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

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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 36,8%, blood pressure 30 ± 5 mmHg). The biosynthetic capacity of lung tissue, as determined by the incorporation of 1-^{14}C-palmitate into total lung lipids, declined significantly with time. This reduction correlates well ($r = 0,99$) with the rate of decline in $^{14}CO_2$ production from 1-^{14}C- and 6-^{14}C-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 $^{14}CO_2$ production from 1-^{14}C-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 $^{14}CO_2$ production from 6-^{14}C-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.

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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, irreparable injury to the mitochondrial inner membrane and many others.[1] 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.[2]

Although irreversible cell injury due to hypovolaemic shock has been characterized at the ultrastructural level,[2] 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 triphosphate (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.[3] In irreversible shock a reduced ability of tissues to derive energy from glycolysis has also been observed.[4] Studies on shocked patients revealed that non-surviving patients had a lower oxygen consumption than survivors.[5] 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 vivo*.

Recently[6] 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 $^{14}CO_2$ production from 1-^{14}C- and 6-^{14}C-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[6] 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 $^{14}CO_2$ production from 1-^{14}C- and 6-^{14}C-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 was noted. Blood was then withdrawn by means of the syringe

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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 periods 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-^{14}C-glucose (56,8 mCi/mmol) or 6-^{14}C-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 $^{14}CO_2$ was trapped in 0,2 ml Carbo-sorb II (Packard Instrument Co.) injected into the centre well of the flask after termination of the incubation period. The remaining CO_2 in the medium was liberated by injecting 2,0 ml 6% perchloric acid into the medium in the flasks, which were then incubated for a further hour. Thereafter the Carbo-sorb II was transferred quantitatively to glass scintillation vials, each containing 10 ml Instagel (Packard Instrument Co.). The radioactivity was measured in a Beckman liquid scintillation counter until a counting error of 1% was obtained.[7]

Triplicate tissue samples from control and experimental lungs were also weighed for measuring the rate of 1-^{14}C-palmitate incorporation into lung lipids *in vitro*, as described previously.[8]

DNA was determined according to the method of Burton[9] and results were expressed as dpm/mg DNA.

Oxygen consumption was determined using the direct Warburg method[10] over 1 hour with air as gas phase. The Krebs-Ringer phosphate medium used and the detailed procedure has been described.[11] Experiments were repeated at least 6 times.

Standard methods were used to compute the mean and standard error of the mean using Student's *t* test.

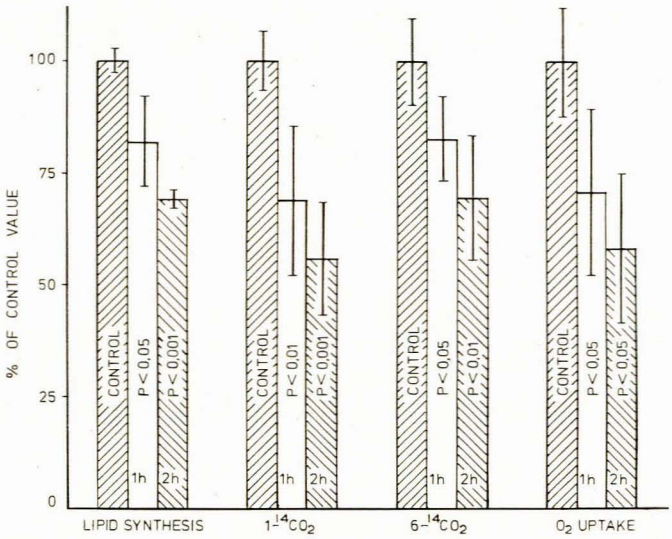

Fig. 1. Percentage declines in the *in vitro* rates of lipid synthesis, $^{14}CO_2$ production from 1-^{14}C- and 6-^{14}C-glucose and oxygen consumption by lung tissue 1 and 2 hours after exposure of the animal to severe haemorrhagic shock (blood pressure 30 ± 5 mmHg). Oxygen consumption is expressed as μl/mg wet tissue and lipid synthesis and $^{14}CO_2$ production as dpm/mg DNA.

Results

The results of the *in vitro* rates of lipid biosynthesis, 1-$^{14}CO_2$ and 6-$^{14}CO_2$ 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 I. The results show that the degree of damage, as measured by the ability of the tissues to oxidize 1-^{14}C-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-$^{14}CO_2$ production (P<0,001).

TABLE I. RATES OF *IN VITRO* GLUCOSE OXIDATION BY LUNG, LIVER AND KIDNEY TISSUE AFTER 1 AND 2 HOURS OF HAEMORRHAGIC SHOCK (BP 30 ± 5 mmHg)*

	$^{14}CO_2$ production from 1-^{14}C-glucose		
Tissue	Control	60 min (% ↓)	120 min (% ↓)
Lung	**6 476,5 ± 425,2**	**4 437,5 ± 1 075,9**	**3 602,2 ± 851,8**
		***P*< 0,01 (31,5)**	***P*< 0,001 (44,4)**
Liver	**3 963,8 ± 504,4**	**2 961,0 ± 440,5**	**2 044,7 ± 306,1**
		***P*< 0,01 (25,3)**	***P*< 0,001 (48,4)**
Kidney	**13 092,0 ± 1 013,6**	**10 544,5 ± 199,0**	**8 724,0 ± 502,0**
		***P*< 0,01 (19,5)**	***P*< 0,001 (33,4)**
	$^{14}CO_2$ production from 6-^{14}C-glucose		
Tissue	Control	60 min (%↓)	120 min (%↓)
Lung	**1 234,4 ± 118,7**	**1 021,6 ± 115,0**	**858,4 ± 175,0**
		***P*< 0,05 (17,2)**	***P*< 0,01 (30,5)**
Liver	**1 451,3 ± 233,3**	**1 279,8 ± 320,8**	**677,3 ± 277,1**
		***P*< 0,05 (11,8)**	***P*< 0,05 (53,3)**
Kidney	**10 808,0 ± 557,7**	**7 872,8 ± 312,8**	**6 675,5 ± 757,0**
		***P*< 0,01 (27,2)**	***P*< 0,01 (38,2)**

*Results are expressed as dpm/mg DNA.

The 6-$^{14}CO_2$ production followed the same tendency. Of the three tissues investigated, liver mitochondria showed the least decline in 6-$^{14}CO_2$ 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,[5] whereas some claimed that glycolysis was reduced, resulting in an inadequate energy supply.[4]

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-$^{14}CO_2$ production was measured to reflect the integrity of the mitochondria whereas 1-$^{14}CO_2$ 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.[12]

Comparing the rates (Fig. 1) of lipid biosynthesis, 1-$^{14}CO_2$ and 6-$^{14}CO_2$ 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.[5]

The production of 1-$^{14}CO_2$ is of special interest because if endotoxins were absorbed from the intestine during hypovolaemia[13,14] an increase in 1-$^{14}CO_2$ 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.*[15] 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-$^{14}CO_2$ 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 deliver the NADPH needed in lipid biosynthesis. The finding that lipid biosynthesis is less markedly affected than 1-$^{14}CO_2$ production may be due to the fact that 1-^{14}C-palmitate incorporation into total lipids, and not only into the phospholipid fractions, was measured. It has been shown[8] that the incorporation of palmitate into the dipalmitoyl-choline fraction is significantly reduced after severe ischaemia, but this decline is partly masked by a simultaneous increase of palmitate in the free fatty acid pool of ischaemic lung tissue.

It is generally accepted that shock lung syndrome is initiated by hypoxia resulting from a low perfusion pressure in the lung which makes it more vulnerable to severe hypovolaemic damage than either the liver or kidney. The results recorded in Table I show a significant decline in $^{14}CO_2$ production from all the organs investigated. Percentage-wise, the capacity of the lung to produce 1-$^{14}CO_2$ from labelled glucose was reduced by 31,5% and that of liver and kidney by 25,3% and 19,5% respectively after 1 hour of severe hypovolaemia. After 2 hours, kidney tissue was least affected, showing a decline of 33,4%, whereas the liver appears to be most sensitive to hypoxic damage. When 6-$^{14}CO_2$ production was measured, the reduction was also most marked in liver tissue (53,3%) followed by kidney (38,2%) and lastly by lung tissue (30,5%) after 2 hours of shock. However, liver tissue showed only an 11,8% decline after 1 hour. This may indicate that liver perfusion is well maintained during the initial stages of shock and that the drastic decrease in $^{14}CO_2$ production during the 2nd hour of hypovolaemia could be due to the effect of endotoxins gradually released from the intestines on liver circulation. It has been shown that intravenous administration of endotoxin causes an increase in the net portal pressure which is associated with a marked fall in the arterial and portal venous flows.[16]

Although the percentage decline in $^{14}CO_2$ production from 1-^{14}C- and 6-^{14}C-glucose differs in lung, liver and kidney tissue from the same animal after severe haemorrhage, our results show that any of these parameters may be used as an index of the severity of the damage incurred.

Comparing the results of Kahng[3] who found a decreased hepatic energy charge of 60% and a drop in ATP levels of 90% in rats during irreversible haemorrhagic shock, the drop in 6-$^{14}CO_2$ production by liver tissue from rabbits was only 53,3% after 2 hours of severe haemorrhage. Although this value is lower than the percentage reduction in ATP levels, the 53,3% reduction in the oxidative capacity of liver mitochondria from rabbits in a near terminal phase correlated well with the 60% decline in hepatic energy charge measured in terminally shocked rats. From these observations, it is concluded that liver damage may contribute to the fatal course of haemorrhagic shock.

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