Mitochondrial Dysfunction in Sepsis

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I Introduction

While the essence of cellular metabolism and respiration are considered to be essential knowledge in medical education, there are few postgraduate specialties that require this information to be omnipresent during the day-to-day duties of the clinician. Convincing evidence exists to suggest that early improvement of oxygen delivery (D02) to the tissues can improve survival in patients with sepsis [1] and with this in mind, it is not hard to imagine the effects that abnormal cellular oxygen utilization could throw into the equation. Recent advances in the scientific literature highlight the need for fluency in the understanding of cellular respiration in order that one can comprehend the pathophysiology in sepsis and the potential for future therapies. The aim of this chapter is to reiterate the physiology of cellular respiration, in particular the role of the tricarboxylic acid (TCA) and electron transport pathways, and then review the recent literature in an attempt to explain where these pathways become dysfunctional during sepsis and why variation may have occurred in clinical studies of improving D02 in critically ill patients [1-3].

I Cellular Energetics

Before considering the intricacies of oxidative phosphorylation, it is worth contemplating the more fundamental aspects of energy dynamics within systems. The first law of thermodynamics states that, during the course of a chemical reaction in a closed system, energy can neither be created nor destroyed but can be converted from one form to another. The second law of thermodynamics states that in a closed system the total amount of usable energy (termed the Gibbs free energy or *deltaG*) is always decreased. In other words, a chemical reaction will only take place spontaneously if the reaction proceeds in the direction that results in a net decrease in the *deltaG*. Take the example:

A+B<->C

If the free energy *(deltaG)* of the forward reaction is positive, the reaction will not take place spontaneously and energy will be required to form C from A and B. It is an 'uphill' process and is said to be 'endergonic'. If however the *deltaG* is negative then the reaction of A with B to form C is an energetically 'downhill' process and will occur spontaneously. Such a reaction is 'exergonic'.

The clinical relevance of the above is that no enzyme or chemical reaction in the body can take place unless the *deltaG* for that reaction is negative (if it is zero, the

reaction will remain in equilibrium). In order to make a reaction with a positive *deltaG* occur spontaneously it must be 'coupled' to another reaction where the *deltaG* is negative such that the sum of their free energies (deltaGtot) is negative. This coupling can occur in several ways, the most common of which is for both the reactions to take place on the same enzyme. Given that so many of the reactions in the body are energy dependant (endergonic) there is a constant requirement for a highly exergonic reaction to which they can be coupled in order to give a negative *deltaGtot* and hence allow the overall reaction to proceed spontaneously. This highly exergonic reaction is provided in the hydrolysis of ATP, where:

ATp4- + H20 -> ADp3- + HPO_- + H+

This process has a *deltaG* -30.5 kJ and therefore occurs spontaneously.

An example of an endergonic reaction is the formation of glycerol-3-phosphate from glycerol:

glycerol + HPO_- -> (glycerol-3-phosphate)z- + *HzO*

This has a *deltaG* +9.2 kJ and thus will not occur spontaneously. However if the two reactions are coupled we get the following reaction:

glycerol + ATp4- -> (glycerol-3-phosphate)z- + ADp3- + H+

This combined reaction has a *deltaGtot* of -30.5 kJ +9.2 kJ=-21.3 kJ and thus will now proceed spontaneously.

It is obvious from the above why ATP has become the 'universal currency' that can be coupled with energy dependent processes. Hence, ATP depletion rapidly results in these processes coming to a halt. Over the past decade there has been increased interest in the effects of sepsis on the production of ATP and cellular respiration. Increasing evidence is coming to light to suggest that these are impaired in septic patients and in order to understand the pathways that are affected it is necessary to revise the basic physiology of cellular ATP production.

I ATP Production from Glucose

To make ATP, energy must be absorbed in the form of food, and must subsequently be released and packaged in a usable form. The simplest pathway to follow in order to analyze this process is the formation of ATP from carbohydrates, in particular glucose.

Glucose contains 15.7 kJ/g of free energy and therefore if 1 mole undergoes complete oxidation it will release 2826 kJ, which if collected could be used for energy-dependent cell functions. If this process were to occur in one step (e.g., combustion) this energy would be released in the form of heat and none would be stored. The challenge for the body therefore is to oxidize the glucose in a manner that allows the free energy to be released in a step-wise fashion so that it can be collected and utilized elsewhere. The breakdown of glucose into carbon dioxide (C02) occurs in two steps: glycolysis and the citric-acid cycle. These two pathways are used to recycle molecules of two important reducing agents, NADH and FADH2, which donate electrons to the electron transport chain with the resultant production of ATP. They are the key elements that transfer energy from the pathways involved in the breakdown of glucose to that involved in the production of ATP.

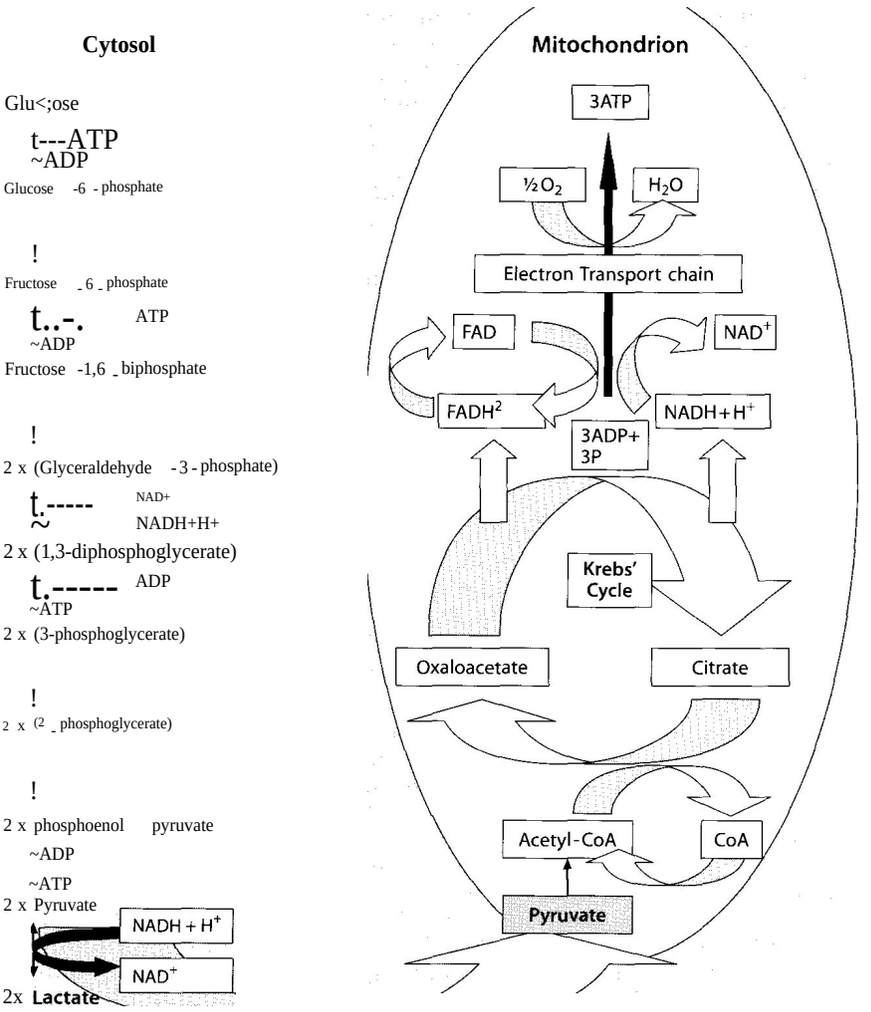

Fig. 1. Aerobic metabolism of glucose. FAD and FADH2=oxidized and reduced Flavin cofactors respectively; CoA = coenzyme A

The process of glycolysis is shown on the left hand side of Figure 1 with the interplay with the TCA cycle (Krebs' cycle) shown on the right hand side. It can be seen that glycolysis utilizes two molecules of ATP and produces four for every glucose molecule metabolized, with a net production of two ATP and two NADH. Here the ATP is formed by a process called 'substrate level phosphorylation' where the energy required to phosphorylate ADP is acquired by direct linkage to the chemical reactions:

1,3-diphosphoglycerate <-> 3-phosphoglycerate

and:

Phosphoenol pyruvate -> pyruvate

These reactions result in the formation of ATP whereas the others in the pathway do not because they are exergonic and contain a *delta*G greater than that required by the endergonic production of ATP from ADP. Indeed this must be a common factor in all reactions involved in the production of ATP.

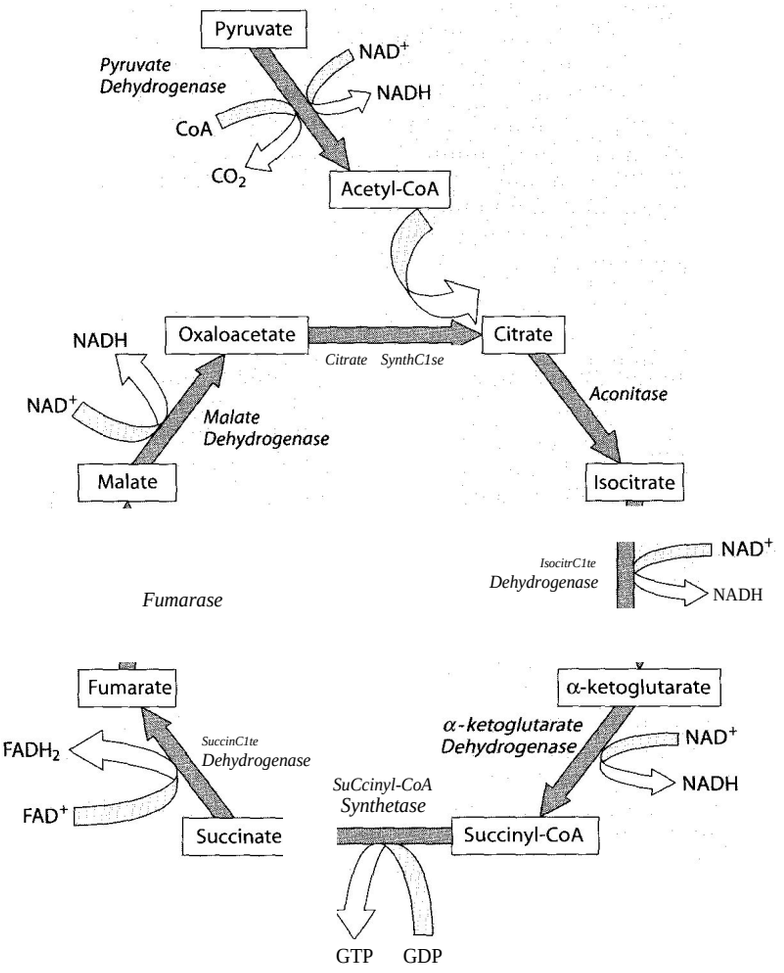

Fig. 2. The tricarboxylic acid (Krebs) cycle

The two pyruvate and two NADH produced by glycolysis subsequently enter the mitochondrion, the former by facilitated diffusion. The fate of the NADH will be discussed later. The pyruvate, meanwhile, is combined with coenzyme A to form acetyl-CoA. The catalyst for this reaction is pyruvate dehydrogenase and is an important enzyme that may be inactivated in sepsis [4]. The resultant acetyl-CoA combines with oxaloacetate to form citrate and so enters the Krebs' cycle depicted in Figure 2. A number of steps in this cycle release free energy that is stored in the reducing agents NADH and FADH2. In addition a molecule of GTP is produced for every cycle. This can be considered as equivalent to ATP in terms of the *delta*G stored in its 'high energy' phosphate bonds. Thus each 'turn' of the Krebs' cycle produces 4NADH + FADH2 + GTP. As two molecules of pyruvate enter the cycle for every glucose undergoing glycolysis, the net product is therefore twice this, i.e., 8NADH + 2FADH2 + 2GTP.

Combining glycolysis and the Krebs' cycle it is apparent that the equivalent of only four molecules of ATP are produced from the complete oxidation of glucose to CO2, These have a net energy content of 122 kJ (4x30.5 kJ), which would make the efficiency of the reaction only 4.5% if all the other energy was lost in heat (122/2826 kJ). Fortunately this latter situation is not the case. Much of the energy released is stored in the reduction-oxidation (redox) potential of the NADH and FADH2. It is the release of this energy via the electron transport chain by what is termed the chemiosmotic theory that results in the metabolism of glucose having an overall efficiency of nearly 45%. Thus the importance of these reducing agents cannot be overemphasized.

I The Electron Transport Chain and Chemiosmotic Theory

The ability to couple the redox potential of the NADH and *FADHz* to the phosphorylation of ADP to form ATP is a stroke of evolutionary genius, the mechanism for which has only recently been adequately elucidated. The essence of the process lies in the ability of the former agents to donate electrons and for the sake of simplicity NADH will be used as the example though the same principles apply to FADH2.

If a chemical element exists in a state that is far from its electrochemical equilibrium, it will have a tendency to achieve equilibrium by gaining or losing electrons. Thus it has an electrical potential that can be termed reduction potential if the element has a propensity to lose electrons. As within a battery, the movement of electrons occurs from areas of low electrical potential (reduction potential) to those with higher electrical potential and in doing so energy is released. They will move in the direction that results in the greatest change in electrical potential and this is the principle behind the one-way movement of electrons through the electron transport chain thus creating an electrical current. The structure of both the mitochondrion and the complexes within the electron transport chain are crucial to the coupling of the redox and phosphorylation reactions in what is termed 'oxidative phosphorylation' .

The mitochondrial membranes are the crucial areas where this coupling takes place. There are two membranes, a convoluted inner membrane, which is largely impermeable and an outer permeable membrane. The inner membrane contains a number of proton channels, pumps, and complexes that are together referred to as the electron transport chain (Fig. 3). Importantly, evidence exists to suggest that a

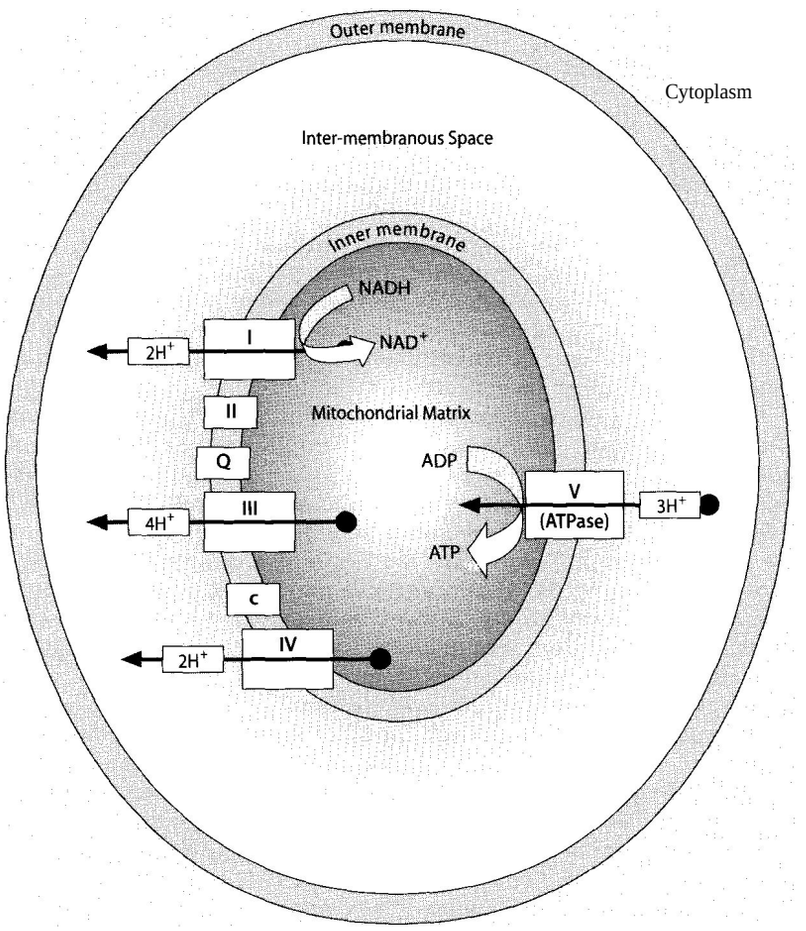

Fig. 3. The electron transport chain. Diagram showing complexes I, II, III, IV and ATP synthetase (V), the mobile electron carriers Coenzyme Q and Cytochrome c, and hydrogen ion movement (solid arrows)

number of these areas within the electron transport chain become dysfunctional in sepsis. All the components of the electron transport chain pathway are proteins with the exception of the NADH and succinate (which are soluble in the mitochondrial matrix) and coenzyme Q (CoQ), the latter of which acts as a mobile carrier of electrons between the primary dehydrogenases and cytochrome b. The electron transport proteins are clustered together to form complexes I, II, III, and IV. Complex I is composed of NADH dehydrogenase, non-heme-iron proteins, and a cofactor and is responsible for transferring electrons from NADH to CoQ. The electrical

energy contained within this transfer is referred to as *deltaE* and is related to the free energy of the reaction by the Nernst equation:

$$\Delta G = -n.\ F\ .\Delta E$$

where n is the number of electrons involved in the reaction and F is the Faraday constant.

Thus it can be established that the transfer of an electron through Complex I is highly exergonic and results in a *deltaG* of -79kJ. This is more than enough to form ATP when coupled with the endergonic reaction between ADP and inorganic phosphate.

Electron transfer through Complex-II, or succinate dehydrogenase, results in a *deltaG* of -10 kJ, which is insufficient to drive the synthesis of ATP. Of note it is at Complex-II that the FADH2 donates its electrons and it is for this reason that the oxidation of one molecule of *FADHz* results in the formation of only two ATP versus the three ATP produced by NADH due to the latter entering the electron transport chain at the more exergonic Complex-I stage.

The hydrophobic CoQ is reduced by Complexes- I and II and diffuses through the membrane to donate its electrons to Complex-III. The principle components of this latter complex are the heme-proteins, cytochromes *band c1,* and a non-heme-protein. The iron within the heme-proteins alternates between the oxidized (Fe^{H}) and reduced (Fe2+) forms as electrons pass within the complex.

After passing through Complex-III, the electrons are transferred by the mobile cytochrome c to Complex-Iv' This complex is also known as 'cytochrome oxidase' as it donates its electrons to the ultimate acceptor, oxygen. The reduced oxygen then combines with hydrogen ions to produce water. Complex-IV contains, amongst other proteins, the heme-proteins cytochromes *a* and *a3.*

The energy released by the passage of electrons through the electron transport chain is used to pump protons through the inner membrane from the matrix to the inter-membrane space. Complexes I, III, and IV achieve this task acting as the proton pumps. Thus a proton concentration gradient is established across the inner membrane and the potential energy so created is termed the 'proton-motive force' or PMF. The movement of these protons down their concentration gradient releases energy that is used by the ATP synthetase (Complex-V) to phosphorylate ADP and produce the desired ATP. The Complex-V is composed of Ft and Fo subunits which, when triggered by the passage of protons, bind ADP and inorganic phosphate at its catalytic site with the resultant product, ATP.

Thus from the above complicated pathway, it can be seen that transfer of electrons from NADH via the electron transport chain results in the production of three ATP and those from FADH2 produce two ATP. Thus the eight NADH, two FADHz and two GTP produced as a result of two pyruvate entering the Krebs' cycle give the equivalent of 30 ATP once they have entered into oxidative phosphorylation. Glycolysis yields two ATP and two NADH equivalent to eight ATP once oxidative phosphorylation has take place and thus the overall oxidation of glucose to water and carbon dioxide yields 38 ATP with an efficiency of nearly 45%, largely as a result of the redox reactions taking place within the chemiosmotic machinery.

I Mitochondrial Dysfunction in Sepsis

The delivery of oxygen to tissues in a state of sepsis has commanded a great deal of attention over the past two decades, and yet evidence to support techniques that enhance such delivery has been inconsistent, if not contradictory [1-3]. This reflects the complexity of the pathophysiology underlying cellular and organ dysfunction in sepsis. Over time, however, a number of authors have highlighted the possibility that cellular utilization of oxygen is impaired in sepsis and subsequently a number of studies have produced evidence to support this [5-14]. This phenomenon has not been replicated consistently though [15, 16] suggesting once again that a complex interplay of factors exists in producing the organ dysfunction that we see clinically.

In tissue that exhibits oxygen consumption at a greater rate than it is being delivered, under normal physiological conditions one would expect to see an increase in the extraction ration for oxygen entering and leaving the cells. Thus the distribution of tissue P02 should move to the left showing more values nearer zero. However, this has not consistently been found to be the case. Whole body and regional oxygen extraction have been shown to decrease or remain static in a number of studies using animal models of sepsis [5-7] though this phenomenon has not been replicated in human models [15]. In one animal study [7], a group of pigs were infused with *Escherichia coli* lipopolysaccharide (LPS) and were resuscitated once the subsequent septic response ensued. Distal ileal mucosa was analyzed for pH, blood flow and P02, and compared with a control group. The surprising finding was that although mucosal blood flow was similar between the two groups, acidosis developed in the septic group in the presence of increased *POz*. Thus, ongoing ATP hydrolysis in the presence of reduced oxygen extraction ratio was observed, inferring that cellular oxygen utilization was dysfunctional.

Subsequent studies have aimed to analyze where the defect in oxygen utilization may lie and whether or not this can be reversed. While considering these studies it is worth referring back to the previously described metabolic pathways involved in ATP production. It is generally accepted that nitric oxide (NO) is upregulated in sepsis [17] and that inducible NO synthase (iNOS) can appear in a number of organs and tissues in animal models of sepsis [18, 19]. A number of studies have highlighted NO and iNOS as contributing to cellular dysoxia [10, 13, 20]. Indeed inhibition of iNOS by the use of amino guanidine has been shown to restore cellular oxygen utilization to normal values in endotoxemic rats [9, 10]. In conditions of raised NO levels, NO has been shown to inhibit Complex I and the cytochrome a,a3 portion of Complex IV [13, 21], thus preventing electron transfer to molecular oxygen and thus inhibiting the electron transport chain. This inhibition is associated with a decrease in reduced glutathione concentrations and while *in vitro* data suggest that this state can be reversed by the addition of exogenous glutathione [21], this was not the case when studied in humans [13]. The transfer of electrons along the electron transport chain may be disrupted by competition between oxygen and NO for binding sites on mitochondrial electron transport chain complexes [22]. Of interest, Levy et al. [11] showed that although the inhibition of cytochrome oxidase was competitive early in the septic phase, this became non-competitive by 48 hours. Such observations may explain the variation seen in clinical trials aiming to optimize D02 to tissues in septic patients depending on the time period at which interventions were initiated [1, 3]. While NO seems a likely culprit in causing cellular dysoxia, it is the formation of peroxynitrite from the reaction between NO and

reduced oxygen that has more potential for toxicity. Peroxynitrite has been shown to inhibit electron transport chain complexes I, II, and V [23], and the enzyme aconitase that converts citrate into isocitrate in the Krebs' cycle [24].

Another possible mechanism for cell dysfunction is the over expression of poly-(adenosine 5'-diphosphate-ribose) synthetase (PARS) in sepsis. This nuclear enzyme is activated in the presence of DNA single-strand breakages, when it triggers the metabolically inefficient transfer of ADP-ribose to nuclear proteins. This results in the rapid depletion of NAD and ATP leading to cell injury and death [25]. It has been shown that PARS production increases in sepsis as a result of increased DNA single-strand breakage caused by reactive oxygen radicals and peroxynitrite. Inhibitors of PARS have been shown to be protective against cell damage in free-radical mediated cell injury [25] and in sepsis [8, 14]. Lastly, as mentioned previously, increased inactivation of the enzyme pyruvate dehydrogenase may occur in sepsis as a result of an increase in pyruvate dehydrogenase kinase levels [4]. This results in reduced conversion of pyruvate to acetyl-CoA and subsequent 'starvation' of substrate for the Krebs' cycle.

I Conclusion

The disruption of cellular oxidation utilization may play an important role in causing organ dysfunction in sepsis. Evidence suggests that the pathways involved may include decreased acetyl-CoA formation from pyruvate, inhibitory effects of NO and peroxynitrite on electron transfer complexes, peroxynitrite inhibition of aconitase, and the over production of PARS resulting in NAD depletion. Of particular interest is that the dynamics of such interference appears to change during the time-course of the septic insult, which may explain the inconsistencies seen in the results from clinical trials aiming to improve *DOz* to tissues in critically ill patients. At present, manipulating these disrupting influences has shown variable results with no clinically useful drugs or techniques materializing. However, in the future this situation may be reversed as our knowledge of the pathophysiology behind sepsis and cellular dysoxia improves. It may yet be that a detailed understanding of cellular respiration proves to be an important factor in improving survival in patients with sepsis.

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