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ALTERATIONS IN ELECTROLYTE AND TRACE-ELEMENT CONCENTRATIONS DURING SIMULATED EXTRACORPOREAL BLOOD CIRCULATION

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In our studies with simulated extracorporeal blood circulation we observe damaging effects on blood cells, especially under oxygenating conditions. In order to characterize these effects we also analysed electrolyte and trace-element concentrations in plasma during and after simulated extracorporeal blood circulation.

Highest resorption effects for magnesium and highest desorption effects for calcium, copper and iron are found with oxygen gas flow in the system. Membrane permeability for electrolytes seems to be induced as well. Cellular damage due only to mechanical stress within the perfusion system can be neglected.

KEY WORDS: Simulated extracorporeal blood circulation, blood cell damage, electrolytes, trace-elements, free radical reactions.

INTRODUCTION

Reperfusion injury is observed e.g. after organ transplantation and open bypass heart surgery. In these cases, lipid peroxidation certainly plays an important role in post-operative damaging effects. The molecular mechanisms of these processes have not yet been fully elucidated.

Damaging effects caused by the extracorporeal blood circulation system are likely to reinforce lipid peroxidation processes or other oxygen free radical mechanisms during reperfusion injury.

In our studies extracorporeal blood circulation was simulated and the conditions for gas flow and blood flow in our system correspond with those of bypass surgery. Damaging effects were studied under different conditions of simulated extracorporeal blood circulation: without gas flow, with argon gas flow and with oxygen gas flow.

Under oxygen gas flow we can observe especially the following effects (details will be published elsewhere[1,4]):

a) plasma can induce vasoconstriction of rabbit carotid artery.[2,3]

b) free arachidonic acid levels in plasma are rising.

c) the platelet number is decreasing and platelet factor IV is activated.

d) the leucocyte number is decreasing and elastase concentrations are rising rapidly.

However, the molecular mechanisms of these damaging effects as well as the effects on the vessels themselves need further investigation. One possibility for obtaining additional informaion on these processes is to look at the plasma concentrations

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of some electrolytes and trace-elements after simulated extracorporeal blood circulation has taken place.

MATERIALS AND METHODS

Fresh blood from healthy donors was used 24 to 48 hours after donation at the Blood Centre of the University of Tübingen. 75 ml ACD-stabiliser was added to every 500 ml donor blood (Biotest Pharma-Dreieich, West Germany). The heparinised donated fresh blood (4.5 units heparin per ml blood) was mixed with 50 ml of a 5% glucose solution, 130 ml of Ringer-Lactate (Eufusol-Ringer-Lactate, Schiwa GmbH, Glansdofr, West Germany), 10 ml of a 8.4% solution of sodium hydrogen carbonate and 10 ml of a 10% calcium solution in the oxygenator reservoir. This is the condition before circulation starts (t = 0 min). Total test volume in our oxygenator system (Shiley S-070) was 650 to 850 ml. The chemicals used — except otherwise stated — are from B. Braun-Melsungen AG, West Germany. Gas flow (oxygen or argon) was 1–2 liters per min, carbon dioxide flow was 0.03 litre per min. The system temperature was adjusted to 28°C (hypothermic), the blood pressure to 60 mm Hg and the pump to 25 revolutions per min. Blood flow, pump-revolution, gas pressure and gas flow were regulated according to heart-lung-machine conditions during bypass operation. Pump-revolution, blood flow and gas flow were modified in relation to decreasing blood volume during the experiments. Thus we obtained comparable blood volume revolution-times and blood flow and gas flow conditions through the whole experiment.

The perfusion mixture (fresh blood + additives) was analysed after 0, 15, 30, 45, and 60 mins. of simulated extracorporeal blood circulation. Plasma was obtained by centrifuging immediately at 3000 rpm for 15 mins. at 5°C. Plasma samples were stored at −20°C.

Electrolytes and Trace elements

Sodium, potassium and chloride concentrations in plasma were determined with ion-selective electrodes (NOVA biomedical, Darmstadt, West Germany). Calcium, magnesium, iron, copper and zinc concentrations in plasma were measured with a Perkin-Elmer 4000 atomic absorption spectrophotometer after special sample procedures:

Calcium. 100 µl plasma was diluted with 4900 µl of a lanthan-standard-solution (20 ml lanthan in 1 litre of distilled water). Magnesium: 100 µl plasma was diluted with 4900 µl of distilled water.

Copper. 1 ml plasma was mixed with 1 ml of 0.1 M nitric acid. The samples were centrifuged, decanted and measured. Zinc: 500 µl plasma was diluted with 2.0 ml of distilled water.

Iron. In order to avoid iron contamination, only fresh plastic devices were used after rinsing with bidistilled water. 1 ml plasma was mixed with 1 ml of 20% TCA. The sample was heated for 15 mins at 90°C. After cooling, the samples were centrifuged (3000 rpm for 10 mins at 20°C), decanted, and measured.

Each sample was measured three times. The results are means of 10 parallel

experiments with standard error. Graphs and regression curves were calculated and designed on a Hewlett-Packard vectra ES/12 computer with Harvard Graphics software.

RESULTS

The results are shown in Figures 1 to 8. The sample number corresponds with the time of simulated extracorporeal blood circulation.

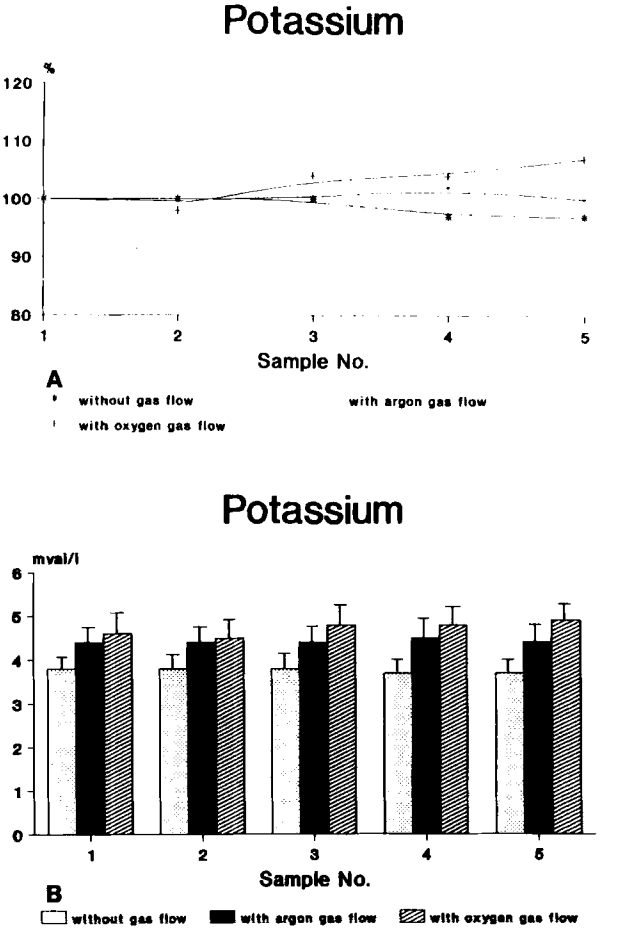

FIGURE 1 Normal plasma level for potassium 3.5–5.0 mval per liter. The sample number in the Figures corresponds with the time of simulated extracorporeal blood circulation. Sample number 1:0 min, sample number 2:15 mins., sample number 3:30 mins., sample number 4; 45 mins., sample number 5:60 mins. In *Part A* of the Figures sample number 1 was set to 100% for each experiment (starting situation in the oxygenator system). In *Part B* of the Figures the results of ten experiments for each condition are shown (means ± standard error).

Potassium (Figure 1)

We detect slight resorption of potassium at the beginning of simulated extracorporeal blood circulation with oxygen gas flow (sample number 2, t = 15 mins.). Then we observe a small increase in potassium concentration. With argon gas flow, and with gas flow in the system, no change was observed. Any alteration in physiological conditions, such as a change in temperature, artificial surfaces, and also induced coagulation processes, can lead to higher potassium levels in plasma. The relatively small increase in potassium concentration during simulated extracorporeal blood circulation indicates that, for example, blood clotting does not seem to be much stimulated.

Sodium (Figure 2)

We find a steady decrease in sodium concentrations of 3–5% below the starting concentrations at t = 0 min. (sample number 1) in all experiments.

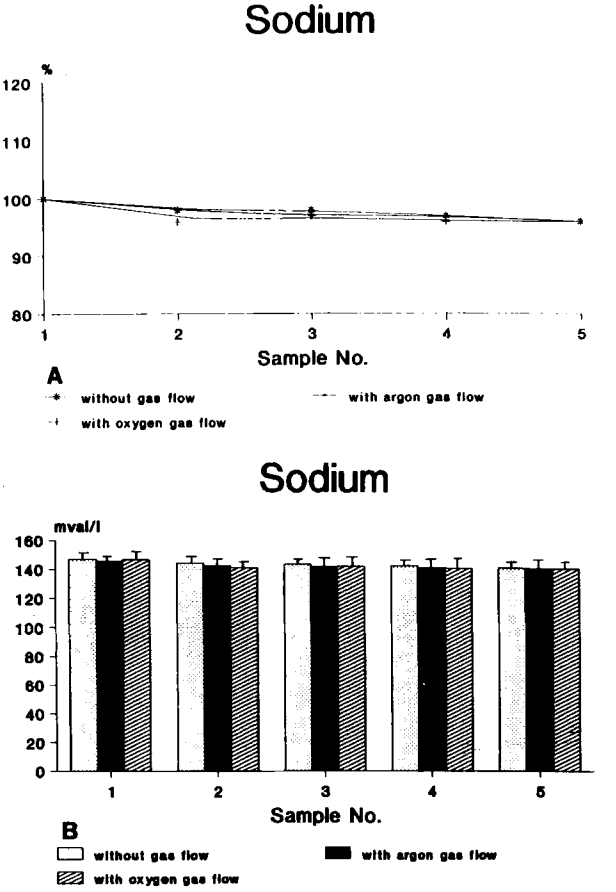

FIGURE 2 Normal plasma level for sodium: 135–150 mval per liter. For sample numbers and Part A and Part B see Figure 1.

Chloride (Figure 3)

In all experiments the chloride concentrations remain around starting values (t = 0 min, sample number 1). The average distribution level is relatively low without gas flow in the system. With argon gas flow we find a slight increase of about 3%. After another 30 mins. this level falls back to starting values. With oxygen gas flow we observe a 4% rise after 15 mins. and the chloride concentrations remain around this level.

Sodium metabolism does not seem to be inhibited or stimulated during simulated extracorporeal blood circulation. We cannot observe great changes in sodium or chloride concentrations.

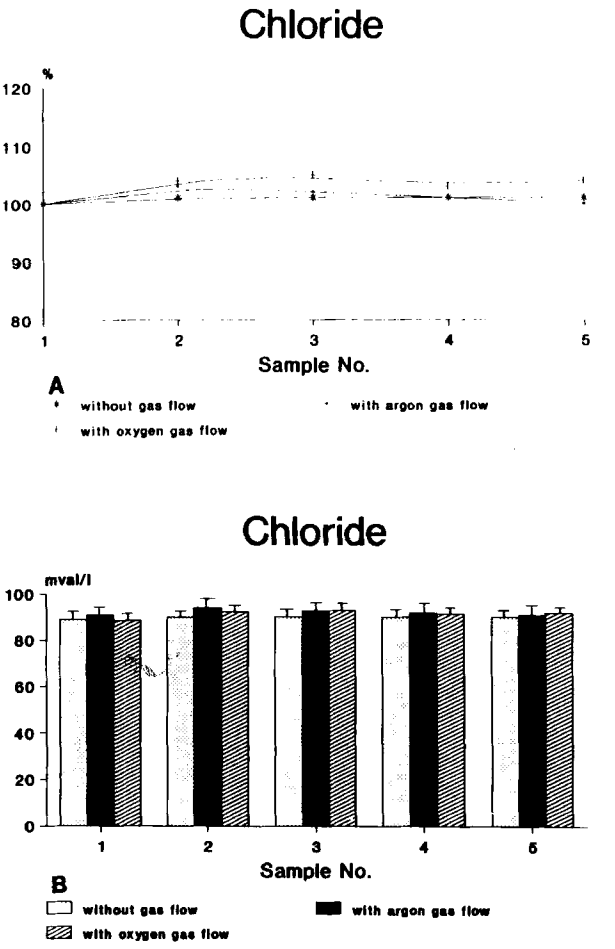

FIGURE 3 Normal plasma level for chloride: 94–110 mval per liter. For sample numbers and Part A and Part B see Figure 1.

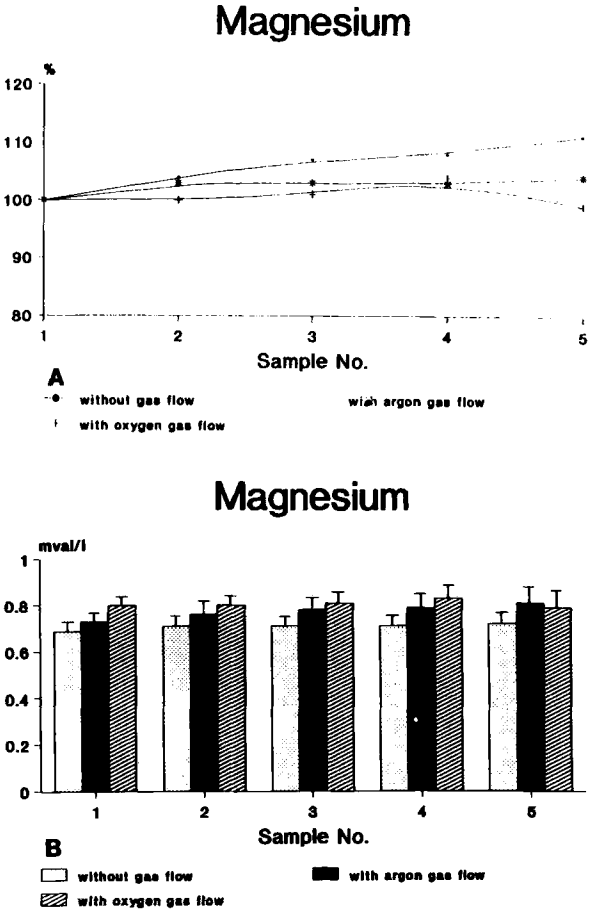

FIGURE 4 Normal plasma level for magnesium: 0.74–1.27 mval per liter. For sample numbers and Part A and Part B see Figure 1.

Magnesium (Figure 4)

With oxygen gas flow, and also without gas flow in the system, only a small increase in magnesium concentrations is found. With oxygen gas flow, however, we can see slight resorption (about 3%) after 60 mins. (sample number 5) of simulated extracorporeal blood circulation. With argon gas flow in the system we observe a rise in magnesium concentrations (see Figure 4A, sample number 5).

Copper (Figure 5)

Steadily rising copper concentrations were detected with oxygen gas flow in the system. During argon gas flow we observe slight resorption (3% at t = 15 mins., sample number 2) and then constantly rising amounts of copper. Without gas flow we detect no changes.

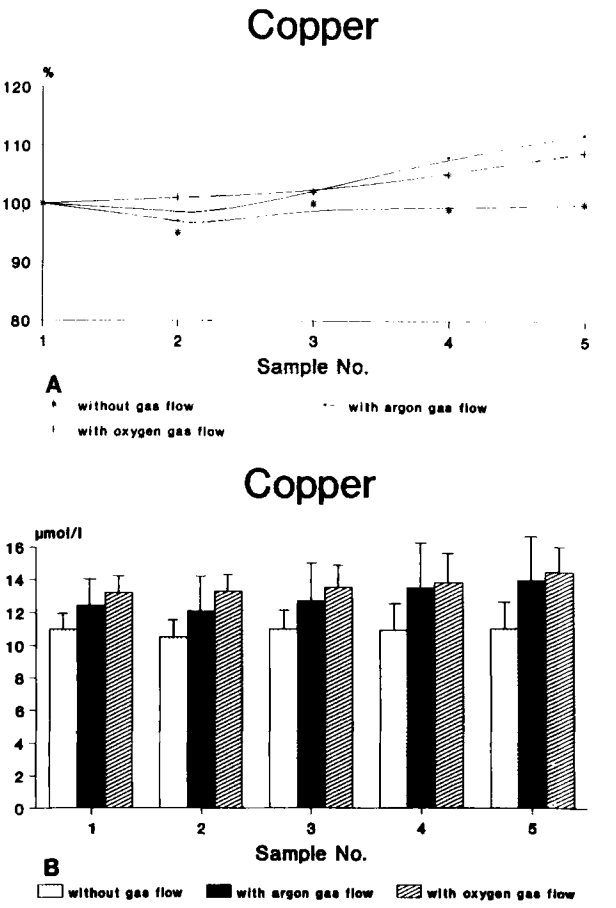

FIGURE 5 Normal plasma level for copper: 11.0–22.0 µmol per liter. For sample numbers and Part A and Part B see Figure 1.

Copper is stored in red blood cells where it is bound to superoxide dismutase. In blood 90% of the copper is attached to caeruloplasmin (8 atoms per molecule). The rest is loosely bound to albumin and amino acids.[5] With gas flow in the system we find more liberated copper in plasma.

Zinc (Figure 6)

Steadily rising zinc concentrations are found with oxygen gas flow and without gas flow in the system (total increase: 20–30% above t = 0 min values). The highest rise in zinc concentrations is observed with argon gas flow in the system (45% above t = 0 min values after 60 min. of circulation, sample number 5), however, following a slight decrease in concentration after 30 min. of circulation (sample number 3). Most of the zinc in blood is loosely associated with albumin (60%). The rest is complexed to α_2-macroglobulin. Also zinc is released from platelets during coagula-

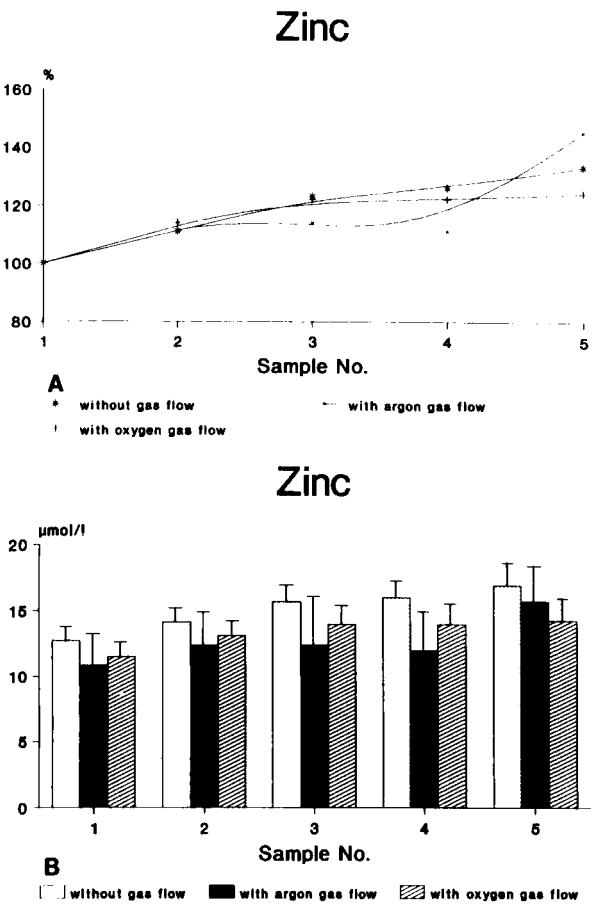

FIGURE 6 Normal plasma level for zinc: 7.65–18.36 µmol per liter. For sample numbers and Part A and Part B see Figure 1.

tion processes. According to physiological conditions, free zinc may be easily mobilised.

No statistically significant differences in zinc mobilisation were found in our experiments. But different trends in zinc concentrations are observed under different conditions of circulation.

Calcium (Figure 7)

The average plasma distribution level of calcium is relatively high in our experiments because we add calcium to the ACD-stabilised donor blood (2.3 mmol to each blood sample). Without gas flow in the system the calcium concentrations remain constantly 10% above the t = 0 min values after 15 mins. of blood circulation, whereas with oxygen gas flow and with argon gas flow we observe slowly rising concentrations. Calcium is concentrated in red blood cell membranes and is important for the

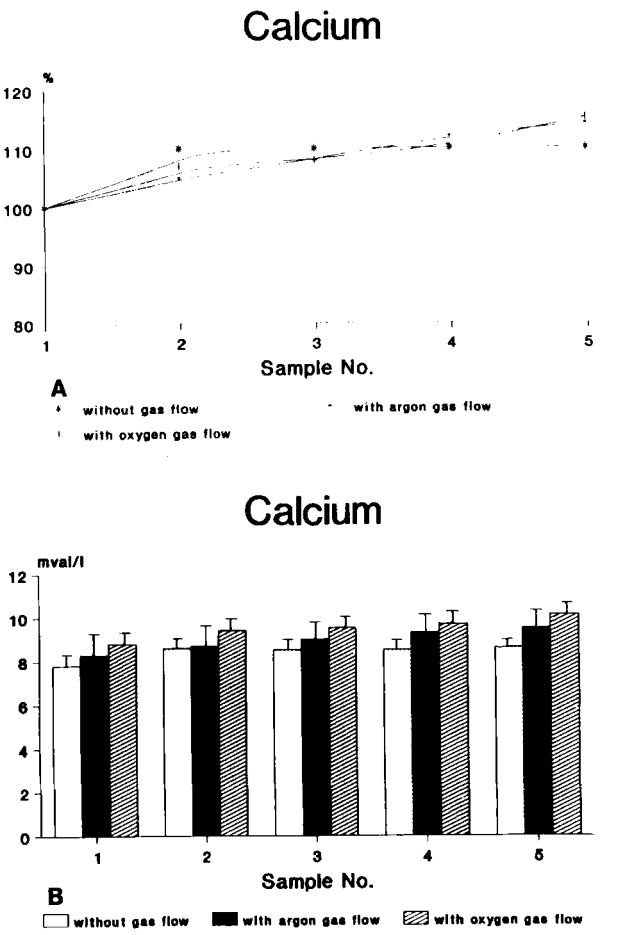

FIGURE 7 Normal plasma level for calcium; 4.5–5.5 mval per liter. For sample numbers and Part A and Part B see Figure 1.

membrane activity. In blood 50% of the calcium present is plasma protein bound, and the rest is so-called "free" calcium. This calcium balance may be influenced during simulated extracorporeal blood circulation, especially with gas flow in the system.

Iron (Figure 8)

We detect highest iron concentrations with oxygen gas flow in the system (about 30% increase). With argon gas flow, iron concentrations are decreasing (40% below t = 0 min values) and slowly rising again to about 80% of the starting values (at t = 0 min). Without gas flow in the system we find nearly constant iron concentrations.

Body iron is mainly bound to intracellularly located proteins (haemoglobin, myoglobin, ferritin). In plasma iron is attached to transferrin (transport iron). Different

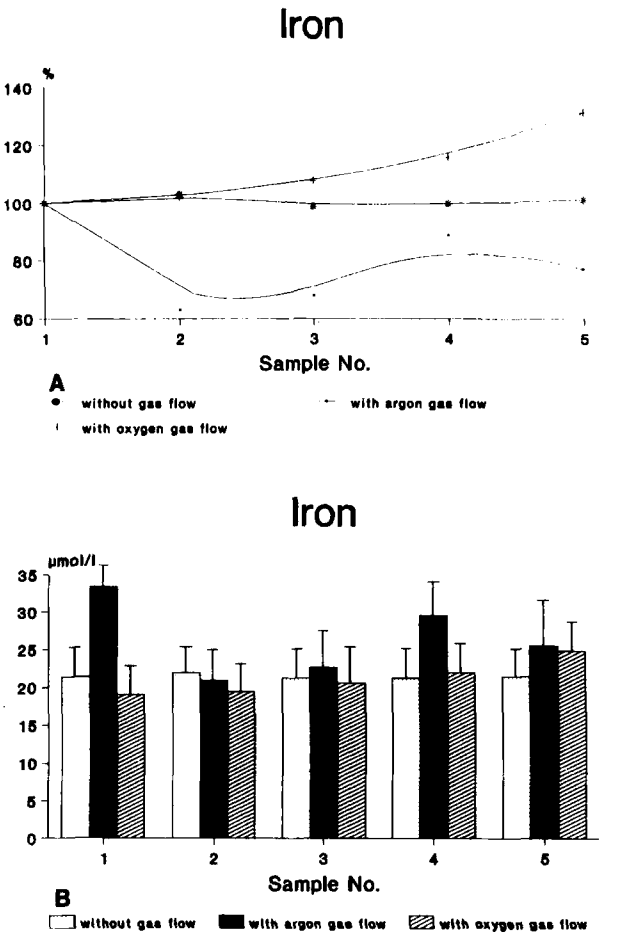

FIGURE 8 Normal plasma level for iron; 11.0–28.0 µmol per liter. For sample numbers and Part A and Part B see Figure 1.

conditions of simulated extracorporeal blood circulation show different influences on the iron binding capacity of cellular blood particles. Iron can obviously be released from its biological surroundings, especially with oxygen gas flow in the system. Resorption and desorption effects are observed during argon gas flow.

DISCUSSION

During simulated extracorporeal circulation of the perfusion mixture in our system under different conditions, potassium, sodium, iron, copper, zinc and magnesium concentrations remain more or less within their normal plasma distribution levels. However, we observe different trends of alterations in electrolyte and trace-element

concentrations. These alterations cannot be explained only by temperature-dependent active transport mechanisms in the membranes of cellular blood particles or their internal organelles: where, for example, potassium is liberated into plasma at low temperatures. The perfusion mixture was circulated at 28°C (hypothermic), and we have to consider a certain adaptation time to these unphysiological conditions. In fact, resorption and desorption effects of potassium show that processes of active transport against the gradient of concentration are obviously being stimulated with oxygen gas flow in the system.

Also compensation effects are possible when no or only little alterations are observed. For example, if blood cells are damaged during circulation and as a consequence electrolytes and trace-elements are liberated, this concentration burst can be neutralised up to a certain extent by the active transport mechanisms of the still intact cellular blood particles. So we cannot assume changes even if we do not see any alterations.

Calcium is added for homoeostatic reasons and its concentration is above normal plasma levels. Although we would expect calcium influx into the cell as a result of the concentration difference between intra- and extracellular calcium after damage to blood cells, we observe a rise in calcium concentration under gas flow.

How can we explain this calcium efflux? During the adaptation period of the perfusion mixture to circulation conditions, the added calcium may be utilised to fill all the intracellular storing capacities (especially mitochondria), which means active transport into the cell and in the cell into internal organelles. After intracellular damage, for example to mitochondria, the stored calcium is liberated again. This leads to a rise in intracellular calcium, which activates the energy-dependent transport of calcium into plasma against the gradient of concentration. Another possibility is induced calcium efflux. Calcium efflux from fat cells induced by hydrogen peroxide has already been observed.[6] And it has been suggested[7] that lipid hydroperoxides are involved in critical physiological processes such as calcium influxes through membranes and activation of eicosanoid biosynthesis.

The following two phenomena show that high iron concentrations in plasma observed under oxygen gas flow cannot be the consequence only of a mechanical damage to red blood cells:

1. the number of red blood cells remains practically constant;
2. no concentration burst of magnesium was found in plasma, which would indicate a damage to red blood cells. So we postulate an energy-dependent transport of iron out of the erythrocytes after the iron has been liberated intracellularly under oxidative conditions.

Concluding, we can say that dominating effects are found with oxygen gas flow in the system and that mechanical stress within the perfusion system can be neglected.

Acknowledgements

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