The effect of acute haemodilution during cardiopulmonary bypass on blood calcium, pH, phosphorus and protein values

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Summary

The changes in total serum calcium, ionized calcium, total serum protein, albumin and phosphate levels during cardiopulmonary bypass were measured. The possible causes of the changes in these parameters are discussed.

The effect of the presence of heparin on serum ionized calcium is evaluated. The metabolic and clinical significance of the changes in blood components measured are discussed, special attention being paid to the use of calcium as an inotropic agent at the termination of cardiopulmonary bypass.

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Ionized calcium (Ca^{2+}), total calcium (Ca_T), albumin, total protein and phosphate levels were measured during hypothermic cardiopulmonary bypass (CPB) using a clear pump prime. At the end of the bypass period, $CaCl_2$ (20 mg/kg) was given to the patients to increase left ventricular function and increase the coronary perfusion pressure. No other inotropic agent was used in weaning patients from CPB.

It has been shown that the Ca^{2+} level decreases during CPB when a clear prime is used and that the decrease is accompanied by a decrease in the serum albumin concentration.[1,2] However, the effect of administering calcium on the blood concentration of calcium, as well as its possible role, if any, in assisting the weaning of patients from CPB was the main purpose of this small clinical study. No attempt was made to investigate the objective inotropic effect of calcium or to compare its effectiveness as an inotropic agent with other cardiosupportive drugs.

Patients and methods

Twenty-two patients scheduled for open-heart surgery took part in the clinical study. Consent was obtained from every patient and the protocol was approved by the Ethics Committee of Tygerberg Hospital. The procedures included aortic and/or mitral valve replacement or coronary artery bypass surgery.

The evening before they were to undergo the procedure, the patients were sedated with lorazepam. Premedication consisted of papaveretum 0,2 mg/kg and promazine 0,4 mg/kg; whenever the haemodynamic status (cardiac catheterization results) permitted, droperidol 0,05 mg/kg was used. All patients received methylprednisolone 30 mg/kg intravenously 2 hours before CPB was started. The anaesthetic consisted of morphine 2 mg/kg, diazepam 0,25 mg/kg, oxygen and pancuronium 0,1 mg/kg. Nitrous oxide was used in 50% concentrations when left ventricular (LV) function was adequate. The patients were mechanically ventilated with slight hypocapnia ($PaCO_2$ 4,2 - 4,6 kPa) and the temperature was initially kept within the normal range. Heparin 2 mg/kg was given before the cannulae were placed in the heart and effective heparinization was ensured by keeping the activated clotting time between 300 and 500 seconds. A dose-response curve was used as a guide to follow-up heparin administration in every patient.

During CPB all patients were cooled to 28°C and the electrolyte, acid-base and blood gas levels were measured at short intervals. Corrections were made for temperature according to the blood gas results.[3]

The CPB priming volume consisted of: 2,3 l Plasmalyte B, $CaCl_2$ 0,3 g/l Plasmalyte B, heparin 2 000 U, insulin 40 U, 4,2% $NaHCO_3$ 150 mmol, albumin 10 g, dextrose 50 g, and mannitol 12,5 g. This was delivered with a Sarns Model 5000/2000 CPB machine using a Bently Boss 10 oxygenator at flow rates of 2,25 - 2,40 l/m²/min.

The cardioplegic solution consisted of: Plasmalyte B at 4°C, Na^+ 130 mmol/l, K^+ 130 mmol/l, Mg^{2+} 3 mmol/l, Cl 108 mmol/l, HCO_3 28 mmol/l (pH 7,4; osmolarity 273 mosm/l), plus KCl 30 mmol/l and $MgSO_4$ 2 g. The solution was given under pressure into the aortic root until asystole developed. The solution was reinfused at 30-minute intervals and/or whenever there was electrical activity on the ECG.

From the time of arrival until CPB was established, all patients received intravenous fluids consisting of Hydrolyte to which was added dextrose 100 g, soluble insulin 30 U, KCl 30 mmol, $CaCl_2$ 2 g, vitamin B complex and vitamin C. The Ca^{2+} concentration in the fluid was 1,78 mmol/l.

Blood was collected for laboratory tests: (*i*) before going on CPB; (*ii*) 5 minutes after initiation of CPB; (*iii*) before the administration of $CaCl_2$ 20 mg/kg, i.e. after the release of the aortic clamp and after the heart had returned to a normal rhythm and the patient's temperature was normal (partial bypass); (*iv*) after the administration of $CaCl_2$ at the time when the increase in perfusion pressure was maximal; (*v*) 5 minutes after termination of CPB. The blood samples for determination of ionized calcium were collected without stasis and transported in tubes completely filled and sealed to ensure anaerobic conditions and kept at 4°C using chipped ice. All samples were analysed within 20 minutes of collection with a Nova 2 ionized calcium analyser.

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The collection and the transport of the samples were as suggested by Ladenhorst and Bowers[4] and Li and Piechocki.[5]

Measurement of the pH was performed on the same sample with an AVL Gas Check Model 937C.

The albumin, total protein, phosphate and total calcium levels were measured with a sequential multiple analyser with computer.

In our laboratory the normal values for Ca_T and Ca^{2+} are 2,1 - 2,6 mmol/l and 1,14 - 1,32 mmol/l respectively. Moore[6] has reported the Ca^{2+} level as being 46,9% of the serum Ca_T level, although the relationship between Ca^{2+} and Ca_T is not constant.

The presence of heparin in blood has an effect on the Ca^{2+} levels.[4,5,7] To evaluate this effect during the experiment, predetermined amounts of calcium were added to blood and saline. The exact concentration of calcium could then be calculated. The pH, temperature and partial oxygen pressure of the blood were controlled at physiological values and the saline was kept at 37°C. Different amounts of heparin were added to the premixed calcium-blood and calcium-saline combinations and the Ca^{2+} value was determined in the samples. If the blood concentration of heparin exceeds 10 U/ml, the calcium values will change significantly. With the heparinization of 2 ml/kg and the heparin added to the prime, we calculated a maximum patient heparin concentration of 4 U/ml. The tight control of the heparin effect with the help of the ACT monitor (although this does not measure the concentration, only the heparin effect) prevented over-heparinization.

For statistical analysis we used the *t* test for unpaired data and a *P* value of less than 0,05 (5% level) was accepted as being significant.

Results

Table I is a summary of the results obtained.

Fig. 1 shows the serial changes in the concentrations of Ca^{2+} and Ca_T plotted against time, i.e. sampling times as specified in the legend. Underneath each set of results the percentage Ca^{2+} of the Ca_T is given.

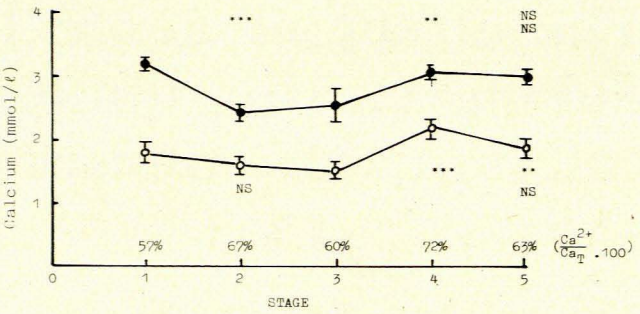

Fig. 1. Changes in calcium concentrations: before CPB (stage 1); 5 min after start of CPB (stage 2); before administration of calcium (stage 3); after administration of calcium (stage 4); 5 min after termination of CPB (stage 5). Statistical comparison was made between stages 1 and 2, 3 and 4, 4 and 5, and 1 and 5 (extra values indicated at stage 5). Significance: $^*P < 0,05$; $^{**}P < 0,025$; $^{***}P < 0,001$; NS = not significant (● = Ca_T; O = Ca^{2+}).

Fig. 2 depicts the total protein and albumin concentrations and Fig. 3 shows the changes in serum phosphate concentration.

Discussion

It is routine in many centres to add $CaCl_2$ to the cardioplegic solution during perfusion and to give calcium to the patient after CPB has been completed. The physiological function of Ca^{2+} in myocardial contraction has been described by McLean and Hastings.[8] Calcium is important for the function of the myofibrillar adenosine triphosphatase and is essential for the normal excitation, coupling and contraction of the myocardial contractile proteins. It also plays a part in cardiac membrane permeability. The availability of Ca^{2+} in the sarcotubular system to a large extent determines the strength of myocardial contraction during systole and the effective re-uptake of the calcium from the contractile proteins is important for the normally occurring increase in myocardial compliance during diastole.[9]

TABLE I. SUMMARY OF RESULTS

	Before CPB	5 min after start of CPB	Before calcium	After calcium	5 min after CPB
Ca^T (mmol)					
Mean	3,14	2,40	2,51	3,04	2,96
SD	0,29	0,41	0,91	0,41	0,50
SEM	0,06	0,09	0,20	0,09	0,11
Ca^{2+} (mmol/l)					
Mean	1,80	1,61	1,53	2,23	1,89
SD	0,40	0,35	0,33	0,51	0,42
SEM	0,09	0,08	0,07	0,11	0,09
Total protein (g/l)					
Mean	63,80	32,75	34,70	34,35	39,30
SD	7,02	3,99	1,39	4,45	8,05
SEM	1,75	0,89	0,31	0,99	1,80
Albumin (g/l)					
Mean	33,90	18,45	20,20	19,55	22,25
SD	4,12	2,32	4,04	3,46	4,63
SEM	0,92	0,52	0,90	0,77	1,04
Phosphate (mmol/l)					
Mean	0,69	0,56	0,47	0,49	0,53
SD	0,41	0,38	0,25	0,26	0,26
SEM	0,09	0,09	0,06	0,06	0,06
pH					
Mean	7,32	7,32	7,33	7,34	7,33

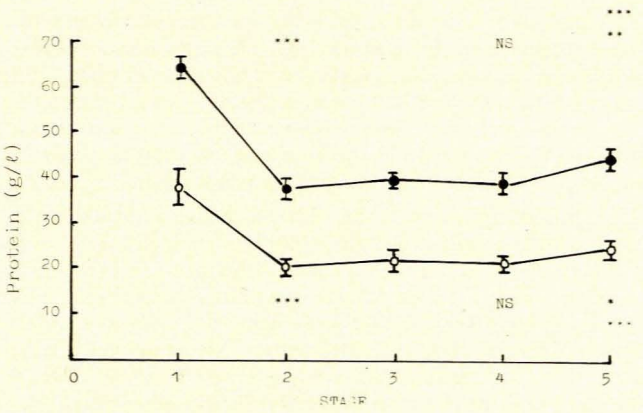

Fig. 2. Changes in total protein and albumin levels: before CPB (stage 1); 5 min after start of CPB (stage 2); before administration of calcium (stage 3); after administration of calcium (stage 4); 5 min after termination of CPB (stage 5). Statistical comparison was made between stages 1 and 2, 3 and 4, 4 and 5, and 1 and 5 (extra values indicated at stage 5). Significance: *$P < 0,05$; **$P < 0,025$; ***$P < 0,001$; NS = not significant (● = total proteins; O = albumin).

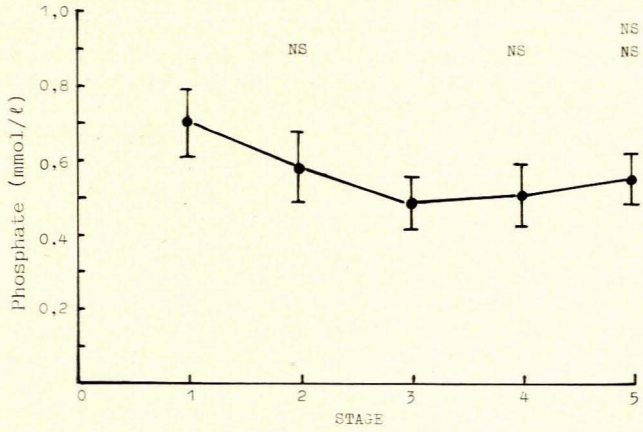

Fig. 3. Changes in phosphate concentration: before CPB (stage 1); 5 min after start of CPB (stage 2); before administration of calcium (stage 3); after administration of calcium (stage 4); 5 min after termination of CPB (stage 5). Statistical comparison was made between stages 1 and 2, 3 and 4, 4 and 5, and 1 and 5 (extra values indicated at stage 5). Significance: *$P < 0,05$; **$P < 0,025$; ***$P < 0,001$; NS = not significant.

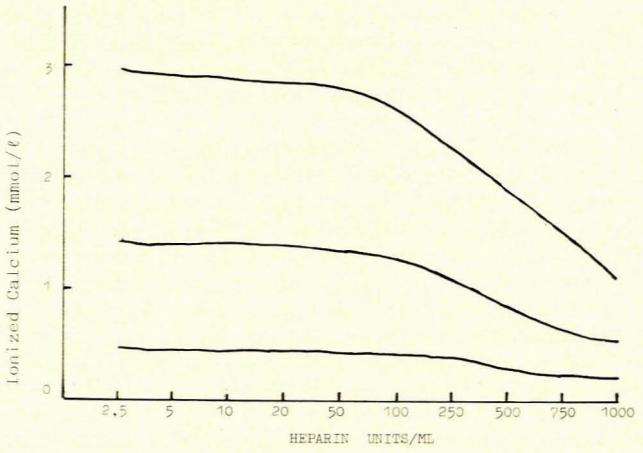

Fig. 4. Effect of heparin on Ca^{2+} concentration (saline plus calcium).

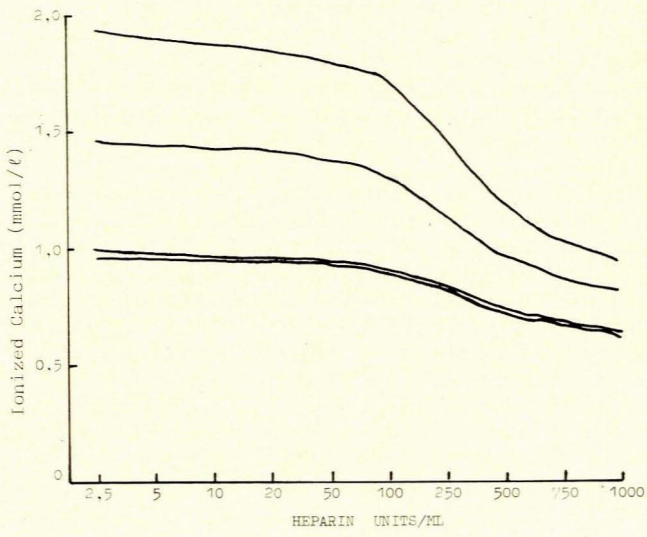

Fig. 5. Effect of heparin on Ca^{2+} concentration (blood plus calcium).

It has been shown previously that ischaemia, as may occur during CPB, can impair both the normal binding and the release of calcium from the sarcoplasmic reticulum.[10] This clearly suggests that the prevention of excessive ischaemia and the control of myocardial Ca^{2+} during CPB can be an important determinant of myocardial function after CPB.

The decrease in the plasma calcium concentration during CPB has been discussed by various authors.[1,2,11,12] During bypass, when the heart is in asystole, the myocardial wall tension and hence oxygen consumption are closely related to the intracellular myocardial calcium concentration.[13] If the myocardial wall tension is allowed to rise during the period when there is no effective coronary blood flow and thus no effective oxygenation, the store of high-energy phosphate molecules will be depleted. This is not only associated with a decrease in myocardial contractility, but is also one of the factors quoted as a possible mechanism for the well-known and fatal 'stone heart' syndrome which can occur after CPB.[14,15] With this in mind the initial decrease in the Ca^{2+} concentration which occurred before exclusion of the patient's coronary circulation from bypass (partial bypass) can be regarded as beneficial for the myocardial oxygen uptake during CPB (Fig. 1).

The addition of $MgSO_4$ to the cardioplegic solution will block the Ca^{2+}-receptor sites on the myocardial cell membrane and could therefore help to lower the diastolic myocardial tension and oxygen demand.[13] Because a potassium ion concentration of greater than 40 mmol/l also gives rise to an increase in the intracellular Ca^{2+} level and thus increases energy demands,[16] the potassium added to the cardioplegic solution was kept to a functional minimum (30 mmol/l).

The difference between sample 1 (pre-bypass) and sample 2 (bypass), is statistically significant ($P < 0,001$) and it is interesting to note that the percentage Ca^{2+} increased from sample 1 to sample 2. This confirms the observations reported by Gray *et al.*[2] but differs from the results reported by Fuchs *et al.*[12] who showed that the ratio of Ca^{2+} to Ca_T stayed at the pre-bypass level.

The 45% reduction in the albumin concentration observed in this series was probably due to haemodilution. Concurrently, the Ca^{2+} level decreased by 10,55% and the Ca_T level by 23,55%. Although these figures differ from those in other published reports[2] the same trend applies in both the experiments. (The real difference may have been due to the different pump primes used.) The calcium is redistributed towards the ionized form

once CPB is started and this may be due to a change in the albumin concentration, stimulation of parathyroid hormone or some as yet unknown mechanism.[2,11]

It has been shown that the Ca^{2+} concentration is influenced by temperature and at 37°C the values obtained were lower than the values obtained at room temperature.[4] As the Ca^{2+} analysis was done at 37°C and the blood samples were from patients with temperatures ranging from 37°C to 28°C, it was assumed that the scatter in the actual calcium values would be greater than that reported. Furthermore, the assumption was also made that the values in the samples taken from patients at lowered body temperatures would have been higher if analysed at that particular temperature.

The increase in the blood perfusion pressure was 3,94 kPa (mean). The standard deviation of 3,19 kPa shows that although a number of patients responded very well to the administration of calcium, a number did not show any significant increase in perfusion pressure. The effect of calcium on perfusion pressure cannot be predicted in individual patients, as judged from this study. Whether calcium administration was accompanied by an increase in flow and thus oxygen delivery to the myocardium can also not be deduced from the results obtained. It is well known that calcium increases myocardial contractility,[17] but whether this is accompanied by an increase in flow will to a large extent be governed by the impedance to ejection of the stroke volume which, in a normal situation, will be 90% because of arterial resistance.

Effective perfusion pressure of the coronary vessels is certainly necessary in normal humans, but there is substantial evidence from current studies and unpublished data that after CPB an effective perfusion pressure is even more important. In the heart which has been ischaemic, there is normally a 100 - 250% increase in coronary blood flow once the coronary flow has been restored. This hyperaemic response is due to the oxygen debt incurred during the period of ischaemia but the temporary loss of distending pressure seems to be of considerable importance.[18] In current (unpublished) experiments the extent of the hyperaemic response when the coronary perfusion pressure was reduced was significantly less than that at normal perfusion pressure. This is most probably related to the dependence of flow in a low-resistance vessel (dilated) on effective perfusion pressure. Another indication that an effective (probably higher than normal) coronary perfusion pressure is needed after a period of myocardial ischaemia comes from the same experiments. The return of segmental function as measured by pressure-length loops and of global myocardial function as measured by conventional parameters, e.g. dp/dt_{max}/instantaneous pressure and aortic blood flow acceleration, needs a higher coronary perfusion pressure after a period of occlusion.

The use of inotropic agents at the end of CPB is often necessary and this is usually due to a number of factors: (*i*) primary myocardial or valve disease, i.e. the reason for surgery; (*ii*) secondary myocardial dysfunction due to distortion of the Laplace law ($T = \frac{P.r}{2h}$), where T = tension, P = pressure, r = radius and h = myocardial wall thickness, which may follow long-standing valve lesions; (*iii*) ischaemia and hypothermia during the period of CPB with the loss of high-energy phosphate molecules.

The use of inotropic agents increases the oxygen demand of the myocardium owing to an increase in the contractile state, i.e. a rightward shift of the force-velocity curve and, more often than not, an increase in heart rate. This combination of increase in demand coupled with inadequate perfusion in the early stages of termination of CPB could increase the extent of the reperfusion injury.[19] Clearly the clinician is caught between the primary goal of terminating CPB, and not increasing reperfusion injury. With this in mind we prefer not to use any inotropic agents unless all the appropriate invasive monitoring has been done and the correct preload and afterload levels have been achieved. Because of the decrease in the calcium concentration during CPB compared with the pre-CPB values, calcium was given and the effect evaluated. At the same time we could obtain a relatively short period of increase in the inotropic state without unduly increasing heart rate and thus oxygen consumption.

The extent of the reperfusion injury to the myocardium is also indirectly dependent on the pH of the coronary blood. The effect of the reperfusion injury, i.e. cellular oedema and the precipitation of calcium in the intracellular space, is increased if the blood pH is decreased. Other workers have indicated that the subendocardial blood flow and the left ventricular compliance were improved if the perfusing blood had a pH of 7,44 at 37°C.[20] Although oedema of the myocardial cells was still present, the function of the left ventricle on measurement of dp/dt was 18% better than that of controls at normal pH. On the other hand, the increase in the pH could adversely affect the delivery of oxygen owing to a leftward shift in the oxyhaemoglobin dissociation curve. This decrease in oxygen delivery could be overcome by an increase in coronary blood flow during the hyperaemic response phase, provided that there was an adequate coronary perfusion pressure and the coronary arteries were normal or relatively normal. The pH values obtained during protocol stages tended towards the lower levels of physiological pH. The effect of this on the heart function was not evaluated but in view of the quoted work,[20] one will have to accept that the pH could safely be adjusted to higher values before termination of CPB.

At the end of the bypass period the pump flow was adjusted to a maximum line pressure of 37 kPa absolute or a pump-patient gradient of not more than 13,3 kPa. This, coupled with a relatively normal blood pH value and the restoration of the calcium levels to pre-bypass levels plus adequate perfusion pressure values, may be the reason why we had to use other inotropic agents at termination of CPB in only 8% of 108 patients to date. We think that the use of inotropic agents other than calcium can be reduced even more once the measurement of total peripheral resistance, pulmonary artery resistance, stroke work index, stroke index, cardiac index and preload has become a more routine procedure in the patient undergoing CPB, as this will allow for the correct measurement and treatment of haemodynamic abnormalities.

Routinely, leads II and V5 are monitored in these patients. In none were there signs of increasing myocardial ischaemia as judged by ST-segment depression in excess of that observed at the end of CPB. This suggests that there may not have been an excess demand for oxygen compared with oxygen delivery after calcium administration, although this is by no means absolute. It has been shown in dogs that increasing Ca^{2+} levels above normal increases the mean arterial pressure by raising systemic vascular resistance, while cardiac output, stroke volume and heart rate remained unchanged.[21] These findings imply an increase in myocardial oxygen consumption. When Ca^{2+} rose from below-normal to near-normal levels mean arterial pressure was increased owing to an increase in stroke volume and cardiac output, while heart rate and systemic vascular resistance remained unchanged. In humans after aortocoronary bypass calcium increased the cardiac index and arterial pressure, but the systemic vascular resistance, coronary sinus blood flow and myocardial oxygen consumption remained unchanged.[22] Unfortunately Moffitt *et al.*[22] did not measure Ca^{2+} levels and it is therefore impossible to say whether their findings agree with or contradict those of Drop and Scheidegger.[21]

Calcium has been reported to cause arrhythmias in patients, especially if there is a low potassium (K^+) value or the patients are on digitalis therapy.[23] None of the 20 patients taking part in the study showed any pathological abnormality in their heart rate and rhythm after calcium had been administered.

In this study the Ca_T and Ca^{2+} values were always higher than those reported as normal. The reason for this is not clear but the calcium in the intravenous fluid and the prime could certainly contribute to this. By simple calculation this does not seem to be the total answer to this observation but calcium homeostasis and changes in body calcium and water content certainly cannot be predicted on the basis of a simple model. The calcium added to the intravenous fluid negates any arrhythmogenic effect of added K^+ on the heart. In view of the slow rate of administration of the fluid and the high values of calcium obtained and keeping in mind the effect of calcium on the basal heart metabolism as discussed above, it would certainly be wise to omit any calcium from the fluid in future. The amount of calcium added to the prime should also be readjusted.

After the termination of CPB there was a further decrease in the Ca^{2+} values compared with the values obtained after calcium administration. Whether this is due to a redistribution of the administered calcium or to some other as yet undetermined process is not clear. It occurred before the administration of blood to the patient so that citrate cannot be implicated as a possible mechanism.

The low phosphate levels encountered in this study (Fig. 3) could be detrimental to the replenishment of the intracellular myocardial adenosine triphosphate (ATP) stores which has an effect on the myocardial stroke work, as shown by O'Connor *et al.*,[24] and it is speculated that hypophosphataemia and the lowered levels of ATP may be a cause for myocardial depression. As we give generous K^+ supplementation during CPB by means of an automated syringe, the use of potassium phosphate instead of the presently used potassium chloride to induce arrest and to replace K^+ loss during CPB seems to be sound. The low K^+ level may be due to a combination of the glucose/insulin therapy we give to all patients on arrival in theatre and the haemodilution effect of CPB. The phosphorylation of glucose is accelerated by the infusion of glucose/insulin, but in view of initially low K^+ levels, and the lack of intra-operative phosphate supplementation, a new study would have to be designed to elucidate the cause of our particularly low K^+ levels.

The fall in the albumin level during CPB is the same as has been reported by other authors,[2,11] and may be due to haemodilution. The decrease in the colloid osmotic pressure which accompanies this decrease in the albumin concentration may have a detrimental effect on the lung water content, especially after CPB which, under certain circumstances, may be associated with an increase in the pulmonary vascular permeability. This effect may be increased in the presence of decreased left ventricular function and can cause hypoxia due to an increase in the number of high ventilation-perfusion lung units. This problem can be overcome with postoperative mechanical ventilation and the application of positive end-expiratory pressure. However, the question is whether or not osmotically 'active' agents, e.g. fresh-frozen plasma, should be used in the CPB prime.

In summary, we confirmed the decrease in the plasma concentration of Ca^{2+}, CA_T, proteins and albumin during CPB using a clear prime. The heart is largely excluded from the CPB 'circulation' during the period of total bypass and the changes during that stage of the procedure are of minor consequence to the heart's metabolic rate and oxygen consumption. The periods of partial bypass, i.e. when the heart and the CPB are responsible for carrying the circulation and the coronary arteries are still perfused, could certainly induce metabolic and functional myocardial changes if the blood perfusing the coronary arteries were biochemically manipulated. Combined with the results shown in this study, this would provide a sensible rationale for the use of the calcium slow-channel blocking drugs in the pre-CPB period.

We also demonstrated a significant increase in the plasma calcium concentration after the administration of calcium chloride, but whether the inotropic effect of the drug alone, the combination of the drug plus careful haemodynamic manipulation or only the latter was responsible for the ease of weaning patients from CPB will need a controlled trial to decide.

Finally, the low K^+ concentration demonstrated may have relevant clinical implications and positive steps should be taken to correct this during CPB.

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