Haemorrhagic shock — metabolic parameters for the assessment of damage in lung, liver and kidney tissue

F. M. ENGELBRECHT, F. J. MATTHEYSE, W. L. MOUTON

Summary

Changes in catabolic and biosynthetic parameters measured in vitro were used as criteria to assess the degree of damage in tissues after an animal was exposed to severe haemorrhagic shock for periods of 1 and 2 hours (blood loss 36.6%, blood pressure 30 ± 5 mmHg). The biosynthetic capacity of lung tissue, as determined by the incorporation of 1-14C-palmitate into total lung lipids, declined significantly with time. This reduction correlates well \( r = 0.99 \) with the rate of decline in 14C02 production from 1-14C- and 6-14C-glucose oxidation as well as with the decline in the rate of oxygen uptake. Any one of these parameters could therefore be used as an index of the degree of tissue damage due to haemorrhagic shock.

Comparing the rates of decline in 14C02 production from 1-14C-glucose by lung, liver and kidney tissue from the same animal after haemorrhagic insult for 1 hour, lung tissue appeared to be the most sensitive to hypoxia and kidney the least so. However, 2 hours after severe haemorrhage, i.e. near the terminal phase, the rate of 14C02 production from 6-14C-glucose by liver tissue decreased dramatically by more than 53% of the control value. Apart from kidney and lung dysfunction, irreparable liver damage probably plays a major role in the fatal course of severe haemorrhage.

Over the past 10 years, numerous experimental studies have been conducted to evaluate the many theories which have been proposed to explain the sequence of events causing irreversible shock due to hypovolaemia. These theories have included lysosomal disruption, deficits in energy production, damage to the plasma membranes, inability of tissue to derive energy from glycolysis, the accumulation of excess lactate, irreversible injury to the mitochondrial inner membrane and many others. Regardless of the type of injury, intracellular calcium accumulation may be responsible for the cytoskeletal changes and activation of phospholipases which probably perpetuate membrane and mitochondrial damage.

Although irreversible cell injury due to hypovolaemic shock has been characterized at the ultrastructural level, it has been difficult to determine the biochemical point of no return in cell injury. Correlative studies in haemorrhagic shock have shown a close relationship between decreased hepatic energy charge and mortality rate. The hepatic energy charge and adenosine tri-phosphate (ATP) levels of rats in irreversible haemorrhagic shock decreased by 60% and 90% respectively, whereas these parameters were 40% and 70% respectively in reversible shock. In irreversible shock a reduced ability of tissues to derive energy from glycolysis has also been observed. Studies on shocked patients revealed that non-surviving patients had a lower oxygen consumption than survivors. It is known that anaerobic pathways cannot supply adequate energy for long periods at normal body temperature and that hypoxia leads to an excessive accumulation of lactate in vitro.

Recently an effort was made to define biochemically reversible and irreversible hypovolaemic shock in lung tissues in terms of a decline in rates of lipid and protein synthesis in vitro after periods of severe hypovolaemic shock. These results demonstrated a progressive decline in the biosynthetic capacity of lung tissue which correlated with the duration of hypovolaemia. Whether catabolic functions are also affected by severe hypovolaemia, is as yet uncertain. The rates at which irreversible changes take place in different organs may also vary and no information is available regarding the point of no return for lung, liver and kidney tissues from the same animal after severe shock.

Two series of experiments were therefore planned. The first was an attempt to correlate the rate of decline in lipid biosynthesis with that of glucose oxidation and oxygen consumption by lung tissue after 1 and 2 hours of severe haemorrhagic shock. In the second series the degree of tissue damage in terms of decline in the rates of 14CO2 production from 1-14C- and 6-14C-glucose in lung, liver and kidney tissue from the same rabbits was measured under identical shock conditions to those in the first series.

Materials and methods

The protocol of these experiments has been described previously and will be summarized here. New Zealand White rabbits, weighing 1.6 ± 0.2 kg, were used. The rates of lipid synthesis, oxygen consumption and 14CO2 production from 1-14C- and 6-14C-glucose by lung, liver and kidney were measured in tissues from the same animal in vitro at different times after the induction of severe haemorrhagic shock. Each rabbit was anaesthetized with 2.5% thiopentone sodium given intravenously (30 mg/kg body weight). It was then transferred to a constant-temperature operating table (38°C); tracheotomy was performed and a cannula inserted into the trachea to ensure an open airway. A polyethylene catheter connected to a T-stopcock was secured in the carotid artery. One opening of the stopcock was attached to a polyethylene tube filled with heparinized 0.9% saline (15 U/ml) from a pressure bottle and connected to a mercury manometer. A 20 ml syringe was attached to the third opening of the stopcock for the withdrawal of blood. The operation lasted 10 minutes.

After a further 10-minute stabilizing period the blood pressure and heart rate were recorded and the stopcock for the withdrawal of blood. The operation lasted 10 minutes. Blood was then withdrawn by means of the syringe...
until the mean arterial pressure was 30 mmHg (zero time). The time taken from the first withdrawal of blood until zero time was 10 minutes. Additional small volumes of blood were withdrawn to maintain a mean arterial pressure of 30 ± 5 mmHg throughout the experimental period of 1 and 2 hours.

On termination of the hypovolaemic period, the rabbits were killed by exsanguination and the lungs perfused in situ with 50 ml cold phosphate-buffered saline (PBS) via the pulmonary artery. The lungs, kidneys and liver were excised and transferred to cold PBS until further processing.

A control rabbit of approximately the same weight and age as the experimental animal was anaesthetized and bled to death. The organs were removed in the same way as from the experimental animal. Tissue blocks were selected from the organs of the control and experimental rabbits and sliced (0.7 mm) with a McIlwain chopper (only renal cortical tissue was used).

Triplicate samples of lung (200 mg), liver (300 mg) and kidney (100 mg) from control and experimental slices were weighed accurately. The samples were incubated separately in metabolic flasks containing 4 ml Krebs–Ringer bicarbonate medium (pH 7.4) and saturated with 95% oxygen and 5% carbon dioxide. As substrate, 5.5 mM cold glucose containing approximately 0.20 µCi 1-14C-glucose (56.8 mCi/mmol) or 6-14C-glucose (56.1 mCi/mmol) (New England Nuclear) was used and the flasks were incubated in a shaking waterbath (90 cycles/min) at 37°C for 60 minutes. The 14CO2 was trapped in 10 ml Instagel (Packard Instrument Co.) injected into the centre well of the flask after termination of the incubation period. The remaining 14C02 was transferred quantitatively to glass scintillation vials, each containing 7.4 ml cold phosphate-buffered saline (PBS) via the pulmonary artery.

The lungs, kidneys and liver were excised and transferred to cold PBS until further processing.

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The results of the in vitro rates of lipid biosynthesis, 1-14CO2 and 6-14CO2 production and oxygen consumption by lung tissue 1 and 2 hours after exposure of the animal to severe haemorrhagic shock (blood pressure 30 ± 5 mmHg). Oxygen consumption is expressed as µl/mg wet tissue and lipid synthesis and 14CO2 production as dpm/mg DNA.

Results

The results of the in vitro rates of lipid biosynthesis, 1-14CO2 and 6-14CO2 production and oxygen consumption by lung tissue from animals subjected to 1 and 2 hours of severe haemorrhagic shock are summarized in Fig. 1. The in vitro biosynthetic and catabolic functions decreased progressively under haemorrhagic shock and the decrease after 1 and 2 hours correlated well (r = 0.99) for all four parameters measured.

The rates of glucose oxidation by lung, liver and kidney tissues in vitro 1 and 2 hours after severe haemorrhagic shock (blood pressure 30 ± 5 mmHg) are given in Table 1. The results show that the degree of damage, as measured by the ability of the tissues to oxidize 1-14C-glucose, was more severe and occurred earlier in lung tissue than in either liver or kidney tissue. However, after 2 hours of severe haemorrhagic shock all tissues showed highly significant decreases in 1-14CO2 production (P<0.001).

### Table 1. Rates of In Vitro Glucose Oxidation by Lung, Liver and Kidney Tissue After 1 and 2 Hours of Haemorrhagic Shock (BP 30 ± 5 mmHg)*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>60 min (%)</th>
<th>120 min (%)</th>
</tr>
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<tbody>
<tr>
<td>Lung</td>
<td>6476.5 ± 425.2</td>
<td>4437.5 ± 1075.9</td>
</tr>
<tr>
<td>Liver</td>
<td>3963.8 ± 504.4</td>
<td>2961.0 ± 440.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>13092.0 ± 1013.6</td>
<td>10544.5 ± 199.0</td>
</tr>
</tbody>
</table>

*14CO2 production from 1-14C-glucose

<table>
<thead>
<tr>
<th>Tissue</th>
<th>60 min (%)</th>
<th>120 min (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>1234.4 ± 118.7</td>
<td>1021.6 ± 115.0</td>
</tr>
<tr>
<td>Liver</td>
<td>1451.3 ± 233.3</td>
<td>1279.8 ± 320.8</td>
</tr>
<tr>
<td>Kidney</td>
<td>10808.0 ± 557.7</td>
<td>7872.8 ± 312.8</td>
</tr>
</tbody>
</table>

*14CO2 production from 6-14C-glucose

*Results are expressed as dpm/mg DNA.
The 6-14CO₂ production followed the same tendency. Of the three tissues investigated, liver mitochondria showed the least decline in 6-14CO₂ production after 1 hour of hypovolaemia (11.8%). Thereafter the capacity of the liver to oxidize glucose deteriorated to reach a value of 53.3% below that of control animals after 2 hours of shock. The same trend was observed in lung and kidney tissue, but to a lesser degree. The significance of these observations is discussed.

**Discussion**

Many efforts have been made to establish measurable parameters to assess the degree of tissue damage after severe haemorrhage and also to evaluate the efficiency of therapeutic regimens. Our laboratory measured lipid and protein biosynthetic capacity of lung tissue after animals had been exposed to severe haemorrhage. Other workers have observed that in patients dying after haemorrhage the total body oxygen consumption was significantly lower than in survivors, whereas some claimed that glycolysis was reduced, resulting in an inadequate energy supply.

In the present study biosynthetic and catabolic functions of tissues from the same animal subjected to shock were measured and compared. Total tissue oxygen consumption was included to quantitate total oxidative capacity. The 6-14CO₂ production was measured to reflect the integrity of the mitochondria whereas 1-14CO₂ production was estimated to gain additional information on pentose shunt activity and perhaps microsomal involvement. In using these parameters as indices of tissue damage, we did not attempt to quantitate any particular metabolic pathway.

Comparing the rates (Fig. 1) of lipid biosynthesis, 1-14CO₂ and 6-14CO₂ production as well as total oxygen consumption in lung tissue from the same animal 1 and 2 hours after subjection to severe haemorrhage a highly significant correlation ($r = 0.99$) was found. It therefore seems reasonable to accept that any one of these indices gives a relative indication of the degree of metabolic damage induced over a period by haemorrhage. This finding supports the observation that declines in haemodynamic and other physiological parameters (including total body oxygen uptake) reflect a multiplicity of changes occurring in irreversible shock.

The production of 1-l4CO₂ is of special interest because if endotoxins were absorbed from the intestine during hypovolaemia, an increase in 1-l4CO₂ production could be expected due to the demand for reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the oxidation of these toxins by microsomal oxygenases. Kuttner et al. demonstrated a 49% increase in glucose-6-phosphate dehydrogenase activity (G-6-PD) in liver tissue after injecting *Escherichia coli* endotoxin 2-3 mg/100 g body weight into rats. As 1-l4CO₂ production is an indication not only of mitochondrial integrity, but also of pentose shunt activity and microsomal involvement, it appears to be a valuable index of cellular function. Our results show a decreased rate of lipid biosynthesis after severe haemorrhage, which could partly be explained by a marked reduction in pentose shunt activity. This pathway must contribute to the fatal course of haemorrhagic shock.

We wish to thank the South African Medical Research Council and the Harry Crossley Fund for financial assistance and Mr L. J. van Schalkwyk, Mr M. Blom and Miss B. E. Anema for technical help.

**REFERENCES**


