THE HEART IN ANOXIA AND ISCHAEMIA

BY

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In its structure and mechanics the heart does not differ greatly from skeletal muscle (Szent-Gyorgyi, 1953). However, unlike skeletal muscle, the myocardium must undergo constant rhythmic contraction. Olson and Schwartz (1951) point out that the heart is admirably designed both structurally and biochemically for continuous aerobic work. The myocardium is three to four times as vascular as skeletal muscle and the blood flow ten to twenty times as great per unit weight of tissue. Coronary sinus blood has a very low oxygen content indicating high oxygen extraction. The oxidative enzymes, cytochrome C, cytochrome oxidase and succinic dehydrogenase are more concentrated in the heart than anywhere else in the body. Also there is a closer adjustment between energy production and energy utilization in the myocardium than in other muscles. Creatine phosphate which is usually considered as a source for reserve energy is less abundant in the myocardium than in skeletal muscles (Lipman, 1941). Recently Sarnoff et al. (1958) were unable to detect any oxygen debt incurred by the heart under physiologic work load.

It has become increasingly important to understand how this normally aerobic organ responds when deprived of its usual supply of oxygen in varied circumstances such as myocardial infarction, haemorrhagic shock and modern heart surgery. In this paper we shall discuss the local compensatory mechanisms for increasing the supply of oxygen, the metabolic changes that occur when these compensatory mechanisms do not keep pace with the demand and finally the survival of the completely ischaemic heart.

I. COMPENSATORY MECHANISMS TO MAINTAIN OXYGENATION.

When the demand of an organ for oxygen increases, the need may be met by increasing the blood flow and by increasing the amount of oxygen extracted from the available blood. The heart usually responds predominantly in the first manner (Bing and Daley, 1951; Edwards et al., 1954). In normal physiologic states oxygen utilization of the heart is almost directly proportional to the coronary blood flow although changes in cardiac output and aortic pressure may affect this relationship slightly (Braunwald et al., 1958). The increase in coronary blood flow in response to oxygen need is brought about by lowering the coronary vascular resistance. The exact stimulus for this coronary vasodilation is unknown. It is not in response to lowered oxygen tension per se (Berne, Blackmon and Gardner, 1957), to increased concentrations of lactic acid or carbon dioxide (Hilton and Eicholtz, 1925), or to high concentration of potassium (Driscol and Berne, 1957); nor is it mediated by the adrenals or sympathetics (Hackel and Clowes, 1956).

Hackel, Goodale and Kleinerman (1954) have demonstrated in intact dogs, breathing low concentrations of oxygen, that increased oxygen extraction occurs and may play a part in maintaining myocardial oxygenation when the oxygen needs of the heart can no longer be met by increased coronary blood flow. However, in their experiments increased oxygen extraction did not take place until the arterial oxygen content had reached 8 volumes per cent. The degree to which this mechanism may be helpful is limited by the already high rate of oxygen extraction that occurs in the normal heart.

II. RESPONSE TO HYPOXIA.

When, despite compensatory mechanisms, the myocardium receives less oxygen than is necessary to maintain its normal aerobic metabolism, the oxygen tension in the myocardium falls. This fall must necessarily be accompanied by a shift in the oxidation-reduction systems towards a more reduced state. The extent to which individual
As a result of failure of oxygen extraction to increase, myocardial oxygen usage declined. In the hypovolaemic phase of shock blood levels of glucose rose to high peaks, but the rise in glucose extraction was statistically not significant. Pyruvate extraction fell and negative coronary A-V differences occurred frequently. Blood lactate rose to very high levels, presumably as a result of anaerobic glycolysis elsewhere in the body. Total myocardial extraction of lactate was actually higher than during control periods, but not as high as one would have expected in a normal, fully oxygenated heart. After retransfusion to normal blood volume the myocardial extraction of lactate and pyruvate was depressed similar to that during the hypovolaemic state. Blood glucose levels fell to normal with increases in the blood volume. Myocardial glucose extraction was statistically not different from that during the control period; however, in several instances concentrations of coronary vein glucose exceeded that in arterial blood. Thus the essential changes in coronary blood flow and myocardial metabolism in haemorrhagic shock persisted during the normovolaemic phase. In both stages myocardial oxygen usage was low and the extraction of glucose, pyruvate and lactate was impaired.

III. RESPONSE TO ISCHAEMIA.

If the oxygen supply to the heart is completely interrupted, the muscle cells die unless blood flow is resumed before irreversible damage occurs.

Scattered reports have suggested that the anoxic heart may survive for long periods (see, for example, Kulako, 1903), but the general clinical and laboratory experience has been that short periods of anoxia result in myocardial death. Blumgart, Gilligan and Schlesinger (1941) noted gross and microscopic evidence of infarction in dog hearts in which the coronary artery was occluded for 25 to 45 minutes but no evidence of infarction when the occlusion lasted less than 20 minutes. Cooley and DeBakey (1956) interrupted the coronary circulation for 31 minutes using cardiac by-pass during aortic surgery. The patient recovered. Wesolowski and his co-workers (1952) arrested coronary perfusion in dog hearts excluded from the general circulation by means of a pump-oxygenator for 30- and 60-minute periods. Only four of the thirteen animals lived more than 3
days. Webb and Howard (1957) performed similar experiments on dogs, but the coronary vessels were flushed out with Ringer's solution to prevent intravascular clotting. After 90 minutes of complete anoxia the hearts were again included in the circulation. Eleven of twelve hearts were able to sustain whole body circulation. There were no long-term survivors, however.

Recent concurrent studies in this laboratory have centred on the survival of the excitability, energy production and energy utilization of the heart (Kardesch, Hogancamp and Bing, 1958b).

A. Survival of energy production.

The normal heart extracts significant amounts of glucose, pyruvate, lactate, fatty acids, ketones and amino acids from the coronary arterial blood (Bing et al., 1953, 1954; Ungar et al., 1955). In the postabsorptive state the myocardium derives 70 per cent of its energy requirement from non-carbohydrate material, chiefly fatty acids (Bing et al., 1954), but the actual amounts of substrate extracted vary directly with the blood level of the substrate. Total myocardial energy production is reflected in the oxygen consumption of the heart providing glycolysis is absent.

Michal, Beuren, Hogancamp and Bing (1958) have studied the survival of energy production in the arrested dog heart. The arrested heart consumes about 25 to 35 per cent as much oxygen as does the normally beating heart (Beuren, Sparks and Bing, 1958). The heart was stopped with potassium chloride and the coronary arteries were perfused. Oxygen consumption and the myocardial extraction of its usual foodstuffs were determined. After control samples had been obtained, coronary artery perfusion was interrupted for a predetermined period of time, following which it was resumed. Oxygen consumption and the extraction of foodstuffs were again determined and these values were compared with control figures.

Results disclosed that oxygen consumption returned to control levels if the period of interruption did not exceed 2 hours (fig. 1). Longer interruption of the perfusion resulted in incomplete return to control levels. After 4 to 5 hours of anoxia the myocardium completely lost its ability to extract oxygen. Myocardial glucose extraction returned to normal even when the oxygen usage was decreased. The extraction of more glucose than could be oxidized by the oxygen taken up indicated that glucose entered metabolic pathways other than oxidative ones. Such would be the case if oxidative enzymes were more sensitive to oxygen lack than were other enzyme groups. Pyruvate extraction fell after a period of anoxia and lactate production by the heart increased even in the presence of adequate oxygen uptake.

When tissue slices were studied rather than the whole heart, oxygen uptake was found to return to only 40 per cent of control values after only 1 hour of anoxia (fig. 2). Rapid falls in oxygen consumption in tissue slices have also been reported by Webb, Saunders and Thienes (1949) and by Fuhrman, Fuhrman and Field (1950). The respiratory quotient (R.Q.) of the heart slices rose to levels above 1. There was a linear relationship between the preceding period of anoxia and the height of the R.Q. Since glucose was the substrate used in the media, the R.Q. should normally be 1. The excess carbon dioxide contributing to the high R.Q. must have originated from the action of acid on the bicarbonate buffer system of the media. These findings favour the production of lactic acid despite adequate supply of oxygen. Thus in heart slices, as in the whole heart, there is evidence for inefficiency of aerobic pathways of metabolism.

The study of the survival of myocardial enzymes after the onset of complete ischaemia is still in an early stage. Dixon (1951) points out that many essential constituents of living matter are unstable substances continually needing resynthesis. This is true of some enzymes and especially true of many coenzymes. It is the oxidative reactions that they themselves catalyze that furnish the free energy for their resynthesis and keep the enzyme-coenzyme systems in being. He reasons that the reactions may continue anaerobically if the oxygen supply is interrupted. Soon the coenzyme concentration starts to fall. If oxygen is supplied before the fall has proceeded too far, the metabolic processes will start up again and rebuild the supply of essential coenzymes. If, on the other hand, the supply of coenzyme has fallen too far, oxidative processes cannot restart and coenzyme cannot be resynthesized. Death is the result.
The nature of the changes in enzymatic activity with anoxia has not been established. Govier (1944) found very little destruction of DPN 1 hour after death. Two hours after coronary ligation the DPN was considerably diminished, but there was no change in the coenzyme (Govier, 1945). Working with tissue slices that had been incubated under anoxic conditions for 30 minutes, Bernheim and Bernheim (1944) found that the heart utilized succinic acid, indicating that the depressed oxygen uptake was not caused by damage to the succinic acid dehydrogenase or the cytochrome-cytochrome oxidase system. Lactic acid and pyruvic acid were also consumed, indicating no significant damage to lactic or pyruvic dehydrogenase or to DPN. Staining techniques have also demonstrated the resistance of succinic dehydrogenase to anoxia (Wachstein and Meisel, 1955).

B. Survival of energy utilization.

The energy obtained from the oxidation of foodstuffs must be transformed into mechanical energy. In the myocardium as in other muscles the energy from metabolic pathways is collected as high energy phosphates (Lipman, 1941). These high energy sources are then drawn upon as needed for mechanical work (Szeg-Gyorgyi, 1953). Adenosine-triphosphate (ATP) is thought by many to be the main energy transfer agent in muscle although other high energy phosphate compounds may also play a minor part (Perry, 1956b).

Many workers using a variety of techniques...
have deepened understanding of the chemistry and physiology of muscle contraction. Extraction techniques have shown that there are at least two contractile proteins in muscle—actin and myosin (Bailey, 1956). Szent-Gyorgyi (1952, 1953) believes that in the normal resting state actin and a myosin-ATP complex exist independently. Part of the myosin molecule (L-meromyosin) is thin and straight. This portion of the protein is kept from folding by repulsive forces between potassium ions attracted by ionized groups in close proximity along the molecule. When a wave of excitation passes this equilibrium is disturbed. Actin combines with the myosin-ATP complex. The characteristics of the myosin molecule are changed and in the presence of ATP increased pliability of the thin L-meromyosin segment occurs. The result is a collapse of this segment and shortening of the myosin molecule. ATP is broken down to ADP and inorganic phosphate during the reaction. For relaxation the heart requires ATP along with creatine phosphate (CP), magnesium and a heat stable protein (Marsh, 1952) identified as myokinase (Szent-Gyorgyi, 1956). In Szent-Gyorgyi's scheme relaxation would require a dissociation of actin and myosin and the regeneration of ATP, presumably in part at least from transfer of high energy phosphate from CP to adenosine diphosphate (ADP). There is some doubt as to the adequacy of this conceptual model (Perry, 1956a). Experiments by Mommaerts (1955) and Fleckenstein, Janke, Davies and Krebs (1954) have demonstrated that under certain conditions muscles can contract without breakdown of either ATP or CP.

H. Huxley and Hanson (1954) and A. F. Huxley and Niedergerke (1954) using morphologic studies came to conclusions that result in a somewhat different picture of muscle contraction and relaxation. They believe that actin filaments arise from either end of the sarcomere or muscle unit and that myosin filaments arise from the

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**FIG. 2**

Per cent of initial oxygen uptake by dog heart slices plotted against hours after death of the animal.
middle of the sarcomere. These filaments then interdigitate. In the relaxed state the filaments slide easily along each other. During contraction this sliding is brought about by internal forces, probably by obliquely oriented cross linkages between the actin and myosin filaments. H. Huxley (1954) found no evidence for actual shortening of myosin filaments until they were crowded by extreme shortening of the sarcomeres.

Szent-Gyorgyi (1953) describes a "plasticizing" effect of ATP. After a change in muscle length has occurred, either by stretching or by shortening, the plasticizing quality allows the muscle to regain a tension almost equal to that originally held. Without this effect a muscle becomes rigid and responds to slow stretch with an increase in tension, finally tearing if the stretch proceeds too far.

This change in muscle property is exactly what happens in rigor mortis. Szent-Gyorgyi (1953) states that rigor mortis is due not only to the failure of actomyosin to dissociate and reverse muscle contraction, but also to the formation of abnormal cross linkages between different filaments. H. Huxley (1956), using low angle X-ray studies, showed well marked equatorial reflections at right angles to the long axis of muscle. In rigor these reflections are changed and secondary rods appear parallel to them. He suggested that in rigor the secondary filaments crystallize out permanently, thus rendering potentially reversible changes in the contractile proteins irreversible.

Under a wide variety of physiologic conditions stiffening of muscle after death is correlated with loss of ATP (Bate-Smith and Bendall, 1956). In the absence of oxygen, energy may be made available for resynthesis of ATP from creatine phosphate and from glycogen via anaerobic glycolysis. Rephosphorylation of ADP then may continue as long as adequate stores of glycogen and CP persist. When the stores are exhausted, ATP levels fall and dynamic resynthesis of ATP is upset. Bendall (1951) has shown that the onset of rigor mortis is more closely correlated with a decreased turnover rate of ATP than with the actual level of ATP.

After death or in complete anoxia, the pH of muscle cells falls with the production of lactic acid. Bendall (1951) points out that the initial pH at death varies the state of the muscle at that time. The end pH is dependent on the total lactic acid production which is in turn a function of the glycogen stores available at the time of death. The relationship of the onset of rigor mortis to the pH has been elucidated (Bendall, 1951). The turnover rate of ATP is affected by the pH. The maximal turnover rate is near a pH of 6.3. Hence, at this pH stores of glycogen and ATP are rapidly depleted. The pH dependence curve of ATP breakdown is very similar to the myosin-ATPase activity curve and suggests a relationship with this enzymatic activity (Bate-Smith and Bendall, 1949). The breakdown of ATP is temperature-dependent. Increasing temperature shortens the delay period of rigor mortis (Bate-Smith and Bendall, 1949).

When one turns from skeletal muscle to heart muscle, one finds that rigor mortis occurs much more rapidly (Lawrie, 1953). This rapid onset of rigor is in keeping with the low CP supply of heart muscle (Fawaz and Hawa, 1953). Being well adapted for continuous aerobic work, the heart has little stored energy.

The survival of contractile proteins of human heart muscle after death has been studied by Kako and Bing (1958). Actomyosin bands were obtained from muscle homogenates prepared at varying intervals after death. Properly prepared actomyosin bands in a suitable ionic environment will contract on the addition of ATP. The contraction of actomyosin bands from normal hearts is influenced by the ionic concentration and the load, but, when these factors are constant, the results expressed as per cent shortening do not vary greatly. There is no significant difference in the response of actomyosin bands prepared immediately after death to bands prepared up to 6 hours after death (Kardesch, Hoganancamp and Bing, 1958).

C. Survival of myocardial excitability.

In order to function, the heart must undergo rhythmic contraction and relaxation. This repetitive cycle is triggered by changes in potential at the cell membrane. The ability of the myocardial cell to respond to stimuli with change of membrane potential and contraction is termed excitability. In the heart a new contraction is dependent on the spontaneous initiation of action potentials by the pacemaker.

Brooks, Hoffman, Suckling and Orias (1955)
review concepts of the cell membrane and the mechanism by which action potentials arise. A brief conceptual framework borrowed largely from Hodgkin (1951) will be reviewed here to aid in considering factors important in survival of the heart. The cell membrane may be considered as a semipermeable membrane that greatly restricts movement of sodium ions and to a lesser extent hinders passage of potassium ions. Inside the cell is an active pump which extrudes Na+. The loss of positively charged Na+ disturbs the electroneutrality. Potassium ions then diffuse in, driven against the concentration gradient by the potential gradient. An equilibrium is eventually reached with a relatively high concentration of K+ and a low concentration of Na+ in the cell. At this equilibrium there is an electrical potential of from 80 to 90 mV across the cell membrane so that the outside of the cell is positive to the inside. The magnitude of this potential depends on the ratio of the K+ outside the cell to the K+ inside the cell.

When the membrane potential is lowered by a suitable stimulus, the permeability to sodium increases. A rapid influx of sodium is brought about by both the concentration and potential gradients. The influx of Na+ is sufficient to neutralize the charge across the cell membrane. In fact, there is a "positive overshoot" that reverses the resting polarity. These changes account for the rapid depolarization phase found when unicellular action potentials are obtained (fig. 3A). At the end of this phase, the membrane again becomes impermeable to sodium and more permeable to K+. K+ then diffuses out of the cell until the equilibrium between the potential gradient and the concentration gradient is restored. The repolarization phase is much slower than the phase of depolarization. At the end of the depolarization-repolarization cycle the cell is left with a small quantity of excess sodium that is removed during the resting phase by the Na+ pump. This conceptualization overlooks the fact that considerable evidence can be marshalled in favour of an active K+ transport as well (Weidmann, 1957).

Much of this knowledge has been obtained in recent years from a technique developed by Ling and Gerard (1949) in which a microelectrode is placed in a single myocardial cell to record unicellular action potentials. Recently a modification of the technique has been used in this laboratory to study the effect of complete ischaemia on the intracellular electrical activity in the left ventricular muscle of rabbits and dogs (Kardesch, Hogancamp and Bing, 1958a). The hearts were perfused and normal ventricular action potentials were obtained. Following a control period the hearts were rendered anoxic. Soon a shortening of the duration of the action potential was noted, suggesting a more rapid influx of K+ during repolarization. There was a progressive loss in the height of the action potentials. After an average of 13 minutes of complete ischaemia the membrane no longer completely depolarized (fig. 3B). After 20 minutes the action potential ceased entirely. At this time the resting membrane potential averaged only 65 per cent of that obtained with full oxygenation. Usually, an action potential can be obtained only if the resting membrane potential is sufficiently high. Weidmann (1957) has shown that if the membrane potential of an exhausted kid Purkinje fibre is raised to normal levels electrically, the ability to respond with an action potential is restored. As the action potential declined during anoxia, the rate and strength of muscle contraction also decreased (Kardesch,
Hogancamp and Bing, 1958a). If the hearts were reperfused after 10 minutes of standstill, the action potentials reappeared and returned rapidly to normal. Repeated episodes of ischaemia over a short time span caused increasingly rapid loss of the action potential.

Similar results have been obtained in the rat atria by Webb and Hollander (1956) and in the cat papillary muscle by Trautwein and Dudel (1956). It seems possible to explain inability to maintain the resting potential in anoxia by a failure of a Na+ pump or a Na+ – K+ pump.

Webb and Hollander (1956) found that 2, 4-dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, affected the atrium in a fashion almost identical to that of anoxia. This suggests that decline of ATP levels may be related to the effects of both DNP and anoxia. Furthermore, Greiner (1952) has shown that decline in force of contraction in electrically stimulated anoxia cat papillary muscles is accompanied by a fall in ATP. In view of the work cited previously one would expect the force of contraction to be correlated with the action potential. Ling and Gerard (1949b) were able to demonstrate that loss of membrane potential paralleled the loss of creatine phosphate and the development of rigor mortis in frog sartorii that had been rendered anoxic or poisoned with iodoacetate.

The simultaneous occurrence of these changes may be fortuitous; however, the movement of ions against concentration and electrical gradients requires energy and is markedly reduced in the rat ventricle by anoxia (Hercus, McDowall and Mendel, 1955), and by metabolic inhibitors in the giant squid axone (Hodgkin and Keynes, 1955). High energy phosphates are a logical source of energy to maintain membrane potential.

To summarize the studies on survival: the completely anoxic heart will continue to function as a pump only as long as the resting membrane potential remains sufficiently high and action potentials are generated and propagated. Action potentials decline and disappear over about 20 minutes of complete ischaemia. At this time neither the metabolic processes nor the contractile proteins are irreversibly damaged.

Myocardial anoxia and ischaemia are important in a variety of clinical entities such as myocardial infarction and haemorrhagic shock. Among today's pressing problems are the determination of safe periods of myocardial ischaemia during surgery and the delineation of the factors that affect the duration of these periods. While much of the work cited above is not yet clinically applicable and much remains to be done, better understanding of the pathologic physiology and biochemistry of the heart must of necessity lead to improved clinical care.

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