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Four Stages of Mitochondrial Deterioration in Hemorrhagic Shock*

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Summary. It is of great importance to define the manner in which cells are damaged and how intracellular derangement becomes irreversible during shock. When supply of both oxygen and substrates to cells is limited during shock, cellular energy metabolism of vital organs is severely depressed. In this experiment, the relationship was clarified between the reversibility of shock and the cellular energy status, from the viewpoint of hepatic energy charge, mitochondrial redox state, ATP synthesis of isolated mitochondria, and fragility of mitochondrial membrane in rat livers. The derangement of energy metabolism passed through a series of four stages during hemorrhagic shock. At Stage I (initial stage), the cellular energy level decreased greatly due to marked energy consumption, without any organic damages in the mitochondria. Stage II (cell distress stage) showed that cellular energy imbalance occurred due to the depressed mitochondrial activity in vivo, although it was reversible when the blood supply was restored. Stage III (transitional stage) was the phase at which mitochondrial fragility increased severely. At Stage IV (terminal stage), mitochondria were markedly damaged organically and cellular energy metabolism was not remedied by any intensive therapies, which inevitably meant the death of vital organs.

Key words: Hemorrhagic shock – Energy metabolism – Mitochondria

Introduction

Many investigations have been made to clarify the mechanisms by which shock results in cell necrosis [1–3]. As oxygen availability inside a cell decreases due to impaired tissue perfusion in shock, the oxidation of NADH to molecular oxygen through the electron carrier system decreases, resulting in the inhibition of the

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Krebs cycle of mitochondria [4]. The energy available to the cell is thus limited to the enhanced production of ATP by the glycolytic pathways. However, enhanced glycolysis is merely a poor yield of ATP in limited duration. With the depletion of ATP, the life of the cell is again endangered as a great number of endergonic biologic reactions cease and cell membrane starts to malfunction [3, 5]. Finally, the energy deficit and acidosis contribute to cellular derangements, some of which are irreversible [6]. In these processes, while certain forming of metabolic aberration in shock is remedied with the restoration of normal perfusion, their relationship to irreversible cell damage remained to be clarified.

It has been found that irreversible cell injury occurs when there is a progressive decline in circulatory homeostasis, which cannot be remedied in spite of the restoration of normal perfusion [7]. Such circulatory failure has been found to occur concomitant with the impairment of liver mitochondrial function [8, 9]. It has also reported that mitochondrial damage resulting from the failure of oxidative phosphorylation is a critical development and may be regarded as a precursor of cell necrosis [5, 8, 10, 11]. Thus, this study will suggest that by careful analysis of the patterns of functional abnormalities and their changes over time, a general pattern emerges by which the magnitude of such impairment can be quantitatively staged, thus providing a key to the clinical classification and staging of the hemorrhagic shock process.

Further evidence will be presented indicating that there are four stages (ranging from normal to irreversible impairment) in mitochondrial functions following hemorrhagic shock. This classification could serve as a pathophysiologic register of the quantitative nature of the severity of the shock process.

Materials and Methods

Male albino rats of Wistar strain weighing 240–260 g were allowed water but no food for about 16 h prior to the experiment. Both sham-operated and experimental animals were anesthetized by i.p. injection of 30 mg pentobarbital/kg of body weight. The left femoral artery was cannulated with polyethylene tubing, and 500 units of heparin sodium was injected. The cannula was connected to a three-way cock; one valve was connected to a syringe used as a reservoir for shed blood, the other to a mercuric manometer for monitoring arterial blood pressure. Animals were fixed in supine position and rapid bleeding was induced for up to 1 min until mean arterial blood pressure had reached 30 mm Hg; the amount of initially shed blood averaged 3,7 ml. For the following 1–2 h, it was necessary to continue extracting small amounts of blood to keep blood pressure at 30 mm Hg. At a certain point, however, small amounts of shed blood had to be reinfused in order to maintain blood pressure at 30 mm Hg.

Five groups of hemorrhagically shocked rats were designated on the basis of reinfusion rate (= amount of reinfused shed blood to maintain blood pressure at 30 mm Hg/maximal amount of shed blood during hemorrhagic shock), since the mortality of the shocked rats corresponds well with the amount of reinfused shed blood, and since the reinfusion rate generally represents the degree of severity in hemorrhagic shock [5].

Five groups were as follows (Fig. 1): Group A, 0% reinfusion; Group B, 25% reinfusion; group C, 50% reinfusion; Group D, 75% reinfusion; Group E, 100% reinfusion. In each group, the following parameters were examined:

[1] *Energy Charge and Mitochondrial Redox State of the Liver*

For the assay of the adenine nucleotides and the calculation of the redox state, 2 g of liver sample was clamped and pressed immediately to about 1 mm thickness with stainless steel tongs

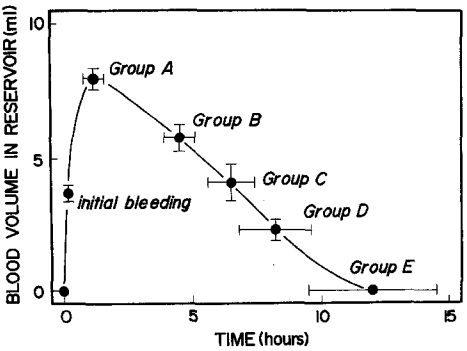


Fig. 1. Relationship between blood volume in reservoir and time course following hemorrhagic shock. Each point represents the mean ± SEM of both the amount of shed blood and time required to maintain the arterial blood pressure at 30 mm Hg. Initial bleeding: the amount of shed blood to induce hemorrhagic shock of 30 mm Hg blood pressure within 1 min. Numbers of rat are as follows: *Group A* (29), B (29), C (8), D (5), E (7)

precooled in liquid nitrogen, and the procedure was completed within 10 s. The frozen tissue was then powdered with a mortar and pestle in a liquid nitrogen bath. The powdered tissue was weighed and homogenized in a cold solution of 6% (W/V) perchloric acid at 0° C. The extract was then centrifuged at 10,000 *g* for 15 min at 0°C. The precipitate was washed with ice-cold 3% perchloric acid to minimize the analytic error due to retention of residual compounds in the pellet, and recentrifuged as described above. The two supernatants were then pooled and adjusted to pH 6.0 with cold 69% K_2CO_3 and recentrifuged at 10,000 *g* for 5 min at 0° C. The amounts of ATP, ADP, and AMP were measured enzymatically [12, 13], and energy charge was calculated according to the formula proposed by Atkinson [14, 15].

$$\text{Energy charge} = (\text{ATP} + 1/2\,\text{ADP}) / (\text{ATP} + \text{ADP} + \text{AMP})$$

The mitochondrial redox state was calculated as follows:

$$\text{Mitochondrial redox state} = \text{Free NAD}^+/\text{Free NADH} = (\text{acetoacetate}/\beta\text{-hydroxybutyrate}) \times 1/\text{K}$$

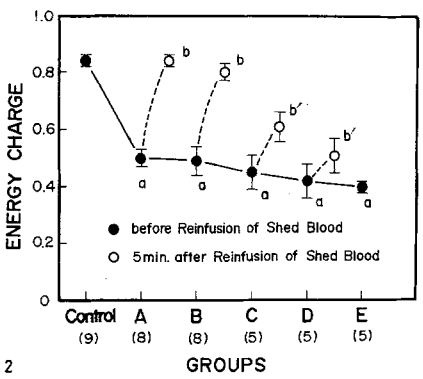
Acetoacetate and β-hydroxybutyrate were determined by the methods of Williamson and Mellanby [16]. Here, the K value of the equilibrium constant was 4.93×10^{-2} for β-hydroxybutyrate dehydrogenase. Energy charge and mitochondrial redox state were examined both before and after the reinfusion of residual shed blood.

[2] *Function of Isolated Mitochondria*

Liver mitochondria were prepared by the method reported previously [17]. Oxygen consumption was measured polarographically according to a previously reported method [18]. The respiratory control ratio (RC = State 3 respiration rate / State 4 respiration rate) was calculated from the polarographical tracings by the method of Chance [19]. Oxidative phosphorylative activity (ATP synthesis = State 3 respiration rate × ADP/O) was calculated. These determinations were made both before and after a 60 min incubation at 22° C. Mitochondrial protein concentration was measured by the method of Lowry et al. [20].

[3] *Swelling and Shrinking of Mitochondria*

Swollen mitochondria have a lower optical density due to water uptake. The swelling and shrinking of mitochondria were determined according to the change in optical density per mitochondrial protein concentration, at 520 nm wave length in KCl medium [21, 22]. Optical density was measured for three points: first, for spontaneous swelling, brought about by incubating 1.4–1.8 mg/ml of mitochondria in the medium at 22° C for 30 min; next, for maximal swelling induced by the addition of phosphate (Pi); and finally, for the reversal effect (shrinking) by the administration of ATP, bovine albumin, and Mg^{++}.



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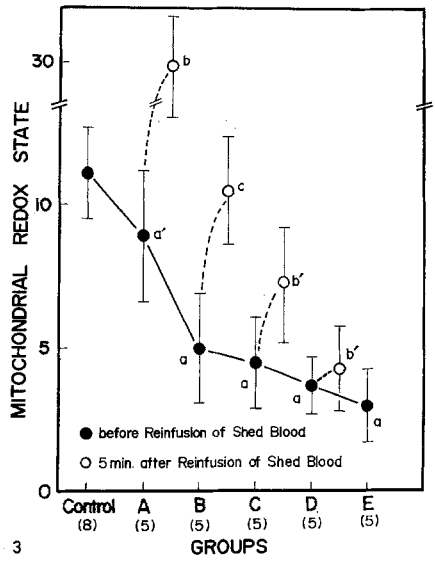

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Fig. 2. Changes in hepatic energy charge levels of shocked rats both before and after reinfusion of residual shed blood. Results are mean ± SEM. Numbers of rat appear in parentheses. $a = P < 0.001$ compared with the control value; $b = P < 0.001$, b' = not significant, compared with the value before reinfusion

Fig. 3. Changes in mitochondrial redox state following hemorrhagic shock both before and after reinfusion of residual shed blood. Results are means ± SEM. Numbers of rat appear in parentheses. $a = P < 0.001$, a' = not significant, compared with the control; $b = P < 0.001$, b' = not significant, $c = P < 0.05$, compared with the value before reinfusion

[4] *Dinitrophenol (DNP)-induced ATPase Activity*

Dinitrophenol (DNP)-induced ATPase activity was determined as the release of inorganic phosphate in a reaction solution containing ATP. Mitochondria were incubated in the reaction medium for 10 min at 22°C, and the reaction was stopped by adding trichloroacetic acid (TCA) solution. Phosphate was determined by the method of Fiske and Subbarow [23].

All results are expressed as mean values and standard errors of the mean (SEM). The statistical significance between the mean values was determined by Student's *t*-test.

Results

Figure 2 shows the changes in the hepatic energy charge before and after the reinfusion of residual shed blood. The energy charge level fell rapidly from 0.84 to 0.50 within 2 h after the onset of bleeding, and gradually continued to decrease, showing 0.4 in Group E ($P < 0.001$). However, energy charge levels were almost completely restored to normal levels immediately when the shed blood was returned in groups A and B ($P < 0.001$). In Groups C and D, the energy charge levels were not restored to normal levels even after reinfusion of residual shed blood. In Group E, the additional therapies, such as infusion of blood, serum albumin, or lactate Ringer solution, did not result in a significant elevation of the energy charge levels.

Table 1. Changes in mitochondrial functions of rat liver following hemorrhagic shock both before and after a 60 min incubation at 22° C

Groups		RC	ST3	ADP/O	PR
Control (7)	before	5.18 ± 0.32	40.8 ± 3.8	2.74 ± 0.07	113.1 ± 13.0
	after	2.44 ± 0.19	33.5 ± 3.9	2.32 ± 0.18	78.2 ± 13.1
Group A (5)	before	6.06 ± 0.58	40.1 ± 3.9	2.72 ± 0.21	109.6 ± 13.2
	after	2.78 ± 0.51	35.8 ± 3.7	2.03 ± 0.18	73.1 ± 10.3
Group B (5)	before	5.51 ± 0.94	39.3 ± 3.8	2.68 ± 0.16	105.3 ± 11.6
	after	2.71 ± 0.32	34.3 ± 4.0	2.37 ± 0.21	79.8 ± 10.8
Group C (5)	before	4.94 ± 0.42	39.8 ± 4.6	2.49 ± 0.17	103.1 ± 16.9
	after	2.63 ± 0.48	U-38.2 ± 4.2	1.60 ± 0.39	62.1 ± 17.0
Group D (5)	before	4.88 ± 0.47	42.9 ± 4.5	2.45 ± 0.16	105.4 ± 12.7
	after	1.35 ± 0.25**	U-30.4 ± 4.3	1.08 ± 0.50*	33.5 ± 17.0*
Group E (7)	before	3.12 ± 0.16***	36.5 ± 4.2	1.62 ± 0.08***	59.7 ± 8.6**
	after	1.34 ± 0.25**	U-31.7 ± 3.0	0.53 ± 0.34***	16.9 ± 9.1***

Mitochondrial activities were measured at 22° C, pH 7.4 in a medium containing 0.3 M mannitol, 0.01 M KCl, 0.004 M $MgCl_2$, 0.01 M Tris-HCl buffer, 0.2 M EDTA, 0.05 M potassium phosphate buffer, and 230 μM ADP. Glutamate was added at a concentration of 4 mM. Values are means ± SEM. Numbers of rat appear in parentheses. RC: Respiratory control ratio, ST3: State 3 respiration rate (nat/mg protein/min), PR: Phosphorylation rate (nmol/mg protein/min), before or after: before or after the incubation at 22° C for 60 min, U: Uncoupling; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the control value

Changes in mitochondrial redox state, both before and after the reinfusion of residual shed blood, are shown in Fig. 3. Before reinfusion, the value of mitochondrial redox state remained unchanged in group A, but had decreased significantly in Groups B, C. D, and E ($P < 0.001$). Immediately after reinfusion, the mitochondrial redox state reached a higher value than normal in group A ($P < 0.001$), and was restored to approximately normal level in Group B ($P < 0.05$). In Groups C and D, while the mean values of mitochondrial redox state were elevated after reinfusion, these values were not significant as compared to those prior to reinfusion. In Group E, the additional infusion of blood, serum albumin, or lactate Ringer solution did not affect the mitochondrial redox state.

In Table 1, the changes in RC, State 3 respiration rate, ADP/O and phosphorylation rate are recorded. These assays were performed both before and after the incubation of mitochondria at 22° C for 60 min. Before incubation, RC, ADP/O, and phosphorylation rate showed significant decrease only in Group E ($P < 0.001$, $P < 0.001$, $P < 0.01$, respectively). As to State 3 respiration, no significant change was observed in any groups, indicating that the decrease of phosphorylation rate was due to the decrease of ADP/O. On the other hand, mitochondrial functions deteriorated for every group after incubation, although to different degrees. In Groups D and E, RC, ADP/O, and phosphorylation rate decreased significantly as compared with the control values (Group D, $P < 0.01$, $P < 0.05$, $P < 0.05$; Group E, $P < 0.01$, $P < 0.001$, $P < 0.001$). In Groups C, D, and E incubated mitochondria were often observed to be in the uncoupling state.

Table 2. Changes in swelling and shrinking of mitochondria of rat liver following hemorrhagic shock

Groups	Before	Sp-S	Pi-S	Shrink
Control (8)	0.512 ± 0.052	0.451 ± 0.062	0.205 ± 0.083	0.487 ± 0.058
Group A (5)	0.525 ± 0.060	0.472 ± 0.060	0.212 ± 0.088	0.480 ± 0.045
Group B (5)	0.501 ± 0.048	0.444 ± 0.050	0.196 ± 0.060	0.471 ± 0.047
Group C (5)	0.480 ± 0.040	0.402 ± 0.039	0.123 ± 0.082	0.353 ± 0.075
Group D (5)	0.452 ± 0.051	0.410 ± 0.058	0.110 ± 0.090	0.333 ± 0.065
Group E (5)	0.381 ± 0.050	0.251 ± 0.065*	0.103 ± 0.085	0.132 ± 0.033**

Test system consisted of 5 ml of 0.125 M KCl–0.02 M Tris, pH 7.4, temperature 22°C. Pi was added at a concentration of 0.01 M as a swelling agent. Additives for shrinking were 0.05 M ATP, 2 mg/ml of bovine serum albumin, and 0.003 M $MgCl_2$. Before: before the onset of swelling reaction, Sp-S: spontaneous swelling after 30 min, Pi-S: Pi-induced swelling after 30 min, Shrink: shrinking 30 min after the addition of ATP, bovine serum albumin, and Mg^{++}. Values are mean ± SEM of optical density per mg protein of mitochondria. Numbers of rat appear in parentheses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the control value

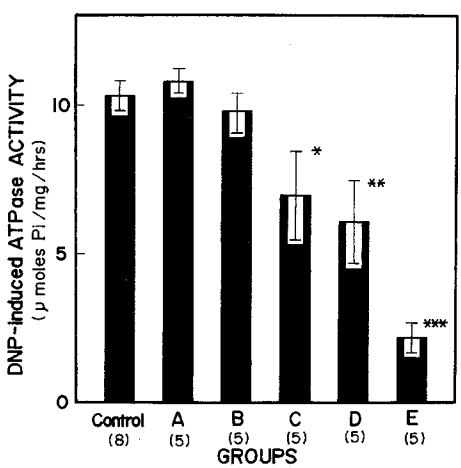

Fig. 4. Changes in DNP-induced ATPase activity of mitochondria of rat liver following hemorrhagic shock. Assay system consisted of 0.25 M mannitol, 0.01 M KCl, 0.2 mM EDTA, 0.01 M Tris-HCl buffer, and 1.5 mM DNP. 25 mmol of ATP was added as a substrate, and released Pi was determiend after 10 min incubation at 22°C. Values are mean ± SEM. Numbers of rat appear in parentheses. * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$ compared with the control

Mitochondrial swelling and shrinking were studied in every group (Table 2). Active swelling of mitochondria was greatly stimulated by Pi, and the swollen mitochondria shrank or contracted again after the addition of ATP, bovine serum albumin plus Mg^{++}. Before the onset of the swelling reaction, the mitochondria from Group E had a lower optical density than those of other groups, although not significantly. In group E, significant spontaneous swelling was observed ($P < 0.05$), whereas it was not as marked in the other groups. As to Pi-induced swelling, there were no significant differences in each group, although mean values were lower than the control in groups C, D, and E. In this assay system, mitochondria seemed to be exhibiting maximal swelling by Pi. In Groups A and B, Pi-induced swelling was completely restored to control values by the addition of ATP, bovine albumin,

and Mg^{++}. In Groups C and D, the shrinking was incomplete, and in Group E no shrinking was observed ($P < 0.001$).

Figure 4 shows the changes in DNP-induced ATPase activity in the mitochondria. DNP-induced ATPase activity remained unchanged in Groups A and B and decreased in Groups C ($P < 0.02$) and D ($P < 0.01$). In Group E the values then dropped markedly to about 20% of the control level ($P < 0.001$).

Discussion

The Wiggars model of hemorrhagic shock is preferable when studying the effect of prolonged arterial hypotention, since this model considers the amount of reinfused shed blood to be representative of the severity of shock, eliminating the factors which comprise affective homeostatic responses [24, 25].

Many investigations have dealt with hemorrhagic shock in terms of the decrease and depletion of hepatic adenine nucleotides [26, 27, 28]. The amounts of adenine nucleotides, however, are not so sensitive indicators as to be able to represent clearly the dynamic alterations of cellular energy metabolism. As reported elsewhere, the restoration of energy charge is possible through the reinfusion of shed blood [29]. In the present experiment, there was a significant decrease in the energy charge level even from the early stage of shock, which progressively declined until the terminal stage. The degree in restoration of energy charge level by reinfusion of residual shed blood is noteworthy. The early stage of shock showed complete recovery of energy charge within 5 min after reinfusion of shed blood. As shock proceeded, however, the restoration of energy charge became incomplete. At the terminal stage, no therapy applied could restore the level of energy charge; all animals died within a few minutes without any restoration of systemic arterial blood pressure. From these results, it seems possible to conclude that the stages represented by Groups A and B are reversible, whereas the stages represented by Group E are irreversible. These data are consistent with the mortality reported by Baue et al. in early and late shock, respectively [5].

Mitochondrial redox state is also a suitable parameter in vivo when discussing the pathophysiology of shock from the energy metabolic point of view. The NAD pool in the mitochondrial compartment becomes further reduced as shock proceeds [30]. Presumably this change is the result of the lower rate of oxidative phosphorylation and the mitochondrial reoxidation of NADH. Mitochondrial redox state remained unchanged in Group A, but dropped in Groups B, C, D, and E. Mitochondrial redox state was restored to a higher than normal value in group A after the reinfusion of shed blood. The reason for this is not clear but it can be hypothetically proposed that mitochondria continued to work in compensation even when sufficient blood was resupplied. In Group B, the restoration of mitochondrial redox state was still complete, whereas it was incomplete in Groups C and D. The final and most serious metabolic derangement associated with progressive shock may be the saturation of the hydrogen shuttle mechanism. Thus, it seems possible to conclude that the recovery of energy charge and mitochondrial redox state is an essential factor when discussing whether or not shock is reversible.

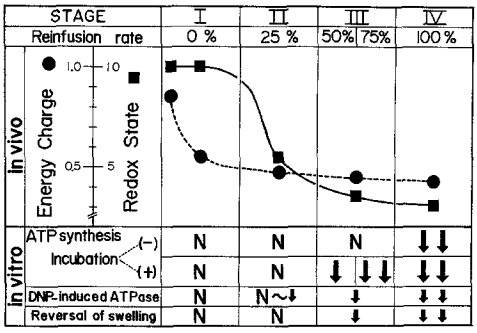

Fig. 5. Four stages of mitochondrial deterioration of rat liver in hemorrhagic shock

There are many investigations reporting that the alterations of mitochondrial function occur at earlier stages [9, 10, 11]. In this experiment, the changes in those parameters showed a nearly identical pattern. ATP synthesis of isolated mitochondria showed no decrease in groups A, B, C, and D, although Group E showed marked decrease. The incubation of mitochondria at 22°C for 60 min was conducted to examine the fragility of mitochondrial membrane. In Groups C and D, RC and oxidative phosphorylative activity showed significant decrease after the incubation, and the fragility of mitochondrial membrane had significantly increased as compared with that of control group.

Mitochondria are highly susceptible to swelling during respiration in the absence of ATP or ADP [31], and in that condition several compounds, such as free fatty acid, Pi, and Ca, tend to accumulate [21]. Vogt et al. reported Ca-induced swelling and shrinking of isolated mitochondria in the ischemic kidney. However, they did not find significant difference in the osmotic properties of these mitochondria [32]. However, in the present experiment, severely damaged mitochondria showed different responses from that of control. It was observed that the mitochondria from Group E showed marked spontaneous swlling and little shrinking by the addition of ATP, serum albumin, and Mg^{++}. Our conjecture is that this is attributable to the accumulation of large amounts of fatty acid which acts as a natural uncoupler [33].

Since DNP-induced ATPase activity correlates with the respiration level of mitochondria [34], it was measured as one of the parameters in vitro in shocked rat livers. In this experiment, such activity showed marked depression at the terminal stage of shock, while showing no alteration at the early stage.

In the light of the results of these parameters, both in vivo and in vitro, a hypothetical classification of the stages of mitochondrial deterioration in the hemorrhagic shock might be as follows (Fig. 5):

Stage I (Initial Stage)

Energy charge decreases significantly, whereas other parameters maintain normal levels. This is the stage at which mitochondrial activity in vivo is not affected but relative increase of energy consumption is predominant as compared to energy-generating reactions. One reason why energy consumption increases relatively is

the increase in metabolic load for the liver from other organs, such as ischemic intestine or muscles. Another reason is that increased metabolites due to accelerated catabolism in hepatocytes accumulate markedly. In this stage, cells fall into the state of energy deficit in spite of no organic changes in the mitochondria. Thus, cellular energy derangement can be said to be reversible at this stage.

Stage II (Cell Distress Stage)

Energy charge and mitochondrial redox state show significant decrease. This indicates that mitochondrial oxidative phosphorylation is being markedly decreased. In this stage, cellular energy imbalance becomes more serious. Usually, hyperglycemia due to hyperglycolysis occurs between Stage I and Stage II; the supply of ATP is provided anaerobically, but the total energy supplied is not sufficient to compensate the decreased aerobical energy supply. However, energy charge and mitochondrial redox state can be restored to normal levels when residual shed blood is reinfused. Other parameters of isolated mitochondria show normal values. Thus, cellular energy metabolism at this stage is decreased due to decreased aerobic energy generating reaction. These changes are also reversible, as at Stage I.

Stage III (Transitional Stage)

It can be said that functional structures of mitochondrial membrane begin to be affected at this stage, as shown by the fact that the reversal effect by addition of ATP, albumin, and Mg^{++} was incomplete. Further, mitochondrial oxidative phosphorylation showed marked decrease after a short period incubation, and finally, DNP-induced ATPase activity is significantly decreased. Thus, mitochondrial fragility is increased markedly at this stage, when some rats die whereas others survive by intensive treatment, such as cardiovascular adjustment or steroid administration.

Stage IV (Terminal Stage)

At this stage, oxidative phosphorylation of isolated mitochondria has decreased even before incubation and to such a degree that energy charge and mitochondrial redox state do not respond to any form of intensive therapy. Here, it is evident that mitochondria have undergone severe damage to their organic structure. Thus, it is difficult to remedy the deficiency in cellular energy metabolism, which inevitably means death for the vital organs.

References

1. Baue AE, Chaudry IH, Wurth MA (1974) Cellular alterations with shock and ischemia. Angiology 25:31
2. Steu I, Cafrita A, Bucur AI (1977) The shock cell. In: Suteu I (ed) Shock pathology, metabolism, shock cell, treatment. Abacus Press, p 313

3. Trump BF (1974) The role of cellular membrane system in shock. In: The cell in shock. Proceedings of Symposium on Recent Research Development and Current Clinical Practice in Shock, p 16
4. Drucker WR, Craig J, Kingsbury B (1962) Citrate metabolism during surgery. Arch Surg 85:557
5. Baue AE (1974) Mitonchondrial function in shock. In: The cell in shock. Proceedings of Symposium on Recent Research Development and Current Clinical Practice in Shock, p 11
6. Mela LM, Miller LD, Nicholas GG (1972) Influence of cellular acidosis and altered cation concentrations on shock-induced mitochondrial damage. Surgery 72:102
7. Lillehei RC (1963) The nature of irreversible shock, experimental and clinical observations. Am J Cardiol 13:599
8. Mela LM, Miller LD, Bacalzo L (1973) Alterations of mitochondrial structure and energy-linked functions in hemorrhagic shock and endotoxemia. In: Arisztid GB, Kovach HB (eds) Symposium on neurohumoral and metabolic aspects of injury. Plenum Publ Corp, New York, p 231
9. Mela LM, Bacalzo LV, Miller LD (1971) Defective oxidative metabolism of rat liver mitochondria in hemorrhagic shock and endotoxic shock. Am J Physiol 220:571
10. Baue AE, Sayeed MM (1970) Alterations in the functional capacity of mitochondria in hemorrhagic shock. Surgery 68:40
11. Depalma RG, Levey S, Holden WD (1970) Ultrastructure and oxidative phosphorylation of liver mitochondria in experimental hemorrhagic shock. J Traumatol 10:122
12. Adam H (1965) Adenosine-5-diphosphate and adenosine-5-monophosphate. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, p 573
13. Lamprecht W, Traushold I (1965) Determination with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, p 543
14. Atkinson DE (1968) The energy charge of the adenylate pool as a regulatory parameter, interaction with feed-back modifiers. Biochem 7:4030
15. Atkinson DE (1970) Enzymes as control elements in metabolic regulation. In: Boyer PD (ed) The enzymes. Academic Press, New York, p 461
16. Williamson DH, Mellanby J (1974) D-(−)-3-hydroxybutylate and acetoacetate. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, p 1836
17. Ozawa K, Kitamura O, Mizukami T (1972) Human liver mitochondria. Clin Chim Acta 38:385
18. Ozawa K, Takasan H, Kitamura O (1971) Effect of ligation of portal vein on liver mitochondrial metabolism. J Biochem 70:755
19. Chance B (1959) Quantitative aspects on the control of oxigen utilization. In: Ciba foundation symposium on regulation of cell metabolism. Little Brown, Boston, p 91
20. Lowry OH, Rosenbrough NJ, Farr Al, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265
21. Lehninger AL (1959) Reversal of various types of mitochondrial swelling by adenosine triphosphate. J Biol Chem 234:2187
22. Lehninger AL (1960) Metabolic and structural state of mitochondria. J Biol Chem 235:242
23. Fiske CR, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66:375
24. Werle JM, Cosby RS, Wiggar CJ (1942) Observations on hemorrhagic hypotention and hemorrhagic shock. Am J Physiol 136:1042
25. Wiggars CJ (1950) Physiology of shock. Commonwealth Fund, New York, p 137
26. Chaudry IH, Sayeed MM, Baue AE (1974) Depletion and restoration of tissue ATP in hemorrhagic shock. Arch Surg 108:208
27. Ida K, Ukikusa M, Yamamoto M (1978) Stimulatory effect of adenosine on hepatic adenine nucleotides and energy charge levels in shocked rat. Circ Shock 5:383
28. Staple DA (1960) Comparison of adenosine triphosphate levels in hemorrhagic shock and endotoxic shock in the rat. Surgery 66:883
29. Ozawa K, Ida T, Kamano J, Garbus J, Cowley RA (1978) Different response of hepatic energy charge and adenine nucleotides concentrations to hemorrhagic shock. Res Exp Med 169:383

30. Yamamoto M, Sato M, Ida T, Ukikusa M, Ozawa K (1978) Obstructive jaundice and hemorrhagic shock. Circ Shock 5:235
31. Lehninger AL (1962) Water uptake and extrusion by mitochondria in relation to oxidative phosphorylation of liver mitochondria. Physiol Rev 42:467
32. Vogt MT, Farber E (1968) On the molecular pathology of ischemic renal cell death, reversible and irreversible cellular and mitochondrial metabolic alterations. Am J Pathol 53:1
33. Lehninger AL, Remmert LF (1959) An endogenous uncoupling and swelling agent in liver mitochondria and its enzymic formation. J Biol Chem 234:2459
34. Azzone GF (1961) Respiratory control and compartmentation of substrate level phosphorylation in liver mitochondria. J Biol Chem 236:1501

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