CHANGES IN SERUM CALCIUM FRACTIONS AND CITRATE CONCENTRATIONS DURING MASSIVE BLOOD TRANSFUSIONS AND CARDIOPULMONARY BYPASS

T. R. ABBOTT

SUMMARY

Total serum calcium, its fractions and serum citrate concentrations were measured in children receiving massive blood transfusion for scoliosis surgery, and in other children undergoing open heart surgery with cardiopulmonary bypass. During scoliosis surgery, although up to 100% of blood volume was replaced, and no additional calcium salts were infused, serum ionized calcium concentration remained within normal limits, provided that infusion rates of blood did not exceed 30 ml kg⁻¹ h⁻¹. During cardiopulmonary bypass, in which citrate-containing blood priming solutions were used, serum ionized calcium concentrations decreased markedly during perfusion associated with extremely high serum citrate concentrations. Serum ionized calcium concentrations returned to normal by the end of bypass, but serum citrate concentrations remained five times the normal concentration 5 h after bypass.

The increase in plasma citrate concentration associated with the transfusion of stored citrated blood has been shown to be related to the rate of transfusion (Ludbrook and Wynn, 1958). More recently, Bunker, Bendixen and Murphy (1962), have demonstrated circulatory depression in association with both increases in citrate concentration and decreases in the calculated concentrations of serum ionized calcium. In contrast, Howland and colleagues (1977) were unable to show any deterioration in haemodynamic function with low serum ionized calcium concentrations during massive transfusion. Kahn and co-workers (1979) concluded that, although massive transfusion decreased the ionized calcium concentration as a result of the administration of citrate, these changes were without haemodynamic significance and that, as long as the circulating volume was maintained, calcium salts need not be administered.

The present study was undertaken to monitor the total serum calcium concentration and its various fractions, and serum citrate concentrations, in children receiving massive blood transfusion for scoliosis surgery and during open heart surgery for congenital heart disease. The purpose of this study was to determine the extent to which these parameters varied during these procedures, whether it was necessary to give increments of i.v. calcium salts during large blood transfusion in children, and if the addition of calcium chloride to citrated blood used in the oxygenator prime resulted in normal serum ionized calcium concentrations during and following cardiopulmonary bypass.

PATIENTS AND METHODS

Five children, aged between 7 and 9 years, undergoing open heart surgery with cardiopulmonary bypass, and three children, aged between 13 and 14 years, undergoing scoliosis surgery were studied (table I).

An initial blood sample was drawn from the central venous line following induction of anaesthesia in each patient (control). Subsequently, blood samples were drawn at 15-min intervals in the patients undergoing spinal surgery. In the group undergoing open heart surgery, samples were taken from the oxygenator prime and from the patient before, during and immediately after cardiopulmonary bypass and then at hourly intervals for 5 h. Serum concentrations of total calcium, ionized calcium, ultrafilterable, protein bound and complexed calcium were measured. Total calcium (Ca) was measured using atomic absorption, ionized calcium (Ca²⁺) using an ionized calcium electrode, ultrafilterable calcium using Worthington anticonvulsant drug filters (Eckfeldt and Koehler, 1980), protein bound calcium calculated following determination of total serum proteins, and complexed calcium calculated by subtracting Ca²⁺ from ultrafilterable calcium. Serum citrate was estimated by a fully enzymatic method.
using citrate lyase. All results are expressed in mmol litre⁻¹.

Patients were premedicated with morphine and atropine and anaesthesia was induced with thiopentone. Tubocurarine was administered, the trachea intubated and the lungs ventilated artificially with nitrous oxide and oxygen (2:1). Morphine was given after the induction of anaesthesia and this brought the total dose, including premedication, to 0.5 mg/kg body weight. In addition, patients undergoing open heart surgery received phenoxybenzamine 1 mg kg⁻¹ i.v. to produce a degree of alpha-adrenergic blockade. All patients received 5-10 ml kg⁻¹ of 0.45% saline in 5% dextrose during surgery and the blood volume was maintained by transfusing blood (citrate phosphate dextrose (CPD)), which was filtered and warmed.

The oxygenator prime consisted of 0.18% saline in 5% dextrose 15 ml kg⁻¹, plus CPD blood which was prepared by adding to each unit heparin 25 mg, and 20% calcium chloride 2.5 ml to produce a concentration of 1 mg/ml of blood. The prime was warmed and oxygenated and the metabolic acidosis corrected by addition of sodium bicarbonate, usually in an amount necessary to correct a base excess of −15 mmol litre⁻¹. During cardiopulmonary bypass the core temperature of the body was decreased to 18-25°C by perfusion cooling to provide good operating conditions and to protect the myocardium. The patient was rewarmed to a core temperature of 35°C before terminating bypass. Thus, all the patients were moderately hypothermic in the period immediately after bypass.

RESULTS
The changes in the serum concentrations of the calcium fractions, and citrate, followed a similar pattern in all the patients who underwent open heart surgery (table II, fig. 1). The ranges of the normal values for total serum calcium, its various fractions and serum citrate concentrations based on an analysis of results obtained in the hospital laboratory, and values reported in the literature (Ludbrook and Wynn, 1958; Rose, 1972; Robertson and Marshall, 1979; Goodman and Gilman, 1980) are presented in tables II and III.

In this study the post-induction and pre-bypass concentrations were within the normal range for all fractions apart from a small increase in citrate concentration (and hence complexed calcium) in the pre-bypass sample, as a result of transfusion of small volumes of blood or plasma. The oxygenator prime showed marked increases in the concentrations of total calcium, citrate and complexed calcium, and low concentrations of ionized and protein bound calcium. During bypass, total calcium, citrate and complexed calcium increased markedly, as a result of the mixing of the prime with the patient's blood. The patients' haematocrits decreased consistently to 25-30% during bypass, and the protein bound calcium was low during this period. Ionized calcium concentrations were half normal during bypass, but returned to post-induction values during the periods after bypass and immediately after operation (6 h). During the period after operation, citrate and complexed calcium and citrate concentrations continued to decrease but were still greater than five times normal after 6 h. Protein-bound calcium concentration approached normal. The response of patient 5 has been plotted separately in figure 1 since, although post-induction and pre-bypass values were normal, citrate concentrations failed to decrease as rapidly as in the other patients, and ionized calcium concentrations remained low for 24 h. This patient
TABLE II. Serum concentrations of calcium and citrate (mmol litre\(^{-1}\)) during cardiopulmonary bypass. Mean values
\((\pm SD)(n = 5)\)

<table>
<thead>
<tr>
<th></th>
<th>Oxygenator prime</th>
<th>After induction</th>
<th>Before bypass</th>
<th>On bypass</th>
<th>After bypass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>6.32 (0.69)</td>
<td>2.12 (0.06)</td>
<td>2.13 (0.07)</td>
<td>4.20 (0.34)</td>
<td>3.47 (0.44)</td>
</tr>
<tr>
<td>Ionized</td>
<td>0.46 (0.12)</td>
<td>1.13 (0.05)</td>
<td>1.05 (0.27)</td>
<td>0.73 (0.09)</td>
<td>1.21 (0.16)</td>
</tr>
<tr>
<td>Complexed</td>
<td>5.67 (0.74)</td>
<td>0.25 (0.06)</td>
<td>0.43 (0.35)</td>
<td>3.24 (0.47)</td>
<td>1.54 (0.41)</td>
</tr>
<tr>
<td>Protein-bound</td>
<td>0.22 (0.27)</td>
<td>0.70 (0.15)</td>
<td>0.66 (0.10)</td>
<td>0.23 (0.12)</td>
<td>0.56 (0.28)</td>
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<tr>
<td>Citrate</td>
<td>13.59 (0.41)</td>
<td>0.09 (0.07)</td>
<td>0.95 (0.63)</td>
<td>6.15 (1.20)</td>
<td>3.12 (0.72)</td>
</tr>
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Time after bypass (h)

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<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>2.98 (0.45)</td>
<td>2.90 (0.31)</td>
<td>2.69 (0.32)</td>
<td>2.59 (0.32)</td>
<td>2.52 (0.28)</td>
</tr>
<tr>
<td>Ionized</td>
<td>1.30 (0.29)</td>
<td>1.35 (0.21)</td>
<td>1.27 (0.15)</td>
<td>1.20 (0.19)</td>
<td>1.15 (0.19)</td>
</tr>
<tr>
<td>Complexed</td>
<td>0.95 (0.37)</td>
<td>0.82 (0.57)</td>
<td>0.60 (0.33)</td>
<td>0.63 (0.33)</td>
<td>0.60 (0.33)</td>
</tr>
<tr>
<td>Protein-bound</td>
<td>0.74 (0.24)</td>
<td>0.73 (0.30)</td>
<td>0.76 (0.30)</td>
<td>0.77 (0.14)</td>
<td>0.72 (0.19)</td>
</tr>
<tr>
<td>Citrate</td>
<td>1.60 (1.40)</td>
<td>0.77 (0.93)</td>
<td>0.90 (1.28)</td>
<td>0.50 (0.18)</td>
<td>0.52 (0.67)</td>
</tr>
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Fig. 1. Changes in serum calcium fraction and citrate concentrations in five children undergoing cardiopulmonary bypass. Mean concentrations and SD are given for each fraction. The low ionized calcium (A: \(\bigcirc\---\bigcirc\)) and high citrate (B: \(\bigcirc\---\bigcirc\)) concentrations of patient 5 are displayed. A: \(\bullet\) = total calcium; \(\bigcirc\) = \(\text{Ca}^{2+}\); \(\nabla\) = protein-bound calcium. B: \(\square\) = citrate; \(\nabla\) = complexed calcium.

had a low cardiac output following operation, required inotropic support and eventually died.

Figure 2 indicates the changes which occurred in serum calcium and citrate concentrations in patients 6, 7 and 8 who underwent scoliosis surgery, during replacement of 95%, 79% and 100% of their blood volumes, over periods of 4 h, and during the periods immediately after operation. In patient 6, blood was replaced continuously as it was lost and all calcium fractions remained stable throughout the operative and postoperative periods. In contrast, patient 7 received two increments of 16% of blood volume over 5 min and patient 8 two increments of 10% of blood volume each over 5-min periods. In these two
TABLE III. Normal serum calcium and citrate concentrations.\n\n| Ultrafiltrable = ionized + complex-bound | Concentration (mmol litre$^{-1}$) |
<table>
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<tbody>
<tr>
<td>Total</td>
<td>2.12–2.50</td>
</tr>
<tr>
<td>Protein-bound, non-diffusible</td>
<td>0.8–1.1 (35–45% of total)</td>
</tr>
<tr>
<td>Ionized</td>
<td>1–1.25</td>
</tr>
<tr>
<td>Complex-bound</td>
<td>0.25</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.09–0.14</td>
</tr>
</tbody>
</table>

patients there were acute increases in the concentrations of complexed calcium and citrate and a coincidental decrease in the ionized and protein bound calcium concentrations. Recovery of these values took 2–3 h, during which time continuing blood loss was replaced at a steady rate of transfusion.

A comparison was made between the calcium binding properties of heparinized plasma, stored CPD blood, fresh frozen plasma (FFP) and human plasma protein fraction (HPPF), by adding equal increments of calcium chloride and determining the resulting Ca$^{2+}$ concentration. The results (fig. 3) show that, in heparinized blood, Ca$^{2+}$ increased linearly as total calcium concentration increased, whereas with all the other blood products there was considerable binding of calcium which resulted in smaller Ca$^{2+}$ concentrations.
When large volumes of citrated blood are transfused, the equilibrium which exists between ionized calcium ($\text{Ca}^{2+}$) protein-bound calcium (Ca-prot.) and the ligand-bound or complexed calcium (Ca-L) is altered. The normal relationship between these fractions can be represented as follows:

$$\text{Ca-L} \rightleftharpoons \text{L} + \text{Ca}^{2+} + \text{prot.} \rightleftharpoons \text{Ca-prot.}$$

The balance between the fractions is also hydrogen ion ($H^+$)-dependent and, therefore, the concentration of $\text{Ca}^{2+}$ will be altered by changes in carbon dioxide tension and metabolic acidosis and alkalosis (Hinkle and Cooperman, 1971; Schaer and Bachmann, 1974). The ligands which normally complex with calcium are anions such as bicarbonate or lactate (Fogh-Anderson et al., 1978) but, as the results of this study have shown, during blood transfusion complexed calcium increased in proportion to the increase in serum citrate concentration, and at the same time there was a decrease in the protein-bound calcium concentration. During steady rates of transfusion, with haemodynamic stability, $\text{Ca}^{2+}$ remains stable as a result of the shift in equilibrium between the various fractions, despite an increase in the serum citrate concentration, even though the whole blood volume may be replaced over a period of 2–3 h—equivalent to a rate of perfusion of about 30 ml kg$^{-1}$ h$^{-1}$. When about 10% of blood volume is transfused over 5–10 min, that is at a rate of approximately 60 ml kg$^{-1}$ h$^{-1}$, $\text{Ca}^{2+}$ concentrations decrease acutely but recover to normal within 10–15 min of termination of the rapid transfusion, thus confirming the work of Kahn and colleagues (1979) who showed that serum $\text{Ca}^{2+}$ concentrations were normal during massive blood replacement, provided haemodynamic stability was maintained.

During cardiopulmonary bypass serum citrate concentrations can reach a peak of more than 6.0 mmol litre$^{-1}$ as a citrate-containing prime solution is pumped into the patient, and this is associated with a marked decrease in $\text{Ca}^{2+}$ concentration to 0.73 mmol litre$^{-1}$, despite the addition of extra calcium salts to the prime which increased its total calcium concentration to more than 6.0 mmol litre$^{-1}$. The in vitro study demonstrated that extremely large amounts of calcium salts would be required to increase $\text{Ca}^{2+}$ concentrations to normal, and that the amounts normally used are inadequate.

Ludbrook and Wynn (1958) studied four patients

![Figure 3](https://academic.oup.com/bja/article-abstract/55/8/753/247533/1234567890)

**Fig. 3.** The relationship between serum total calcium and ionized calcium when increments of calcium chloride were added to the following blood products: fresh heparinized plasma; stored CPD plasma (CPD) (heparin 5 mg% added); human plasma protein fraction (HPPF); fresh frozen plasma (FFP).
undergoing surgery to the abdominal aorta and liver, under hypothermic conditions, and found serum citrate concentrations increased to 40–140 times normal. These workers presented clinical and experimental evidence that the clearance of citrate from the blood was impaired during hypothermia and when the hepatic circulation was decreased or interrupted. A combination of both these factors led to marked increases in serum citrate concentrations even when relatively small volumes of blood were infused. In two patients they showed that systemic hypotension was associated with increases in serum citrate and decreases in Ca\(^{2+}\) concentrations, which responded to an infusion of calcium chloride in one patient. They concluded that, in man, metabolic destruction is the most important means of removing citrate from the plasma, and showed that plasma citrate concentrations decreased to normal over a period of about 1 h following transfusion. In the present study, citrate concentrations took about 2 h to return to control in the spinal surgery patients.

During cardiopulmonary bypass using an oxygenator, a large proportion of the prime may consist of citrate containing blood products, and the body is suddenly exposed to a much greater concentration of citrate than during normal blood transfusion, resulting in a greater decrease in Ca\(^{2+}\) concentration. Despite this, serum Ca\(^{2+}\) concentrations had returned to normal by the end of bypass, but serum citrate concentrations were still five times greater than normal 5 h following operation. In these patients, clearance of citrate from the blood is likely to have been decreased, since all were moderately hypothermic and, since they had undergone complex intracardiac surgery, cardiac output may have been decreased also. The rate of clearance of serum citrate was decreased in one patient and was associated with a persistently low Ca\(^{2+}\) concentration for more than 24 h following surgery, despite the continuous infusion of calcium salts. This patient had required considerable inotropic support to maintain cardiac output and eventually died. Drop and Laver (1975) reported that, in patients who required extensive pharmacological support of the circulation, Ca\(^{2+}\) concentrations were in the range 0.21–0.53 mmol litre\(^{-1}\). They suggested that the Ca\(^{2+}\) mobilization from body stores may be inadequate, secondary to an abnormal distribution of blood flow, and that in order to increase serum Ca\(^{2+}\) concentrations, infusion of calcium chloride at rates up to 1.5 mg kg\(^{-1}\) min\(^{-1}\) may be necessary.

A decrease in Ca\(^{2+}\) concentration during transfusion of citrated blood in man is associated with depression of ventricular contractility (accentuated in the presence of beta-adrenergic blockade with propranolol) and a decrease in peripheral vascular resistance (Stulz et al., 1979; Moffitt et al., 1982). These findings explain the clinical observation that there is often a sudden decrease in perfusion pressure at the start of cardiopulmonary bypass, as a result of a decrease in peripheral resistance. However, the effect on myocardial contractility is not important at this stage and, since serum Ca\(^{2+}\) concentration has usually returned to normal by the end of bypass, ventricular contractility should not be affected.

In conclusion, one can assume that, when blood transfusions are given at about 30 ml kg\(^{-1}\) h\(^{-1}\) and haemodynamic stability is maintained, compensatory mechanisms ensure that serum Ca\(^{2+}\) concentrations remain within normal limits. Faster rates of transfusion temporarily depress Ca\(^{2+}\) concentration, which recovers within 10 min if the infusion rate is decreased again. However, clearance of citrate from the blood will be decreased during hypothermia if liver function is impaired, for example because of pre-existing disease, in which case rapid transfusion of relatively small volumes of citrate-containing blood products will cause depression of serum Ca\(^{2+}\) concentrations. This may be recognized clinically as a sudden decrease in arterial pressure in association with the rapid infusion of blood or plasma, which responds transiently to i.v. injection of calcium salt. Clearance of citrate from the blood will be increased if hypothermia is corrected, systemic and hepatic blood flow increased and urine output increased, since approximately 20% of citrate can be excreted in the urine (Ludbrook and Wynn, 1958). The oxygenator prime solutions in paediatric practice can contain large amounts of citrate and the Ca\(^{2+}\) concentrations can be low despite a high total calcium concentration (from added calcium salts). Thus, consideration should be given to minimizing the total amount of citrate-containing blood products given, especially in infants, since this results in high citrate concentrations which, in critically ill patients, may produce a sustained depression of Ca\(^{2+}\) concentration. This study has confirmed again that total serum calcium concentrations do not necessarily reflect Ca\(^{2+}\) concentrations and that when patients receive large blood transfusions, or require cardiopulmonary bypass (especially children in whom it is necessary to include blood products in the prime solution because of the size of the
oxygenator circuits) it is essential to measure serum Ca\(^{2+}\) concentration directly, and at frequent intervals. Serum citrate concentrations can be assessed rapidly using a citrate lyase method and should also be estimated in these critically ill patients.

ACKNOWLEDGEMENTS

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REFERENCES


**SERUM CALCIUM AND CITRATE CONCENTRATIONS**

**LES MODIFICATIONS DES FRACTIONS CALCIQUES DU SERUM ET DES CONCENTRATIONS DE CITRATE AU COURS DES TRANSFUSIONS SANGUINES MASSIVES ET DES CIRCULATIONS EXTRACORPORELLES**

**REFERENCES**


escoliosis, así como en otros niños sometidos a intervención quirúrgica con desviación cardiovascular. Durante la operación por escoliosis, y aunque se sustituyó hasta el 100% del volumen de sangre sin que se administraran sales de calcio complementarias, la concentración de suero de calcio ionizado permaneció dentro de los límites normales, en tanto en cuanto los regímenes de administración de sangre no superaran los 30 ml kg⁻¹ h⁻¹. Durante el desvío cardiovascular, en el que se utilizaron soluciones de cebado de sangre que contenían citratos, las concentraciones de suero de calcio ionizado disminuyeron notablemente durante la perfusión asociada con concentraciones muy elevadas de suero de citratos. Las concentraciones de suero de calcio ionizado volvieron a su normalidad al término de la desviación, pero las concentraciones de suero de citrato permanecieron cinco veces por encima de la concentración normal después de cinco horas de dicha desviación cardiovascular.