol/L were necessary to prolong the whole blood or plasma clotting time.[74]

Standard tests of blood coagulation such as the prothrombin time and activated partial thromboplastin time would not reflect the effects of depressed ionized Ca + + because reagent calcium is added to the test sample at the beginning of the test. Platelet aggregation as routinely performed is also not designed to detect abnormalities induced by decreased ionized Ca + + since this in vitro test is generally done using citrated platelet rich plasma.

Ionized Ca + + plays an essential role in hemostasis and is necessary in nanomolar, micromolar, and millimolar concentrations for different aspects of normal platelet function and normal fibrin formation. Platelet adhesion to subendothelium is dependent on ionized Ca + +.[75,76] Signals for platelet activation are relayed to the cell's interior by a calcium dependent second messenger system common to many types of cells.[77] Platelets then undergo a characteristic shape change from disc to spiny sphere with central clustering of platelet granules. This shape change is mediated by the actomyosin system of proteins in a manner analogous to the events which occur in skeletal muscle.[78] Ionized Ca + + is released from an intracellular storage pool in the dense tubular system to stimulate this response.[79] The platelet release reaction and secretion of procoagulant alpha granule contents as well as calcium-rich dense granule contents are also signaled by the sudden rise in intracellular ionized Ca + + following platelet activation.[80] Platelet activation also involves changes in platelet membrane receptors most notably a conformational change in the GPI-Ib-IIIa heterodimer which serves as a calcium dependent receptor for fibrinogen binding and platelet-platelet aggregation.[81] Taken together these events constitute the basic platelet reaction which is coupled to an increase in the concentration of ionized Ca + + in the platelet cytosol from the 40 to 100 nmol/L range to 2 to 10 μm, a value which is still 1000 fold lower than the millimolar concentration of ionized Ca + + found in the plasma during citrate toxicity. Thus, citrate toxicity would not be expected to have any significant effect on the basic platelet reaction.

Despite the well known ability of platelets to activate and aggregate in vitro when suspended in ethylenediamino-tetra-acetate (EDTA) or solutions with micromolar concentrations of ionized Ca + +, there is indirect evidence that some aspects of platelet function may be, nevertheless, dependent on a higher concentration of extracellular ionized Ca + +. Platelet adhesion and thrombus formation on collagen or on aortic subendothelium was significantly decreased in citrated blood compared to native blood as the level of extracellular citrate reached 15 mmol/L.[82] Platelet adherence to subendothelial matrix via von Willebrand's protein was shown to require a concentration of at least 0.3 mmol/L ionized Ca + +.[76] This level of Ca + + is still well below

the level of ionized Ca+ + seen in most patients undergoing massive transfusion.

Still higher concentrations of ionized Ca+ + (in the millimolar range) appear to be essential for the normal function of several coagulation proteins. The stability of fibrinogen, factor V, and factor VIIIc are dependent on calcium. Of these factor VIIIc has the highest threshold requirement which is estimated to be 0.3 to 1 mmol/L ionized Ca+ +.[83] The binding factors II, VII, IX, and X to phospholipid membranes is also calcium dependent with a requirement for ionized Ca+ + in the millimolar range. It has been determined that at physiologic concentrations of ionized Ca+ + approximately 50% of potential platelet binding sites for prothrombin are saturated. Binding of factor X to the surface of membranes requires an even higher concentration of ionized Ca+ + than prothrombin.[84] Ionized Ca+ + is also an essential cofactor for several interactions in the coagulation cascade including activation of factor IX; activation of factor X by IXa, VIIIa, and phospholipid; activation of factor X by tissue factor and VIIa; cleavage of prothrombin to thrombin by prothrombinase; and crosslinking of fibrin by factor XIIIa.

Whether any of the complex interactions of coagulation is affected by levels of ionized Ca+ + resulting from massive citrate infusion is not completely established. We have observed depression in ionized Ca+ + resulting in levels from 1.3 mmol/L to 0.2 mmol/L during massive transfusion in the setting of liver transplantation. This level of hypocalcemia could possibly affect a number of coagulation processes which may require near normal millimolar concentrations of ionized Ca+ + including platelet adhesion, the stability of factor VIIIc, the binding of vitamin K dependent coagulation factors such as factor X and prothrombin to the platelet surface and the crosslinking of fibrin by factor XIII.

Recent preliminary reports from two laboratories investigating ultramassive transfusion during liver transplantation have suggested that extreme citrate toxicity during this surgical procedure may affect coagulation as measured in vitro. Kang, et al demonstrated an effect on the thromboelastogram at levels of ionized Ca+ + in the 0.2 mmol/L range. A progressive increase in the reaction time and a minor decrease in the maximum amplitude of the thromboelastogram were observed.[85] We have

noted a dramatic prolongation of the whole blood activated clotting time in normal blood samples carefully citrated to produce a level of ionized Ca+ + seen during liver transplantation with massive transfusion.[86] Although no abnormalities in coagulation would be expected at levels of ionized Ca+ + occurring during most clinical situations of rapid transfusion, these observations raise the possibility of an adverse effect on coagulation during extreme citrate toxicity. Future studies may define more clearly which aspects (if any) of coagulation are most affected by a severe reduction in ionized Ca+ +.

Metabolic Effects

Numerous studies have documented that metabolic alkalosis frequently develops in patients receiving massive transfusion.[87-95,31,52] This alkalosis is due in large part to metabolism of citrate. Several other factors in addition to citrate metabolism may promote alkalemia following massive transfusion. Hypokalemic metabolic alkalosis may develop as a result of movement of plasma potassium into transfused red cells or as a result of excessive urinary or nasogastric losses. Hypovolemia signals intense renal proximal tubular resorption of sodium with accompanying bicarbonate resorption and alkalosis. Overtreatment with sodium bicarbonate infusions and excessive minute ventilation can provoke metabolic and respiratory alkalosis respectively.

Citrate is an effective alkalinizing agent. At physiologic pH, sodium citrate is almost completely dissociated and citrate anions predominate over citric acid in a ratio that exceeds 99 to 1. The metabolism of citrate involves several biochemical pathways (Fig 2). Complete metabolism consumes three H+ ions. Complete metabolism therefore results in the net production of three bicarbonate anions and the production of metabolic alkalosis. The concentration of citrate in CPDA-1 is 6.7 mmol/L, the complete metabolism of the administered citrate would yield 20.1 mEq/L (3 × 6.7) of bicarbonate.

The rate of citrate metabolism has been studied in renal promimal tubular cells and found to be dependent upon intracellular pH. Alkalemia slows citrate metabolism. The proposed mechanism for this slowing is based on changes in the mitochondrial H+ ion gradient.[41] Mitochondria establish through active transport an electrochemical

gradient across their membrane with increased H+ ions on the exterior relative to interior. This gradient provides a driving force for the transport of citrate and isocitrate into the mitochondria. As cytoplasmic pH rises in alkalemia, the H+ ion gradient across the mitochondrial membrane is reduced and citrate transport into the mitochondria decreases. A rise in pH of only 0.3 U is sufficient to cause a 50% reduction in citrate oxidation.[41] Thus the complete metabolic clearance of citrate may be slowed once metabolic alkalosis from prior citrate metabolism becomes established.

The fractional renal excretion of citrate increases in the setting of alkalemia.[41] The development of alkalemia following massive transfusion would serve to reduce blood citrate levels in patients with good renal function. In contrast, patients with renal failure or low glomerular filtration rates would be expected to be more prone to develop high blood citrate levels during prolonged rapid blood transport. Furthermore, decreased renal citrate excretion should increase the burden of citrate to be metabolized and thus prolong posttransfusion metabolic alkalosis. Clinical observations in dialysis patients undergoing massive transfusion support this.[93,95]

Recognition of metabolic alkalosis as a delayed consequence of prolonged citrate infusion is valuable in the management of patients in selected clinical circumstances. In one study, patients undergoing hepatic transplantation requiring an average of 45 U of citrated blood per procedure developed a mean arterial pH of 7.51 during the first ten hours following surgery.[29] Because measurement of citrate levels are not routinely available in the clinical setting, the presence of unmetabolized citrate anions may be overlooked. One clue to the presence of extreme elevation of unmetabolized citrate following prolonged massive transfusion is the development of an unusual electrolyte disturbance, metabolic alkalosis with an increased anion gap.[29] Treatment of marked metabolic alkalemia can be accomplished with hydrogen chloride solutions. Restoration of near normal pH would be expected to improve ventilatory drive and decrease the time required for post-operative ventilator support.[29]

Excessive unmetabolized cytoplasmic citrate may also influence other aspects of cellular metabolism. Cytoplasmic citrate is known to inhibit glycolysis at the level of phosphofructokinase, stimulate cytoplasmic acetylCoA decarboxylase and fatty acid synthesis, and inhibit phosphodiesterase decreasing breakdown of cAMP in a manner analogous to the action of theophylline. The significance of these regulatory effects in the setting of citrate infusion accompanying massive transfusion is unknown.

Neuromuscular Effects

Most patients requiring massive transfusion are in a surgical setting where administered paralytics and anesthetics mask or prevent the neuromuscular signs and symptoms of hypocalcemia. Large amounts of citrate may also be administered during apheresis procedures making this the most common setting for observing neuromuscular reactions to citrate. Patients with citrate induced hypocalcemia often first report a vague uneasiness or a bitter taste in the mouth. As the pheresis continues, shivering, light headedness and perioral or mild peripheral paresthesia may develop, followed by diffuse vibratory parasthesias, muscle cramps, fasciculations, carpopedal spasm and nausea.[30,31,96] These later signs are referred to as tetanic equivalents. True seizures may result from depressed ionized Ca++, but are rare. With marked depression of ionized Ca++, severe tetanic episodes without loss of consciousness, incontinence, or true tonic clonic movements may occur and be mistaken for seizures.[96] After prolonged severe depression of ionized Ca++, hypoxia may result from respiratory insufficiency due to laryngeal stridor and chest wall or diaphragmatic spasms.[96] Stimulating the facial nerve by tapping it in front of the ear (Chvostek's sign) or blood pressure cuff induced ischemia of the forearm (Trousseau's sign) lead to facial twitching and carpal spasm respectively. These maneuvers may not always elicit a response even with a low ionized Ca++ accompanied by other symptoms.[30] These maneuvers are also less reliable in infants.[96]

The neuromuscular manifestations of hypocalcemia are a result of disturbing the interactions of calcium and nerve membranes. These interactions are complex and not completely understood but it is generally agreed that the overall result of low ionized calcium is to heighten the excitability of nerve membrane leading to multiple and spontaneous depolarizations of the membrane.[97,98] The depolarization of the axonal membrane resulting in

conduction of an action potential is due to a rapid local influx of Na+ through channels in the membrane selective for Na+ ions. The interior of the axon has a negative change and low Na+ concentration when compared to the extracellular fluid so when the Na+ channels are open, Na+ flows into the cell with the electrical potential and down the concentration gradient. These channels open sequentially along the length of the axon, allowing propagation of the action potential. Because depolarization is a phenomenon of the membrane, the role of calcium must involve the membrane or at least certain components of the membrane.

The mechanism proposed for the action of calcium on the excitable membrane involves calcium being bound to the external side of the membrane when the axon is at rest.[98] According to one mechanism, positively charged Ca++ ion hyperpolarizes the membrane making depolarization more difficult. When the neuron is bathed in fluid low in calcium, the potential between the outside of the membrane and the inside is less, making depolarization easier including spontaneous depolarization.[99] Other mechanisms propose a direct action on the Na+ channel with Ca++ acting as sort of a gatekeeper, changing the permeability of the channel to sodium by causing a conformational change in the channel or the membrane surrounding the channel. Thus, when Ca++ is low, the channels remain open and the axon is maintained in a continuously active state.[98,99]

Ionized Ca++ is also responsible for allowing transport and fusion of synaptic vesicles at the sympatic junction, an effect inhibited by high Mg++ levels. At the synapse, low Ca++ levels would be expected to inhibit neural transmission, but this effect appears to be overpowered by the effect on excitable membrane stability. Lowered Mg++ levels during citrate toxicity may also partially explain this lack of synaptic inhibition but no direct evidence supports this.[97]

Most of the work mentioned above deals with the peripheral nervous system. The action of low ionized Ca++ on the central nervous system is probably similar in mechanism but more diverse in effect. In animals lowering the level of ionized Ca++ in the cerebral ventricles directly resulted in increased vasomotor reflexes and increased arterial pressures.[99] There is evidence that reduced CSF ionized Ca++ may increase the CNS sensi-

tivity to CO_2 leading to increased respiration, respiratory alkalosis, and thus even lower serum ionized Ca++ levels.[100] Low levels of ionized calcium in CSF can result in all types of seizure activity and frequently produce mental status changes. CSF effects are not commonly seen in adults. True seizures are more likely to occur with chronic hypocalcemia as occurs in hypoparathyroidism. This may be a result of blunted acute fluctuations in Ca++ due to the blood brain barrier.[99] Seizures are more likely to occur in patients with previous seizure disorders or cerebral injury, and in infants.[96,97,99]

A number of studies have been done to correlate neuromuscular symptoms with ionized calcium levels.[13,21,30,31,52,101] These studies are hampered by the lack of a standard reference method for ionized calcium determination with subsequent disparity between normal values between laboratories. Nevertheless, some general inferences can be made. Minimal symptoms such as perioral numbness or paresthesias usually require ionized Ca++ levels below 3.5 mg/dL. Below 3.2 mg/dL, some patients experience more severe symptoms with nausea and light headedness requiring slowing of the citrate infusions. In children, seizures and tetany may occur with levels <2.5 mg/dL.[21] These levels of calcium are by no means absolute and patients may have lower levels with little or no symptoms. Differences in patient sensitivity to low calcium may be due in part to differences in magnesium levels,[21,13] serum protein levels,[13] low serum glucose levels,[52] and the rate of the drop in ionized Ca++.

Similar neuromuscular signs and symptoms occur with hypomagnesium. Low ionized magnesium may play an important part in the neuromuscular manifestations of citrate toxicity but the extent of this role is presently difficult to ascertain due to lack of Mg++ ion specific electrodes needed to routinely measure ionized Mg++ levels.

MANAGEMENT OF CITRATE TOXICITY

Although nearly all patients undergoing massive transfusion do not require supplemental administration of calcium to treat citrate toxicity, for some patients such treatment is essential. Old guidelines suggesting supplemental calcium after every so many units of blood (though clearly unfounded) are nonetheless well established in the

minds of many physicians and surgeons. The proper use of supplemental calcium is best guided through monitoring of the ionized Ca+ +.[102]

Both calcium gluconate and calcium chloride are suitable for intravenous correction of acute citrate induced hypocalcemia. It must be recognized that one gram of calcium chloride provides four times the amount of calcium as found in one gram of calcium gluconate. One study which compared different calcium preparations found more reliable levels of ionized Ca+ + after administration of calcium chloride.[102] Repeated doses of 100 to 500 mgm of calcium chloride are typically administered to patients with symptomatic citrate induced hypocalcemia during massive transfusion. Indications for supplemental calcium administration during massive transfusion are generally limited to specific clinical settings in the treatment of decreased ventricular performance and/or extreme derangement of the ECG due to documented hypocalcemia. Neonatal massive or exchange transfusions may also require supplemental calcium for the treatment of seizures. In neonates the rate of citrate delivery per kg recipient per minute can reach extremely high levels and the ability to defend against acute sudden hypocalcemia is reduced. In adults massive transfusion in the setting of severe hepatic ischemia or interruption of hepatic blood flow such as occurs during surgery for multiple trauma or during liver transplantation is an important setting for the development of symptomatic hypocalcemia due to citrate toxicity. Although guidelines for

therapy of hypocalcemia due to citrate must be individualized for each patient, we have used a 50% reduction in the level of ionized calcium as a guide to replacement in the setting of hepatic transplantation and massive transfusion in adults. The importance of frequent monitoring of the ionized Ca+ + cannot be overemphasized.[103] Results of calcium measurements should be available within the shortest possible turnaround time. Values are best interpreted in the context of simultaneous measurements of cardiac performance and ventricular filling pressures.

Treatment with excessive supplemental calcium is dangerous. We have observed fatal cardiac arrest in an adult in the setting of massive transfusion, rapid calcium replacement, and an ionized calcium of 18 mg/dL. Fatal hypercalcemia (over 50 mg/dL) developed in a 14 month-old boy after repeated doses of calcium chloride during transfusions.[104] In another report two adult patients undergoing transfusion developed junctional rhythms and hypotension coincident with bolus injections of calcium administered over one minute.[105] The course of a patient overtreated for citrate toxicity during massive transfusion is shown in Table 3. The patient was a 49 year-old man undergoing orthotopic liver transplantation. During a period of rapid transfusion the ionized calcium fell to 2.86 mg/dL. Repeated injections of calcium chloride were administered despite gradually rising ionized calcium measurements. Once the administered citrate was metabolized by the revascularized hepatic graft, ionized calcium

Table 3. Development of Hypercalcemia in a Patient Undergoing a 13.5 h Orthotopic Liver Transplantation

OR Time (hours)	Units of Blood Products		IV CaCL$_2$ (grams)		Ionized Calcium
	Per Hour	Cumulative	Per Hour	Cumulative	
2	4	4	0	0	4.27
3	5	9	.2	.2	4.24
4	2	11	.3	.5	3.91
5	10	21	2.5	3	3.48
6	10	31	3.0	6	4.90
7	16	47	5.0	11	4.06
8	12	59	5.0	16	2.86
9	17	76	5.0	21	7.56
10	5	81	5.5	26.5	9.93
11	2	83	3	29.5	9.10
12	2	85	0	29.5	9.35
13.5	1	86	0	29.5	11.05

Notes: Hours 6 to 8 represent the anhepatic phase. The patient received 86 U of blood products and 29.5 g of supplemental calcium chloride.

levels rose dramatically peaking at 11.05 mg/dL. Blood samples for laboratory testing collected in commercially prepared EDTA or citrated tubes clotted before processing. The patient's early postoperative course was complicated by hypercalcemia, lethargy, pancreatitis, and renal failure requiring treatment with calcitonin, mithramycin, and dialysis. He made a gradual recovery and was discharged with normal renal function. The patient died 1 year later of other causes. At autopsy diffuse calcifications were present in the lung and pancreas.

During the past century citrate has proved to be a practical and effective blood anticoagulant. With the increase in the number of trauma centers and programs in liver transplantation, the number of patients affected by citrate toxicity is expected to increase. Numerous areas of clinical and basic investigation in the pathophysiology of acute citrate induced hypocalcemia merit further investigation. New information on the fate and distribution of citrate, its metabolism in man, its effects on cardiovascular performance, endocrine balance, and coagulation should be of interest to all involved in the care of patients needing massive transfusion.

ACKNOWLEDGMENT

REFERENCES

1. Hustin A: Note sur une nouvelle methode de transfusion. Bull Soc R Sci Med Nat Brux 72:104-111, 1914

2. Agote L: Nuevo Procedimiento pava la transfusion de la sangre. An Inst Modelo Clin Med. Buenos Aires 1:25-29, 1915

3. Weil R: Sodium citrate in the transfusion of blood. JAMA 64:425-426, 1915

4. Lewisohn R: A new and greatly simplified method of blood transfusion. Med Rec 87:141-142, 1915

5. Rous P, Turner JR: The preservation of living red cells in vivo: I. Methods of preservation. J Exp Med 23:219-237, 1915

6. Robertson OH: Transfusion with preserved red blood cells. Br Med J 1:691-695, 1918

7. Wain SL: The controversy of unmodified versus citrated blood transfusion in the early 20th century. Transfusion 24:404-407, 1984

8. Adams WE, Thornton TF, Allen JG, Gonzalez DE: The danger and prevention of citrate intoxication in massive transfusion of whole blood. Ann Surg 120:656-669, 1944

9. Bunker JP, Stetson JB, Coe RC, et al: Citric acid intoxication. JAMA 157:1361-1367, 1955

10. Collins JA: Massive blood transfusion. Clin Hematol 5:201-222, 1976

11. Zauder HL: Massive transfusion. Int Anesthesiol Clin 20:157-70, 1982

12. McLellan BA, Reid SR, Lane PL: Massive blood transfusion causing hypomagnesemia. Crit Care Med 12:146-147, 1984

13. Toffaletti J, Nissenson R, Endres D, et al: Influence of continuous infusion of citrate on responses of immunoreactive parathyroid hormone, calcium, and magnesium components and other electrolytes in normal adults during plateletpheresis. J Clin Endocrinol Metabol 60:874-879, 1985

14. Walser M: Ion association, VI. Interactions between calcium, magnesium, inorganic phosphate, citrate, and protein in normal human plasma. J Clin Invest 40:723-730, 1961

15. Meyer JL: Formation constants for interaction of citrate with calcium and magnesium ions. Anal Biochem 62:295-300, 1974

16. Gordon EE, Craigie A: Effect of intravenous glucose on splanchnic and peripheral metabolism of endogenous pyruvate and citrate in patients with cirrhosis and in subjects without liver disease. J Lab Clin Med 55:841-848, 1960

17. Henry RJ, Cannon DC, Winkelman JW (eds): Clinical Chemistry, Principles and Technics. (ed 2). Harper & Row, Hagerstown, MD, 1974, p 1348

18. McLean FC and Hastings AB: The state of calcium in the fluids of the body: I. Conditions affecting the ionization of calcium. J Biol Chem 108:285-322, 1935

19. Perkins HA, Snyder M, Thacher C, and Rolfs MR: Calcium ion activity during rapid exchange transfusion with citrated blood. Transfusion 11:204-212, 1971

20. Farrell EC: Calcium. in Pesce AJ and Kaplan LA: Methods in Clinical Chemistry, Mosby, St Louis, 1987, pp 1003-1009.

21. Sorell M, Rosen JF: Ionized calcium: Serum levels during symptomatic hypocalcemia. J Pediatrics 87:67-70, 1975

22. Kost GJ, Jammal MA, Ward RE, et al: Monitoring of ionized calcium during human hepatic transplantation. Am Jo Clin Path 86:61-70, 1986

23. Conceicao SC, Weightman D, Smith PA, Luno J, Ward MK, Kerr DNS: Serum ionized calcium concentration: measurement versus calculation. Br Med J 1:1103-1105, 1978

24. Kahn RC, Jascott D, Carlon GC, et al: Massive blood replacement: correlation of ionized calcium, citrate, and hydrogen ion concentration. Anesth Analg 58:274-278, 1979

25. Denlinger JK, Nahrwold ML, Gibbs PS, et al: Hypocalcemia during rapid blood transfusion in anesthetized man. Br J Anaesth 48:995-999, 1976

26. Howland WS, Bellville JW, Zucker MB, et al: Massive blood replacement V. Failure to observe citrate intoxication. Surg Gynol Obstet 105:529-540, 1957

27. Bunker JP, Bendixen HH, Murphy AJ: Hemodynamic effects of intravenously administered sodium citrate. New Engl J Med 266:372-377, 1962

28. Abbot TR: Changes in serum calcium fractions and ci-

trate concentrations during massive blood transfusions and cardiopulmonary bypass. Br J Anaesth 55:753-759, 1983

29. Driscoll DF, Bistrian BR, Jenkins RL, et al: Development of metabolic alkalosis after massive transfusion during orthotopic liver transplantation. Crit Care Med 15:905-908, 1987

30. Olson PR, Cox C, McCullough J: Laboratory and clinical effects of the infusion of ACD solution during platelet pheresis. Vox Sang 33:79-87, 1977

31. Szymanski IO: Ionized calcium during plateletpheresis. Transfusion 18:701-708, 1978

32. Miller RN, Englehardt R, Collins JA, et al: Observations on the biochemical effects of transfusion of citrate-phosphate-dextrose stored in blood in man. Laryngoscope 86:1272-1279, 1976

33. Klee GG, Shikagawa J, Trainer TD: CAP survey of parathyroid hormone assays. Arch Pathol Lab Med 110:588-591, 1986

34. Kaneshige J: Studies on citrate metabolism in liver injuries, 1. Fasting blood citrate level in chronic hepatitis and liver cirrhosis. Acta Med Okayama 29:1-8, 1975

35. Vang JO, Zemel R, Davidson M, Drapanas T: Citrate metabolism by the isolated perfused liver. Arch Surg 93:142-147, 1966

36. Marquez J, Martin D, Virji MA, et al: Cardiovascular depression secondary to ionic hypocalcemia during hepatic transplantation in humans. Anesthesiol 65:457-461, 1986

37. Gray TA, Buckley BM, Sealey MM, et al: Plasma ionized calcium monitoring during liver transplantation. Transplantation 41:335-339, 1986

38. Martensson J: On the citric acid metabolism in mammals. Acta Physiol Scand 1, 2:37-96, 1940 (suppl)

39. Herndon RF, Freeman S: Renal citric acid utilization in the dog. Am J Physiol 192:369-372, 1958

40. Baruch SB, Burich RL, Eun CK, King VF: Renal metabolism of citrate. Med Clin North Am 59:569-582, 1975

41. Simpson DP: Citrate excretion: a window on renal metabolism. Am J Physiol 244:223-234, 1983

42. Simpson DP: Influence of plasma bicarbonate concentration and pH on citrate excretion. Am J Physiol 206:875- 882, 1964

43. Colletti RB, Pan MW, Smith EWP, et al: Detection of hypocalcemia in susceptible neonates: The QoTc interval. N Engl J Med 290:931-935, 1974

44. Estes EH: Electrocardiography and vectorcardiography, in Hurst JW, Logue RB, Schlant RC, et al (eds): The Heart. (ed 3). McGraw-Hill, New York, 1974, p 299

45. Rumancik WM, Denlinger JK, Nahrwold ML, et al: The QT interval and serum ionized calcium. JAMA 240:366-368, 1978

46. Surawicz B: The QT interval and cardiac arrhythmias. Ann Rev Med 38:81-91, 1987

47. Krautwald A, Dorrow H: Tolerance of greater amounts of sodium citrate infusions. Arch Exp Pathol Pharm 194:691-700, 1940

48. Corbascio AN, Smith T: Hemodynamic effects of experimental hypercitremia. Anesthesiol 28:510-516, 1967

49. Nakasone N, Watkins E, Janeway CA, et al: Experimental studies of circulatory derangement following massive transfusion of citrated blood. J Lab Clin Med 43:184-195, 1954

50. Scheidegger D, Drop LJ: The relationship between duration of QT interval and plasma ionized calcium concentration: experiments with acute, steady-state, Ca+ + changes in the dog. Anesthesiol 51:143-148, 1979

51. Howland WS, Schweizer O, Carlon GC, et al: The cardiovascular effects of low levels of ionized calcium during massive transfusion. Surg Gynol Obstet 145:581-586, 1977

52. Ladenson JH, Miller WB, Sherman LA: Relationship of physical symptoms, ECG, free calcium, and other blood chemistries in reinfusion with citrated blood. Transfusion 18:670-679, 1978

53. Howland WS, Boyan CP, Schweizer O: Ventricular fibrillation during massive blood replacement. Am J Surg 92:356-360, 1956

54. Ludbrook J, Wynn V: Citrate intoxication: a clinical and experimental study. Brit Med Jo 2:523-528, 1958

55. LeVeen HH, Schatman B, Falk G: Cardiac arrest produced by massive transfusions. Surg Gynol Obstet 109:502-508, 1959

56. Vassalle M: Electrogenesis of the plateau and pacemaker potential. Ann Rev Physiol 41:425-440, 1979

57. Ringer S: Concerning the influence exerted by each of the constituents of the blood on the contraction of the ventricle. Am J Physiol 3:380-393, 1882

58. Ringer S: A further contribution regarding the influence of different constituents of blood on contractions of the heart. Am J Physiol 4:29-42, 1883

59. Drop LJ: Ionized calcium, the heart, and hemodynamic function. Anesth Analg 64:432-542, 1985

60. Howland WS, Schweizer O, Boyan CP, et al: Physiologic alterations with massive blood replacement. Surg Gynol Obstet 101:478-482, 1955

61. Hubbard TF, Neis DD, Barmore JL: Severe citrate intoxication during cardiovascular surgery. JAMA 162: 1534-1535, 1956

62. Watkins I: Experimental citrate intoxication during massive blood transfusion. Surg Forum 4:213-219, 1953

63. Cookson BA, Costas-Durieux J, Bailey CP: Toxic effects of citrated blood and search for suitable substitute for use in cardiac surgery. Ann Surg 139:430-438, 1954

64. Senning A: Risk of rapid and large citrated blood transfusions in experimental hemorrhagic shock. Acta Chir Scand 110:394-402, 1956

65. Baue A, Hermann G, Shaw RS: A study of bank blood toxicity. Surg Gynol Obstet 113:40-46, 1961

66. Cooper N, Brazier JR, Hottenrott C, et al: Myocardial depression following citrated blood transfusion. Arch Surg 107:756-763, 1973

67. Olinger GN, Hottenrott C, Mulder DG, et al: Acute clinical hypocalcemic myocardial depression during rapid blood transfusion and postoperative hemodialysis. J Thor Card Surg 72:503-511, 1976

68. Smith NT, Hurley EJ: Citrate infusion in dogs following cardiac autotransplantation. Arch Surg 98:44-48, 1969

69. Stultz PM, Scheidegger D, Drop LJ, et al: Ventricular pump performance during hypocalcemia. J Thor Card Surg 78:185-194, 1979

70. Kovalik SG, Ledgerwood AM, Lucas CE, et al: The cardiac effect of altered calcium homeostasis after albumin resuscitation. J Trauma 21:275-279, 1981

71. Geffin GA, Drop LJ, O'Keefe DD, et al: Global and regional function in the regionally ischaemic left ventricle

related to plasma ionized calcium. Cardiovasc Res 17:415-426, 1983

72. Gray TA, Buckley BM, Sealey MM, et al: Is calcium important for hemodynamic stability during liver transplantation? Trans Proc 17:290-292, 1985

73. Wu AHB, Bracey A, Bryan-Brown CW, et al: Ionized calcium monitoring during liver transplantation. Arch Path Lab Med 111:935-938, 1987

74. Stefanini M: Studies on the role of calcium in the coagulation of blood. Acta Med Scand 136:250-266, 1950

75. Baumgartner HR: Platelet interaction with collagen fibrils in flowing blood. I. Reaction of human platelets with alpha chymotrypsin digested subendothelium. Thromb Haemost 37:1-16, 1977

76. Sakariassen KS, Ottenhof-Rovers M, Sixma JJ: Factor VIII-von Willebrand factor requires calcium for facilitation of platelet adherence. Blood 63:996-1003, 1984

77. Rasmussen H, Barett PQ: Calcium messenger system: an integrated view. Physiol Rev 64:938-984, 1984

78. Nachmias VT, Kavaler J, Jacubowitz S: Reversible association of myosin with platelet cytoskeleton. Nature 313:70-72, 1985

79. Cutler L, Rodan G, Feinstein MB: Cytochemical localization of adenylate cyclase and of calcium ion, magnesium ion-activated ATP-ases in the dense tubular system of human blood platelets. Biochim Biophys Acta 542:357-371, 1978

80. Papahadjopoulos D, Vail WJ, Newton C, et al: Studies on membrane fusion. III. The role of calcium-induced phase changes. Biochim Biophysol Acta 465:579-598, 1977

81. Fitzgerald LA, Phillips DR: Calcium regulation of the platelet membrane glycoprotein IIb-IIIa complex. J Biol Chem 260:11366-11374, 1985

82. Baumgartner HR: Effects of acetylsalicyclic acid, sulfinpyrazone, and dipyridamole on platelet adhesion and aggregation in flowing native an anticoagulated blood. Haemostasis 8:340-352, 1979

83. Rock GA, Cruickshank WH, Tackaberry ES, et al: Stability of VIII:C in plasma: The dependence on protease activity and calcium. Thromb Res 29:521-535, 1983

84. Nelsestuen GL, Broderius M: Interaction of prothrombin and blood clotting factor X with membranes of varying composition. Biochem 16:4172-4177, 1977

85. Kang YG: Monitoring and treatment of coagulation, in Winter PM, Kang YG (eds): Hepatic Transplantation: Anesthetic and perioperative management, New York, Praeger, 1986, p 159

86. Dwarakanath S, Jacques S, Dzik WH: The effect of low ionized calcium on coagulation. Blood 70:329a, 1987 (suppl)

87. Litwin MS, Smith LL, Moore FD: Metabolic alkalosis following massive transfusion. Surgery 45:805-813, 1959

88. Povey MJC: pH changes during exchange transfusion. Lancet 2:339-340, 1964

89. Bunker JP: Metabolic effects of blood transfusion. Anesthesiol 27:446-455, 1966

90. Miller RD, Tong MJ, Robbins TO: Effects of massive transfusion of blood on acid-base balance. JAMA 216:1762-1765, 1971

91. Collins JA: Problems associated with the massive transfusion of stored blood. Surg 75:274-295, 1974

92. Schweizer O, Holland WS: The effect of citrated bank blood on acid-base balance. Surg Gynecol Obstet 114:90-96, 1962

93. Barcenas CG, Fuller TJ, Knochel JP: Metabolic alkalosis after massive blood transfusion. JAMA 236:953-954, 1976

94. Pearl DG, Rosenthal MH: Metabolic alkalosis due to plasmapheresis. Am J Med 79:391-393, 1985

95. Howland S: Calcium, potassium, and pH changes during massive transfusion, in Nusbacher J, Fletcher J (eds): Massive Transfusion. Arlington, VA, American Association of Blood Banks, 1978

96. Frame B: Neuromuscular manifestations of parathyroid disease, in Vinken PJ, Bruyn GW (eds): Handbook of Clinical Neurology, Vol 27 (ed 1). North-Holland Publishing, Amsterdam, 1976, pp 283-314

97. Katzman R and Pappins HM: Brain Electrolytes and Fluid Metabolism, ed 1. Baltimore, Williams & Wilkens, 1973, pp 209-217

98. Abood LG, Hoss W: Excitation and conduction in the neuron, in Seigel GJ, Albers RW, Katzman R, et al (eds): Basic Neurochemistry, ed 2. Boston, Little Brown, 1976, pp 103-124

99. Davis FA, Schanf CL: Neurological manifestations of calcium imbalance, in Vinken PJ and Bruyn GW (eds): Handbook of Clinical Neurology, Vol 28 (ed 1). Amsterdam, North-Holland Publishing, 1976, pp 527-543

100. Edmondson JW, Brashear RE, Li T: Tetany: quantitative interrelationships between calcium and alkalosis. Am J Physiol 228:1082-1086, 1975

101. Watson DK, Penny AF, Marshall RW, Robinson EAE: Citrate induced hypocalcemia during cell separation. Br J Haematol 44:503-507, 1980

102. White RD, Goldsmith RS, Rodriguez R, et al: Plasma ionic calcium levels following injection of chloride, gluconate, and gluceptate salts of calcium. J Thor Card Surg 71:609-613, 1976

103. Kost GJ: The challenges of ionized calcium. Arch Path Lab Med 111:932-984, 1987

104. Wolf PL, McCarthy LJ, Hafleigh B: Extreme hypercalcemia following blood transfusion combined with intravenous calcium. Vox Sang 19:544-545, 1970

105. Carlon GC, Howland WS, Goldiner PL, et al: Adverse effects of calcium administration. Arch Surg 113:882-885, 1978